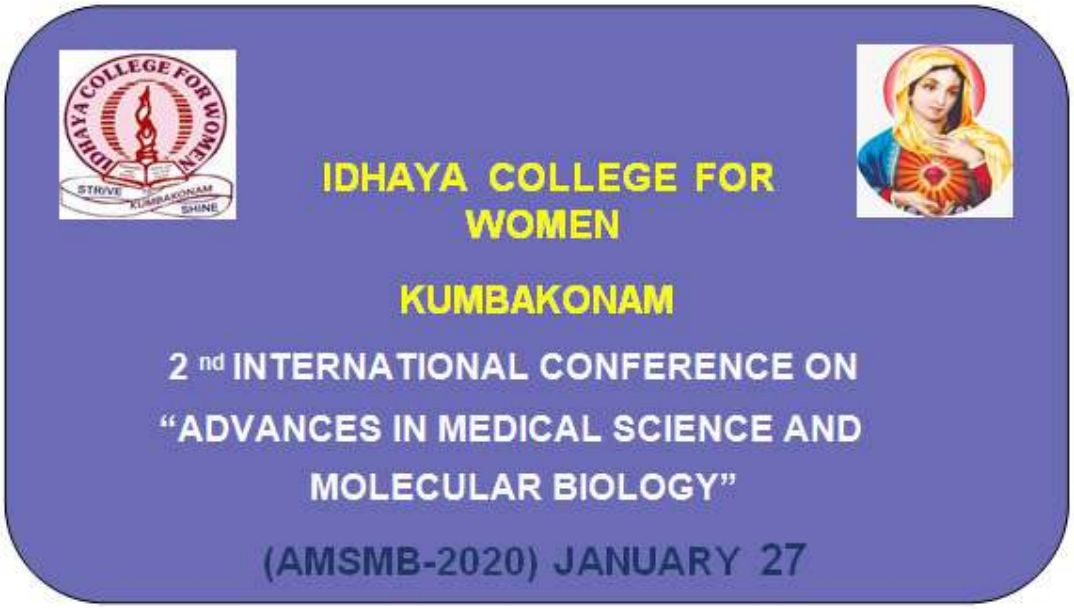


*A Special issue on*

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# "Advances in Medical Science and Molecular Biology-AMSMB-2020"

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## 2nd INTERNATIONAL CONFERENCE ON "ADVANCES IN MEDICAL SCIENCE AND MOLECULAR BIOLOGY"

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# ABOUT THIS SPECIAL ISSUE

Greetings to all from PG & Research Department of Microbiology, Idhaya College for women, Kumbakonam.

It gives me intense pleasure to publish this journal of our 2nd International Conference on "Advances in Medical Science and Molecular Biology" (AMSMB-2020) January 27". The special issue focuses on Molecular and Medical techniques. The journal comprises of various topics like Molecular Biology, Genetics and Medical Microbiology. It includes interesting features that make connections between scientific concepts and everyday world. Molecular medicine is wide field helps to understand the normal body functions and diseases pathogenicity at the molecular level by using physical, chemical, biological and medical techniques. It enhances the maximum utilization of scientific knowledge about Molecular Biology. The conference intends to focus on challenges and it brings new ideas to the students and scholars for their future platform in advanced fields.

The articles published in the special issue with developing field of biological sciences which has many important applications. This publication would not be possible without the tremendous contribution of authors and reviewing committee. Their contribution and efforts are greatly appreciated.

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# CONTENT (ORIGINAL RESEARCH / REVIEW ARTICLE

SP-01

# DETERMINE AND ANALYZE THE GENETIC DIVERSITY OF MITOCHONDRIAL DNA FROM SEPIA SPECIES BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM" (RFLP)

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## ABSTRACT

The nucleotide divergence between sequences of one gene or a portion of a gene from the mitochondrial genome can be analyzed phylogenetically. Mitochondrial DNA (mtDNA) sequence diversity has been used in several studies to differentiate between organisms and to establish relationships between them. Nucleotide differences between individuals can be identified by using restriction endonucleases which cut the DNA at specific recognition sequences. Fragments of different sizes are generated when individuals differ in their DNA sequence; these are called restriction fragment length polymorphisms (RFLPs). RFLPs of mtDNA have proven to be useful for determining relatedness among the closely related organisms. The findings of the existence of mitochondrial DNA in all animals and plants and the isolation and molecular analysis of the same have emerged to answer many intra-species relationships between different organisms.

**KEYWORDS**: mtDNA, Sepia, RFLP, Electrophoresis

## I. INTRODUCTION

Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. The population will continue for more generations because of the success of these individuals[1]. High genetic diversity results in difficulty in designing targeted vaccines and allows for viruses to quickly evolve resisting vaccination lethality. For example, malaria vaccinations are impacted by high levels of genetic diversity in the protein antigens[2]. In addition, HIV-1 genetic diversity limits the use of currently available viral load and resistance tests[3]. The human mitochondrial genome is a circular DNA molecule of about 16 kilobases[4]. It encodes 37 genes: 13 for subunits of respiratory complexes I, III, IV, and V, 22 for mitochondrial tRNA (for the 20 standard amino acids, plus an extra gene for leucine and serine), and 2 for rRNA. One mitochondrion can contain two to ten copies of its DNA[5]. The near-absence of genetic recombination in mitochondrial DNA makes it a useful source of information for scientists involved in population genetics and evolutionary biology[6]. Structural analysis of mitochondrial DNA (mtDNA) by restriction endonuclease digestion and agarose gel electrophoresis has proven to be of wide importance for the assessment of genetic relatedness in systematic and population genetic studies[7&8]. DNA sequencing techniques have made the sequencing of large stretches of DNA (e.g. mitochondrial genomes) both quick and economical, and mitochondrial genome-based analyses are playing an increasingly important role in phylogenetic and population genetic studies[9].

## II. MATERIALS AND METHODS

The sepia species were collected from the coastal regions of Bay of Bengal, Indian Ocean, and the Arabian Sea and were processed for Mitochondrial DNA (mtDNA) isolation. Briefly, 2 gm of tissue samples were homogenized with 10 volumes of TE Buffer and stored at -20°C. The mitochondrial DNA was then isolated by the CTAB method with little modification and the mtDNA was analyzed for the quality by 1%

agarose gel. The mtDNA was digested with 5 different restriction enzymes such as BamH1, EcoR1, HaeIII, Hind III, and Taq1. The restriction fragment Length Polymorphism (RFLP) of mtDNA was analyzed by 12% non-denaturing polyacrylamide gel.

## III. RESULTS

### 1. Isolation and size determination of mtDNA

The presence of mtDNA after the extraction procedure was checked by 1% Agarose gel electrophoresis. The mtDNA was seen at the position of 16 Kb as shown in Figure 2. The size of the mtDNA was compared and determined with the use of the DNA markers which were run along with the Sepia mtDNA samples. The first DNA marker was lambda DNA digested with Hind III restriction enzyme, which has the bands corresponding to the following base pairs (i.e. 23130, 9416, 2322, 2027, 564, 125 bp) and the second DNA marker was lambda DNA digested with Bam HI restriction enzyme, which would have the bands of 16841, 7233, 6770, 6527, 5626, 5505 base pairs. In figure 2, it is evident that the isolated mtDNA was located in the region corresponding to 16841bp of lambda / BamHI digestion.

### 2. RFLP analysis of mtDNA with Taq1 restriction enzyme

The restriction digestion pattern of mtDNA of 5 different Sepia species with the Taq 1 restriction enzyme yielded 8 different bands ranging from (5-7) in each species with an average of 6 bands per sample. The RFLP pattern revealed significant genetic variation among different individuals as evidenced by the difference in banding pattern and banding position as shown in Figure 3. Each band was considered as different loci and each locus was labeled from L1 to L8.

### 3. RFLP analysis of mtDNA with HaeIII restriction enzyme

Hae III restriction enzyme yielded 6 different bands ranging from (4-6) in each species with an average of 4.6 bands per sample. The RFLP pattern revealed significant genetic variation among different individuals as evidenced by the difference in banding pattern and banding position as shown in Figure 3. Each band was considered as different loci and each locus was labeled from L1 to L6.

### 4. RFLP analysis of mtDNA with Hind III restriction enzyme

Hind III restriction enzyme yielded 4 different bands ranging from (3-4) in each species with an average of 3.2 bands per sample. The RFLP pattern revealed no significant genetic variation among different individuals as there is no significant difference in banding pattern and banding position except the genotype CM as shown in Figure 4. Each band was considered as different loci and each locus was labeled from L1 to L4.

### 5. RFLP analysis of mtDNA with EcoRI restriction enzyme

EcoRI restriction enzyme yielded only 2 bands ranging from (1-2) in each species. The RFLP pattern revealed no significant genetic variation among different individuals as there is no significant difference in banding pattern and banding position except the genotype CM as shown in Figure 4. Each band was considered as different loci and each locus was labeled from L1 & L2.

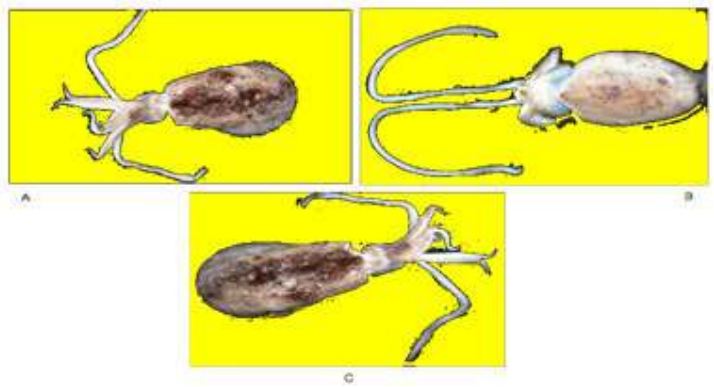

**Figure 1: Morphology of Sepia Species**

**M1 M2 S1 S2 S3**


**Figure 2: Agarose Gel Electrophoresis Analysis of Undigested mtDNA: M1; Lamda DNA/Hind III digest, M2; Lambda DNA/EcoRI digest, S1, S3; MtDNA samples isolated from Sepia, S2; Genomic DNA isolated from blood as negative Control**

**Taq1 Dgestion HaeIII Digestion**
**L ER KM CM KL VZ CM ER KL VZ KM**

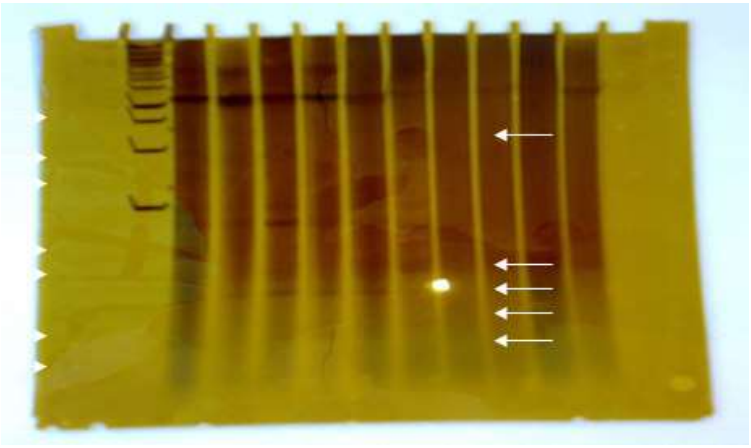

**Figure 3: RFLP - Lane 1 (L): 1Kb DNA ladder, Lane 2,3,4,5, & 6; mtDNA digested with TaqI enzyme, Lane 7,8,9,10, and 11; mtDNA digested with HaeIII enzyme**

**E VZ CM KL KM ER L VZ CM KL KM ER H**

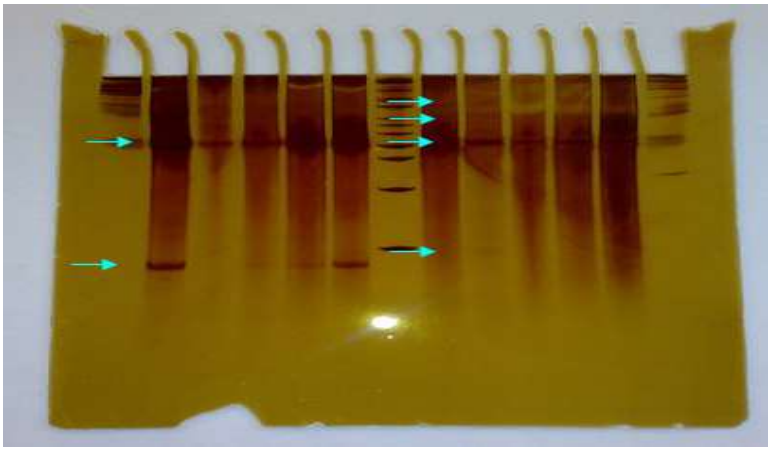

**Figure 4: RFLP - Lane 1 (E): Lambda/DNA EcoRI marker, Lane 2,3,4,5 and 6; mtDNA digested with EcoRI enzyme, Lane 7 (L); 1Kb DNA ladder, Lane 8,9,10, 11 and 12; mtDNA digested with HindIII enzyme, Lane 13 (H); Lambda/DNA Hind III1 marker**
**ER KM CM KL VZ CM ER KL VZ KM**

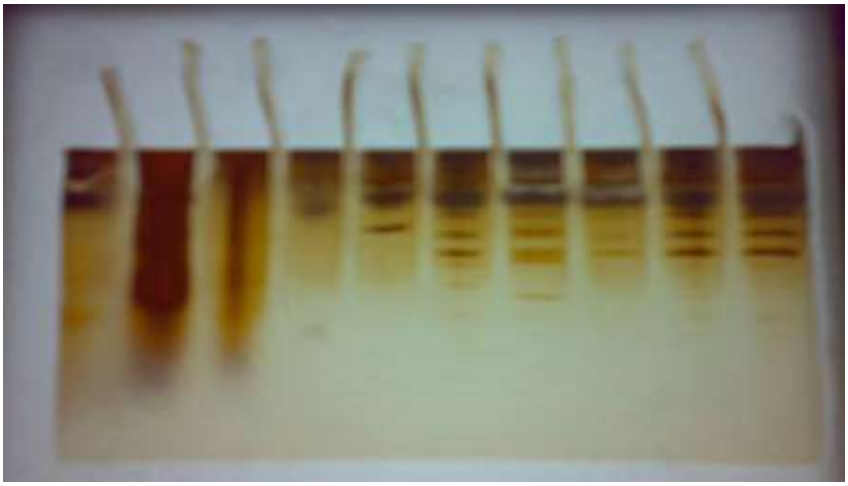

**Figure 5: RFLP Double Digestion - Lane 1 (L): 1Kb DNA ladder, Lane 2,3,4,5, & 6; mtDNA digested with $pBR^{322}$ and TaqI enzyme, $pBR^{322}$ and BamH1, Lane 7,8,9 and 10**

## IV. DISCUSSION

In the present study utilized a newly emerged methodology rather than the conventional Cesium Chloride gradient centrifugation method for the isolation of mtDNA. As the conventional isolation technique requires more sophisticated instruments as well as those techniques utilize more expensive consumables. The method utilized in this procedure is a simple CTAB lysis procedure, where the mtDNA can be precipitated even at the centrifugal force of 10,000 rpm, whereas in conventional Cesium chloride centrifugation, the centrifugal force required for the separation of mtDNA from the nuclear DNA pool is about more than 1,00,000 rpm. Moreover, the time required for the separation of mtDNA is about 16-24 hours in the case of the conventional method, whereas in the CTAB procedure it is only 30 minutes. The purity of the isolated mtDNA has been checked by 1% agarose gel as shown in Figure 2. The presence of mtDNA after the extraction procedure was checked by 1% Agarose gel electrophoresis. The mtDNA was seen at the position of 16 Kb as shown in Figure 2. The size of the mtDNA was compared and determined with the use of the DNA markers which were run along with the Sepia mtDNA samples. The first DNA marker was lambda DNA digested with Hind III restriction enzyme, which has the bands corresponding to the following base pairs (i.e. 23130, 9416, 2322, 2027, 564, 125 bp) and the second DNA marker was lambda DNA digested with Bam HI restriction enzyme, which would have the bands of 16841, 7233, 6770, 6527, 5626, 5505 base pairs. In figure 2, it is evident that the isolated mtDNA was located in the region corresponding to 16841 bp of lambda / BamHI digestion. Initially, the mtDNA had small RNA contamination, which was removed by treating the DNA sample with RNase A at 37°C for 10 minutes, which resulted in the pure form of mtDNA. Loss of genetic diversity in domestic animal populations has also been studied and attributed to the extension of markets and economic globalization[10&11]. The more genetic diversity a population has the more likely the population will be able to adapt and survive. Conversely, the vulnerability of a population to changes, such as climate change or novel diseases will increase with a reduction in genetic diversity[12]. This approach presented here provides selectively enriched fractions and eliminates the need to separate the extraction of mtDNA. Suitable processes like PCR amplification and DNA sequencing may prove to be useful for the people. Studying population genetics and evolution using molecular markers maximizing the available sources, especially in cases where a large database needs to be generated from a limited amount of tissue sample. Fragments of different sizes are generated when individuals differ in their DNA sequence; these are called restriction fragment length polymorphisms (RFLPs).

## V. CONCLUSION

Rapid advances in molecular biology have had profound ramifications for other branches of biology, including the biotechnology and environmental biology. For many years the only readily available tool for the genetic analysis of population structure was allozyme electrophoresis. Now it is possible to examine several classes of genes, mitochondrial and nuclear, coding and non-coding regions, to arrive at a more

robust understanding of how contemporary natural populations are structured. Expanding genetic databases coupled with versatile techniques such as PCR and gene sequencing make it possible to undertake sophisticated genetic analyses in species for which no previous genetic information is available. The primary challenge now to the Marine Biotechnologist is to integrate information derived from a suite of molecular markers with an understanding of the evolutionary history of the species, as well as the physical and biotic forces affecting its demography.

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SP-2

# EXPLORING THE ANTIMYCOTOXIGENIC POTENTIAL OF *ACALYPHA INDICA* AND *BIDENS PILOSA*

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## ABSTRACT

Aflatoxins are highly toxic and immunosuppressive secondary metabolites produced mainly by fungi, *Aspergillus flavus* and *Aspergillus parasiticus* on food and agricultural commodities. Detoxification of these mycotoxins has been considered as one of the best strategies to prevent contamination and to retain the nutritive properties of feedstuffs and agricultural products while storage. The objective of the study is to evaluate the antifungal and antiaflatoxigenic potency of the *Acalypha indica* and *Bidens pilosa* crude leaf extracts concerning *Aspergillus species*. The minimum concentration required to inhibit the fungal mycelial growth were determined separately for each plant extract and compared with each other in maize sample. Further, the aflatoxin detoxification efficacy of the two plant extracts was screened by thin-layer chromatography. The inhibitory effect on the mycelial growth of *A. flavus* was measured for each plant extract. Among the two leaf extracts, *Bidens pilosa* showed higher inhibitory effect and detoxification potential when compared with the extract of *Acalypha indica*. Thus, this study concludes that phytoconstituents derived from medicinal plants could be used as a substitute for synthetic fungicides and can act as natural preservatives.

**KEYWORDS:** *Bidens pilosa*; *Acalypha indica*; antifungal activity; aflatoxin; detoxification.

## 1. INTRODUCTION

Fungal spoilage and mycotoxin contamination are of major economic and health problem worldwide as they lead to various food-born diseases. Mycotoxins are toxic secondary metabolites produced by fungal, contaminate various agricultural commodities, and hurt human health, animal productivity.[1] Aflatoxin contamination is most common in food products, especially in maize and groundnut. The fungal species *Aspergillus flavus* and *Aspergillus parasiticus* have been focused with more attention due to the production of highly toxic secondary metabolites, aflatoxins (AFs) in feeds and foods. AF has been well-known to induce adverse effects such as immune suppression and liver cancer in humans[2]. In a man-made ecosystem, there is a demand for food decontaminating compounds from natural resources to maintain a minimum level of food safety under storage in which quality and nutritive changes occur utilizing various physical, chemical, and biological factors. Most of the synthetic fungicides which have been used to control the growth of the storage fungi were found to be associated with carcinogenicity, teratogenicity as well as cytotoxicity.[3, 4] Plant nutraceuticals are considered to be of more interest as they are safe and more effective than synthetic antimicrobial agents for food preservation. Bioactive compounds derived from various parts of the plants have been used traditionally as alternative food preservatives in countries like Japan, Russia, and India.[5] Many species and herbs and their extracts have been reported to suppress the growth of toxigenic fungi thereby prevent the spoilage of food due to fungi.[6] Over the years numerous studies have screened and tested the efficacy of array of compounds isolated from plants to search for natural fungicides and among which essential oils and phenols are inhibitory.[7] Besides, it becomes essential to profile biologically active chemicals and metabolites from natural sources, which provoke an inhibitory response on the growth of various bacterial and fungal organisms and makes its use in the miscellaneous application. Thus, plant-based ingredients of the predictable antimicrobial spectrum are of interest concerning their possible use in the process of food preservation.

*Acalypha indica* (family *Euphorbiaceae*) and *Bidens pilosa* (family *Asteraceae*) are used as traditional remedies to treat various diseases and thus their extracts and isolated compounds are much studied for the pharmacological activities. Several studies have addressed the biological potency of different parts of these two plants extracts and metabolites for their antimicrobial and antifungal activities.[8-10] However, there are no reports about their fungicidal effect on food commodities. The present study aims to evaluate the inhibitory potential of *A. indica* and *B. Pilosa* aqueous leaf extracts on mycelia growth of *A. flavus* and antimycotoxigenic effect.

## 2. MATERIALS AND METHODS

**2.1. Chemicals and Apparatus:** Sabouraud dextrose agar (SDA) medium was procured from Hi-Media Laboratories, Mumbai, India. All chemicals used in the experiments were of analytical grade.

**2.2. Collection of sample and processing:** The fungal contaminated maize samples were collected from the market in Pudukkottai district, Tamil Nadu. The samples were washed with distilled water to remove dust and debris and later subjected to surface sterilization.

**2.3. Preparation of plant aqueous extracts:** The healthy leaves of *Acalypha indica* and *Bidens pilosa* were collected from various places in the Pudukkottai district, Tamil Nadu. The leaves were washed under running water and air-dried in the laboratory for 10 days at room temperature and powdered. The aqueous extracts were prepared by mixing 10g of each dried and powdered plant leaf material with 100 ml sterile distilled water with constant stirring for 30 min and then were kept in the shaker at 200 rpm for 24 hr at room temperature. Then the contents were centrifuged for 20 min at 10,000 rpm to remove particulate material. The supernatants were collected and stored in a refrigerator (4°C) until further usage.

**2.4. Characterization of *Aspergillus flavus* and sub-culture:** Single-spore isolates of fungi originally isolated from contaminated maize samples were grown on Sabourad's dextrose agar (SDA) at 28 ± 2°C for 5 to 7 days. Fungi were characterized by staining with lactophenol cotton blue. Using morphological references the identified species were confirmed as *Aspergillus flavus*.

**2.5. Effect of plant extracts on mycelium growth of *Aspergillus flavus* (*In vitro* study):** Antifungal activity of plant extract against *Aspergillus flavus* was determined by fungal growth inhibition assay as described by Suman Singh (2010).[11] 100 ml of SDA media in each of 30 conical flasks were autoclaved for 20 min at 121°C. After cooling different volumes (0.5 ml, 1 ml, 2 ml, 3 ml, and 5 ml) of sterilized aqueous leaf extracts of each plant was added to the above conical flasks separately, and gently mixed for 2 min to allow proper mingling of extract. 20 ml aliquots of the extract added media were dispersed into individual Petri-dishes (9 cm). Each plate was supplemented with streptomycin (100µg/ml) to prevent bacterial growth.1 ml of *A. flavus* spore suspensions at concentration $1x10^6$/ml was placed onto the center of the treated Petri dish and incubated at room temperature (28 ± 2°C) for 10 days. The experiments were performed under aseptic conditions in triplicates. A plate was included without the extract as a negative control.

Average radial growth was determined by measuring the diameter of a colony. The inhibition zone percentage (P) of the fungal growth about the control treatment was calculated using the formula of Francisco (2010)[12] as given below:

$$P = \frac{\text{Mycelial growth (control)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth (control)}} \times 100$$

**2.7. Heat stability and antifungal effects of the plant extracts:** To study the effect of heat on antifungal properties of *Acalypha indica* and *Bidens pilosa* extracts, 1 ml of each plant extract was exposed to boiling temperature (100°C) for 10 min, cooled to room temperature and the impact of the extracts on the inhibition of colony growth of fungus *A. flavus* was investigated as described earlier after 5th day and 10th day of the incubation period.

**2.8. Effect of *Acalypha indica* and *Bidens pilosa* on aflatoxin production by *A. flavus*:** *A. flavus* was cultured in a 250 ml conical flask containing 100 ml of SDA broth supplemented with various volumes (0.5 ml, 1 ml, 2 ml, 3 ml, and 5 ml) of each plant extract for 10 days. The control flask was maintained only with *A. flavus.* After that, the mycelia mats were removed by filtration through Whatman No.1 filter paper and the filtrate was extracted with chloroform (1:1 v/v) and evaporated to dryness. Then the pellets were redissolved in a known volume of chloroform and subjected to thin-layer chromatography (TLC) to observe the formation of aflatoxin and to examine the antiaflatoxin effect of *Acalypha indica* and *Bidens pilosa* extracts.

**2.9. Aflatoxin detoxification efficacy of the plant extracts:** 5 ml of 10-day old culture filtrate of *A. flavus* was mixed with various volumes (0.5 ml 1 ml, 2 ml, 3 ml, and 5 ml) of *A.indica* and *B.pilosa* leaf extracts and incubated at room temperature for 5 days. Following the incubation period, aflatoxin remaining in a solution of each test tube was repeatedly (3 times) extracted by mixing equal volume of chloroform followed by centrifugation. The chloroform fractions were pooled, dried, and redissolved in a known volume of chloroform and analyzed by TLC. The developed spots were observed under UV light.

## 3. RESULTS AND DISCUSSION

Consumption of mycotoxins through agricultural products remains a major health issue in many parts of the world as it is associated with adverse toxic effects in the human system.[13,14] Human food can be contaminated with mycotoxins at various stages in the food chain and the major class of mycotoxins include a metabolite of *Aspergillus flavus*, aflatoxin $B_1$.[15] Many plants and plant products have been reported to possess antifungal and antimycotoxigenic properties.[16] Several scientific reports have been implicated in the *in vitro* antibacterial and antifungal activity of *Acalypha indica*[17] and *Bidenspilosa.*[18,19] Although most of investigations supported their pharmacological role, their antifungal activity against food-borne fungi and antimycotoxigenic effect has not been studied yet. The present study aims to reveal the effect of *A. indica* and *B. pilosa* leaf extracts for controlling fungal growth and mycotoxin production by *A. flavus* cultures grown on a liquid medium. The antifungal activity was studied for the aqueous leaves extracts of *Acalypha indica* and *Bidens pilosa* and the observed pattern of colony diameter was shown in Table 1. The size of the colony diameter was decreased with an increase in the concentration of each leaf extract. The extract of *Bidens pilosa* was found to possess a higher antifungal effect when compared to *Acalypha indica* leaf extract. The percentage of the zone of inhibition studied at different volumes against fungal mycelium growth of *A. Flavus* also suggested the antifungal efficacy of *A. indica* and *B. pilosa* (Figure 1). The *B. pilosa* extract has been shown to exhibit a maximum percentage (70%) of inhibition when compared to *A. indica*. Also, a gradual increase in the percentage of inhibition was observed with an increase in the concentration of the leaf extract. The aliquots of *B. pilosa* extract effectively inhibited the mycelia growth of *A. flavus* by the production of secondary metabolites. It has been previously reported that the secondary metabolites of crude extracts of *B. pilosa* were responsible for its antimicrobial effect.[10] The heat stability of the antifungal compounds of *Acalypha indica* and *Bidens pilosa* leaf extracts was determined on treatment with heat exposed samples after 5 days and 10 days of incubation (Table 2). The antifungal activity of both extracts was markedly decreased on heating which implies the temperature sensitivity property of the extract constituents. Table 3 depicts the effect of *A. indica* and *B. pilosa* leaf extracts on aflatoxin production that was analyzed by TLC. The resolved spots were detected under UV light and the $R_f$ values were calculated. The cultured broth of *A. flavus* amended with different volumes of *A. indica* and *B. pilosa* extracts have been shown to inhibit aflatoxin production completely at all concentration when compared with control (*A. flavus*) as evidenced by their respective $R_f$ values. The results revealed the ability of the leaf extracts to suppress the growth of *A. flavus* and subsequently prevented the formation of aflatoxin. The aflatoxin detoxification potential of the leaf extracts was studied by TLC and the chromatogram was presented in Figure 2. The extract of *B. pilosa* (Figure 2c) demonstrated significant aflatoxin detoxification efficacy compared to the extract of *A. indica* (Figure 2b). Indeed the effect was equally observed in different volumes of the extracts. All these results suggested the antimycotoxigenic properties of these two plants. *Bidens pilosa* extract showed promising inhibitory action against food-borne fungi *A. flavus*. The beneficial effect might be due to the combination of antifungal metabolites present in these plants and can be used in food commodities to minimize fungal contamination.

Table 1. Effects of extracts of *Acalypha indica* and *Bidens pilosa* on the inhibition of colony growth ($cm^2$) of *Aspergillus flavus* grown on SDA media incubated at 25°C for 10 days. Each data is the mean from at least three independent experiments.

| Aqueous extracts of plants | Control | Comparison of volumes of water-soluble extracts (ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0.5 | 1.0 | 2.0 | 3.0 | 5.0 |
| | Size of the Colony diameter (cm) | | | | | |
| *Acalypha indica* | 8.3 | 4.8 | 4.5 | 3.9 | 3.6 | 3.1 |
| *Bidens pilosa* | 8.8 | 4.3 | 3.9 | 3.4 | 3.0 | 2.2 |

Table 2. Heat stability of the plant extracts *Acalypha indica* and *Bidens pilosa* and the inhibitory effect against the growth of *A. flavus* after 5 days and 10 days of incubation at 25°C. Each data is the mean from at least three independent experiments.

| Treatment (Heated at 100°C) | Colony diameter (cm) | |
|---|---|---|
| | 5 days | 10 days |
| Control | 4.7 | 8.2 |
| *Acalypha indica* | 4.5 | 7.6 |
| *Bidens pilosa* | 4.2 | 7.9 |

Table 3. Inhibition of aflatoxin production by *A. indica* and *B. pilosa* extracts. *A. flavus* was cultured and supplemented with various volumes (0.5 ml, 1 ml, 2 ml, 3 ml, and 5 ml) of each plant extract for 10 days. Each data is the mean from at least three independent experiments.

| Treatment groups | Volume of the spot | $R_f$ value |
|---|---|---|
| Control | 0.25 µl | 0.7 |
| *Acalyphaindica* (0.5 ml, 1 ml, 2 ml, 3 ml & 5 ml) | 0.25 µl | - |
| *Bidenspilosa* (0.5 ml, 1 ml, 2 ml, 3 ml & 5 ml) | 0.25 µl | - |

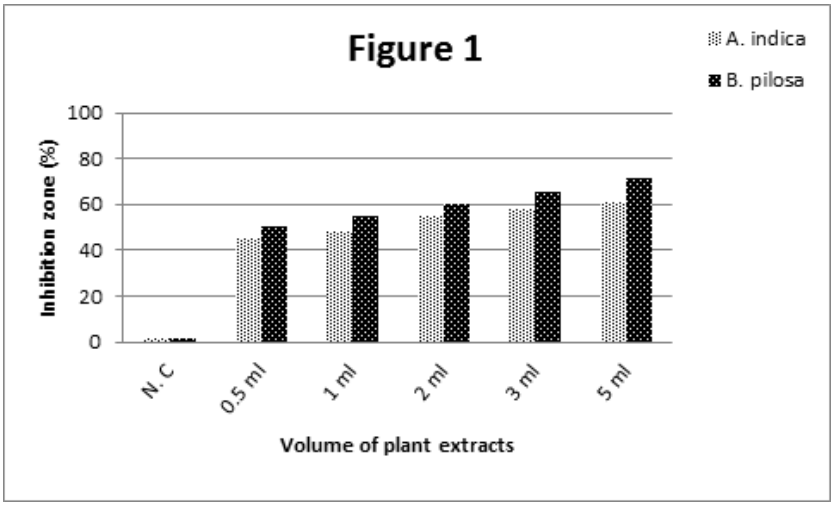


Figure 1. Effect of extracts of *Acalypha indica* and *Bidens pilosa* at different volumes in inhibition of fungal mycelium growth (*Aspergillus flavus*) in SDA media after 10 days at 25°C compared with the negative control. Each data is the mean from at least three independent experiments.

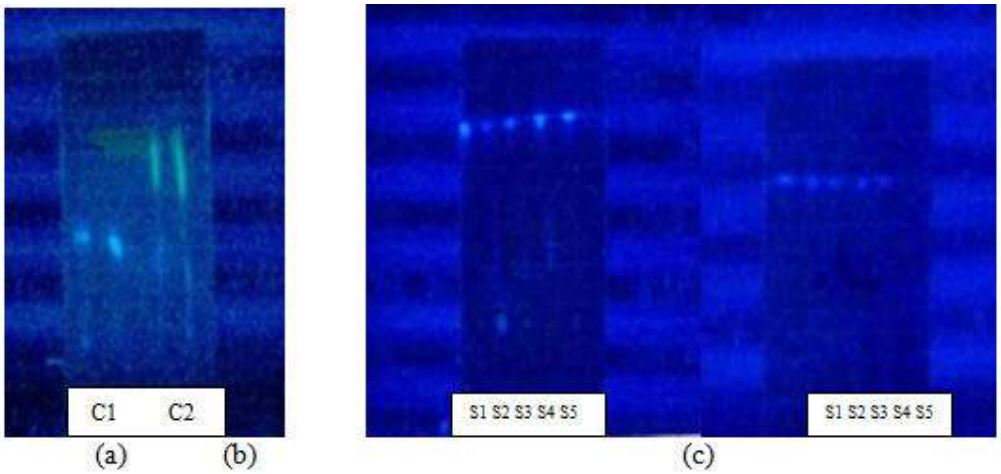


Figure 2(a):C1 (Spot 1and 2) - Contaminated maize sample 1
C2 (Spot 3 and 4) - Contaminated maize sample 2
Figure 2(b): S1, S2, S3, S4 and S5 - Treated with *A.indica*0.5, 1, 2, 3 & 5 ml respectively
Figure 2(c):S1, S2, S3, S4 and S5 - Treated with *B.pilosa*0.5, 1, 2, 3 & 5 ml respectively

Figure 2. TLC Analysis for aflatoxin detoxification effect of the plant extracts at different volumes (0.5 ml, 1 ml, 2 ml, 3 ml and 5 ml). (a) Control(b) On treatment with *A. indica* extract (c)On treatment with *B. pilosa* extract

Table 4. Inhibitory action of *Acalypha indica* and *Bidens pilosa* aqueous extracts on the growth of *A. flavus* after 0, 3, 6, 9, 12 and 24 h of incubation at 30°C. Each data is the mean from at least three independent experiments.

| Aqueous extracts of plants | Inhibitory activity at the following incubation time (h) Dosage (500 µl) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 24 |
| *Acalypha indica* | - | - | - | - | - | - |
| *Bidens pilosa* | - | - | + | ++ | ++ | +++ |

[a] Determined by an agar diffusion assay and scored as follows: -, no inhibition; +, weak inhibition; ++, low-level inhibition; +++, strong

## 4. CONCLUSION

The present results concluded that *Bidens pilosa* plant extract was more efficient in inhibiting the growth of food spoilage, aflatoxin-producing fungi *A. flavus*. However, more investigation is needed to explore the active metabolites of the extract and the possibility of using them as fungal decontaminants in agricultural products.

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SP-3

# PATHOGENIC IMMUNE RESPONSE IN CAENORHABDITIS ELEGANS DURING *ACINETOBACTER INDICUS* INFECTION

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## ABSTRACT

A better understanding of microbial interaction with the host has a direct impact. Among the animal model used for the host-microbial interaction study, *Caenorhabditis elegans* may be one of the best animal model because it has short life cycle, easy maintenance in lab, and easy accessibility of different genetic mutants. This study deals with *Caenorhabditis elegans* interaction with pathogenic bacteria *Acinetobacter indicus to provide a* comprehensive review of the physiological and innate immune response of infected C. elegans. *Acinetobacter sp.* is opportunistic bacteria that play a major role in the occurrence of infections worldwide in recent decades. The physiological response of *Caenorhabditis elegans* infected with *Acinetobacter* by survival and behavioral assay reveals that it causes host mortality by persistent infection. The candidate genes *tol*1, *clk*2, *lys*7 were used to study the innate immune response in *Caenorhabditis elegans.* The physiological response of *Caenorhabditis elegans* infected with *Acinetobacter indicus* reveals the mortality in worms by persistent infection.

**KEYWORDS:** *Caenorhabditis elegans*, Innate immune response, *Acinetobacter indicus*

## INTRODUCTION

The host-microbe interaction occurs in all the animals, and the symbiotic bacteria may be helpful, harmful, or with no effect. For example, the gut microbe harmless *Escherichia coli* strains found give advantage to their host by producing vitamin K and not allowing the pathogenic bacteria to colonize in the intestine.[1,2,3] In contrast, some of the bacterial strains of *E.coli* O26 can cause diseases in the host[4]. The interaction between the host and the microbe forms a complicated network. To cure the diseases efficiently and to immunize good health, it is necessary to understand the interaction and relationship of pathogens. The behavioral study on *Caenorhabditis elegans* (*C. elegans*) with pathogenic bacteria has uncovered that *they* can smell and sense the pathogens and also responds to learning behaviors.[5,6] Such studies provide a deep insight about the nervous system play an important role in pathogen defense, and various cellular stress response may be related to pathogenic defense.[7,8,9] The *Acinetobacter* is a Gram-negative, coccobacillus, obligate aerobe, and major causative agent for nosocomial infections.[10] Due to the overuse of antibiotics, the *Acinetobacter* has emerged as multidrug-resistant strain and causing serious issues of pathogenicity in the hospitals environment.[11] According to WHO Carbapenem-resistant *Acinetobacter sp.* is emerging worldwide as a critical pathogen for which new therapeutics are urgently required.[12] To study the gene expression and pathogenicity of bacteria, the C. elegans as animal model has been extensively used. *C. elegans* has become a widely used model for studying human pathogens due to its functional similarity with humans.[13] Many reports were demonstrated that some genes were induced when *C. elegans* were infected with pathogenic bacteria.[14,15] Therefore, this study aimed to evaluate the role of candidate innate immune genes against *Acinetobacter indicus* infection.

## MATERIALS AND METHODS

### Bacterial strains and growth condition

The strain *Acinetobacter indicus* was isolated from the contaminated hospital site. The bacterial strain was grown on Luria–Bertani (LB medium) at 37° C incubator. The bacterial strain was stored in 50% glycerol stock in -80°C for further studies.

**_Caenorhabditis elegans_ maintenance**

*C. elegans* wild type strains were fed with *E.coli* OP50 as a food source and maintained on nematode growth medium (NGM) at 20°-22°C.[16] The worms were age synchronized by bleaching with commercial bleach. The age synchronized adult worms were collected and used for various assay as described.[17]

**Age Synchronization of _Caenorhabditis elegans_**

A large population of worms was collected by culturing them on NGM solid media plates. The worms were then washed with M9 buffer three times and freshly prepared 1ml bleaching solution (Sterile water 8.25 ml + 1M NaOH 3.25 ml + Sodium hypochlorite 3.0 ml) was added. The worms were vortex vigorously 2-5 min until worms lysis occurs. Later again 1ml of M9 buffer was added to stop the bleaching reaction, and eggs were centrifuged. The supernatant was discarded and synchronized eggs were placed onto to new *E.coli* OP50 NGM plate.

**Detection of bacterial accumulation in nematode intestine**

The bacterial accumulation assay was performed to determine bacterial load (*Acinetobacter indicus)* in the gut of *Caenorhabditis elegans*. The *C. elegans* were grown on bacterial containing plates for 2 hours then the plates were washed with M9 buffer containing 1mM sodium azide to prevent the discharge of bacteria from their intestine. Approximately 10 numbers of *C. elegans* were transferred to the 1.5ml micro-centrifuge tube, and then silicon carbide particles (40mg) were added to the tube and vortexed vigorously. *C. elegans* get disrupted, and the suspension was serially diluted and plated on LB medium to determine the colony-forming unit (CFU).

**Chemotaxis Assay**

In this assay, 100µl bacterial culture of *Escherichia coli* OP-50, and *Acinetobacter indicus culture* (0.5OD) was inoculated 2.5 cm apart from the center of NGM plate (90mm) and named as zone A and zone B respectively. The wild type *C. elegans* were washed three times with M9 buffer, and 30 worms were placed at the center of the NGM plate. The no of worms towards zone A and B were counted every 6 hours for 24 hours. For the control plate, both the zone A and B were inoculated with *E.coli* OP50, and 32 worms were added at the center.

**Short-Time Exposure Assays**

The L4 staged *C. elegans* were infected with *Acinetobacter indicus* for the short time duration (2, 4, 6, 8, 10, 12 h) and worms physiological changes were observed.[17]

**Pharyngeal Pumping Assay**

To examine the pharyngeal pumping rate, wild type worms were transferred to NGM plates seeded with *E.coli* and *Acinetobacter* sp. The pharyngeal pumping rate observed was noticed under a light microscope for 30 consecutive seconds at 10X magnification.[17]

**Total RNA isolation**

To study, the *C.elegans* were infected with *Acinetobacter indicus* for two hours, and later worms were used for total RNA isolations. The worms were pooled together and bacterial contamination as removed by washing three times with M9 buffer and two times with DEPC treated M9 buffer. To the worms pellet, one ml TRIzol (RNAios Plus) was added and vortex for 10-15 min or until worms lysis. Then 400µl chloroform was added, mixed by inversion for two min and centrifuged 12000 rpm for 15 min. Later supernatant was collected in a new tube and 700 µl ice-chilled isopropanol was added for RNA precipitation and again centrifuged 12000 rpm 10 min. The next supernatant was discarded and the pellet was washed 2-3 times with DEPC treated 70% ethanol. Finally, the pellet was air-dried, and sterile DEPC water (25 µl) was added and RNA was run in 0.8% agarose gel (Fig3). The total RNA was quantified by nanodrop (BioSpec-nano) for its purity and transcribed using a cDNA kit (Takara).

**Semiquantitative PCR**

Semi-quantitative PCR was carried out to analyze the gene expression pattern of antimicrobial genes (*tol*-1, *lys*-7, clk-2) using gene-specific primers. The conditions for semi-quantitative PCR were 94°C for 6 min, 94°C for 40s, annealing at 57 °C for 60s, and extension at 72 °C 40s.

## RESULTS AND DISCUSSION

The *C.elegans* eggs gradually developed to adults via L1, L2, L3, L4 phases of the life cycle. The eggs after hatching took about 7-8 h to reach L1 stage, and from L1 to L2, L2 to L3, L3 to L4, L4 to the adult stage they took 10 h, 8h, 8h, 10h (Fig 1). The worms were found to be healthy.

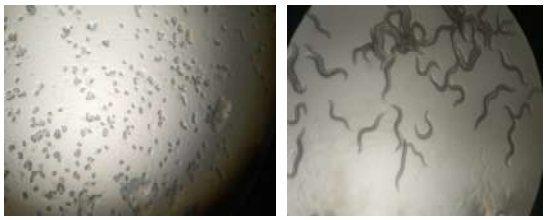

**Figure 1: Age synchronization of C. elegans and development**

To find out the bacterial accumulation inside the infected *C. elegans*, the CFU assay was performed and accumulation of *Acinetobacter sp.,* inside the worms, was found to be $1.5*10^2$ cfu/ml. Whereas live bacteria was not observed in the case of *E.coli* OP50 control worms. This suggests that the accumulation of *Acinetobacter* sp. was gradually increased during infection, thus found to be pathogenic to worms(Fig 2a, 2b, 2c).

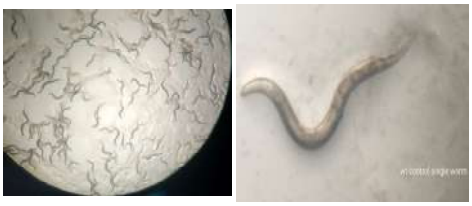

**Fig 2a. Wide type *C. elegans* fed with E.*coli* OP-50**

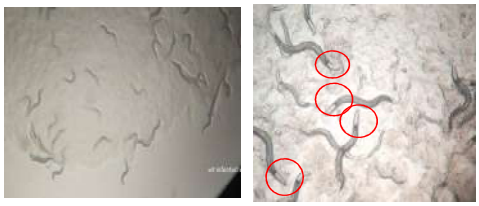

**Fig 2b. Wild type *C. elegans* infected with *Acinetobacter sp.***

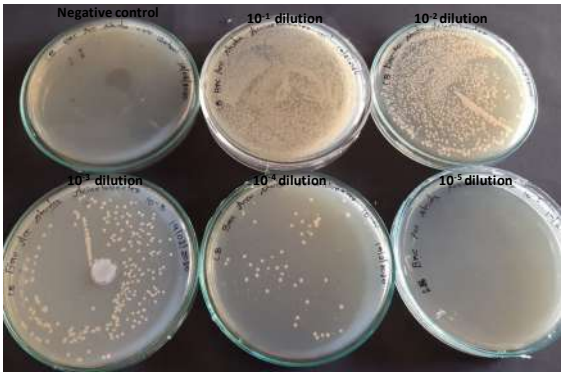


**Fig 2c *C. elegans* gut spread plate on LB medium**

*C. elegans* detects the pathogens by their olfactory neurons and response by avoiding the bacterial pathogen, so it is necessary to analyze the chemotaxis behaviors of worms towards *Acinetobacter*. In case of *Acinetobacter* infection, significant avoidance was observed as there were more no. of worms towards *E.coli* OP-50 lawn as compared to *Acinetobacter* lawn after 6 h(Table 1a, 1b)

**Table1a: Chemotaxis assay: Test experiment**

| Time (h) | *C.elegans* towards Zone A (*E.coli* OP50) | *C.elegans* towards Zone B (*Acinetobacter sp*) | Worms In the center region |
|---|---|---|---|
| 6 | - | - | 30 |
| 12 | 12 | 8 | 10 |
| 18 | 15 | 8 | 7 |
| 24 | 16 | 6 | 8 |

**Table1b: Chemotaxis assay: Control experiment**

| Time (h) | *C.elegans* towards Zone A (*E.coli* OP50) | *C.elegans* towards Zone B (*E.coli* OP50) | Worms in the center region |
|---|---|---|---|
| 6 | - | - | 30 |
| 12 | 8 | 10 | 14 |
| 18 | 12 | 14 | 6 |
| 24 | 10 | 9 | 13 |

In a short time exposure study, the life span of *C.elegans* exposed to *Acinetobacter* was periodically observed. The worms were active up to 10h, but a later stage, their movement becomes slower as compared to control worms(Table 2).

**Table 2: *C. elegans* during the short time exposure assay**

| Time (h) | Infected worms |
|---|---|
| 2 | *C.elegans* were live and producing the next generation |
| 4 | *C.elegans* were live and producing the next generation |
| 8 | *C.elegans* were live for 60 hours and not producing the next generation |
| 12 | *C.elegans* were live for 48 hours and not producing the next generation |

In the pharyngeal pumping assay, the pumping rate in infected *C.elegans* found to be decreasing in a time-dependent manner as compared to control worms. The pharyngeal pumping is directly associated with healthiness of the worms and this result demonstrates that *Acinetobacter* infection damage the pharyngeal part of the host (Table 3).

**Table 3: Pharyngeal pumping rate of wild type and infected *C. elegans***

| Time (h) | Control wild type worms | Infected wild type worms |
|---|---|---|
| 0 | 38 | 35 |
| 6 | 37 | 36 |
| 12 | 39 | 34 |
| 18 | 40 | 30 |
| 24 | 37 | 32 |
| 30 | 41 | 30 |
| 36 | 39 | 29 |
| 42 | 35 | 28 |
| 48 | 38 | 28 |

| | | |
|---|---|---|
| 54 | 40 | 26 |
| 60 | 37 | 25 |
| 66 | 39 | 24 |
| 72 | 36 | 23 |
| 78 | 38 | 22 |
| 84 | 41 | 24 |
| 90 | 37 | 22 |

**RNA isolation**

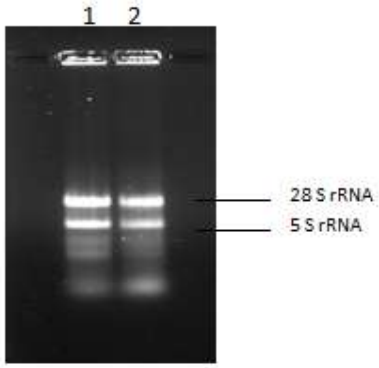


**Fig 3 RNA gel image, lane 1 RNA from wild type, and lane 2 RNA from infected worms.**

*C. elegans* are a eukaryote thus showing multiple RNA bands. Regulation of candidate immunogenic genes (*lys*7, *tol*1, *clk*2) were analyzed during *Acinetobacter* infection. In the present study level of expression of *tol*1, *clk*2, *lys*7 were analyzed. The expression of *lys*7 was down-regulated while *tol*1 and *clk*2 genes were up-regulated (Figure 1). The lysozyme plays an important role in both vertebrate and invertebrate immunity. In the *C. elegans*, *lys*7 gene expression was found to be down-regulated in infected condition with gram-negative bacteria *Acinetobacter sp*. In contrast, the *lys*7 gene-expression was up-regulated when infected with gram-negative bacteria *Serratia marcescens*.[18]

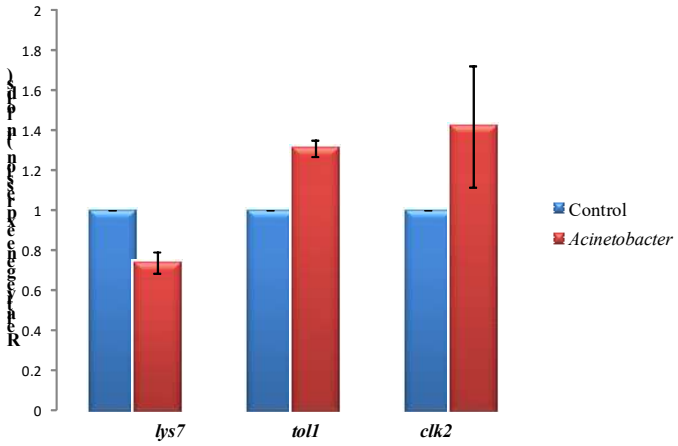


**Figure 4: Relative gene expression of *lys*7,*tol*1, and *clk*2 genes in control and infected *C. elegans* by semi-quantitative PCR analysis.**

## CONCLUSION

In the present study, we found that the life span of *Caenorhabditis elegans* was reduced considerably upon infection with *Acinetobacter sp*. The microscopic, and bacterial accumulation assay had shown the colonization of *Acinetobacter sp*. in the gut region when compared to wild type worms. We also found that pharyngeal pumping was decreased in infected worms and they preferred *E.coli* OP-50 rather than *Acinetobacter* as a food source. In the case of chemotaxis assay, no significant difference was observed. The gene expression of *tol*1 and *clk*2 was increased while *lys*7 was found to be down-regulated. Furthermore, an in-depth study is required to understand *C. elegans* response against pathogens.

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SP-04

# ANTIDIABETIC STUDIES OF PM (23NM) NANOCRYSTALS

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## ABSTRACT

Nanocrystal memory properties are influenced by nanocrystal size, shape, and configuration. Nanocrystals are uncontaminated drug crystals using sizes in the range of manometers. Nanocrystals can be made either by top-down or bottom-up technologies. High-density nanocrystals can store more charge in a memory device and mitigate the influence of fluctuations between individual devices because there are more nanocrystals in each memory cell. Generally, in material applications, nanomaterials and nanocrystals are widely used and many utilization in all fields of science and technology and they have some special properties. In this study we converted macrocrystal into nanocrystal by ball milling method, Crystals are analyzed by the XRD method for parameters. For macrocrystal single XRD are used, for nanopowder XRD are used and the crystal is converted into nanocrystals which are used for filter applications and -an anti-diabetic study of the nano. Crystal inhibition values are increased with a proper increase in the value of concentration, $IC_{50}$ values are 20.23for nanocrystal of PM.

**KEYWORDS:** PM nanocrystal, -anti-diabetic properties, ball milling, XRD method

## INTRODUCTION

Nanocrystal is obtained from macro crystals. The crystal is a solid whose molecules are arranged in repeating patterns and have geometrical shapes. Generally, crystals are brittle, stable, and strong. In nanocrystal, the range is nearly 100 nm level. The nanocrystal has numerous applications in electronics, biology, and industries. Nanocrystals have some special properties when compared to macro crystals. Small nanocrystals do have not such strong ordering as the large crystal has. Imperfect structures of nanocrystals have been studied experimentally[1]. To improve the dissolution and absorption of the drug bexarotene, nanocrystal was surface-modified by folate-CS (FA-CS)[2]. After being directed by a magnetic field gradient towards the target site, magnetic NPs held great potential for clinical applications and the delivery of therapeutic molecules. Colloidal nanocrystals have been proven to be attractive tools due to the ease to alter their physical properties. Nanocrystals are aggregates of molecules that can be combined into a crystalline form of the drug surrounded by a thin coating of surfactant. They have extensive uses in materials research, chemical engineering, and quantum dots for biological imaging[3&4], but less so in nanomedicine for drug delivery.

## ANTIDIABETIC CHARACTERISATION

The PM crystal is prepared by the solution growth method, by using a ball milling method the crystal is converted to a nanoscale, the PM nanocrystals are having -anti-diabetic, -anti-microbial properties.

## XRD

**TABLE:1** The table represents the XRD analysis for the picolinium maleate crystal. Here $IC_{50}$ values for the macro scale are given below.

| COMPOUND NAME | MACROSCALE $IC_{50}$VALUES |
|---|---|
| PM crystal (macro size) | 20.26 |

Picolinium maleate crystal having a=14.656Å, b=10.385Å, c= 9.13Å, and α=γ=90°, β=102.4°. And the system is monoclinic and crystal having space group as $P2_1/C$.

**TABLE:2** PM crystal for -anti-diabetic properties. Here $IC_{50}$ values for PM nanoscale are given below.

| SL. NO | COMPOUND NAME | CONCENTRATION $\mu g / ml$ | INHIBITION VALUES | NANOSCALE $IC_{50}$VALUES |
|---|---|---|---|---|
| 1 | PICOLINIUM MALEATE 23 nm NANO CRYSTAL | 10 | 25.1 | 20.23 |
| 2 | | 20 | 50.0 | |
| 3 | | 30 | 56.5 | |
| 4 | | 40 | 76.8 | |
| 5 | | 50 | 77.4 | |
| 6 | | 60 | 79.4 | |
| 7 | | 70 | 79.8 | |
| 8 | | 80 | 80.9 | |

The inhibition values are increased with an increase in concentration values. Picolinic acid is an organic compound, under the inflammatory conditions acid is produced. Neuroprotective, immunological, antiproliferative are the some of implicated effects in picolinic acids. Maleic acid is also an organic compound. When compare to fumaric acid it is less stable molecules. here the presence of picolinium acid and maleate acid have a 1:1 ratio.

## RESULT & DISCUSSION

For the past few decades, there is significant research attention in the area of drug delivery systems. Biodegradable nanoparticles have been used frequently used as drug delivery vehicles due to their high bioavailability, good encapsulation properties, and relative lack of toxicity[5]. Different nano-sized carriers, such as nanoparticles, polymeric micelles, liposomes, surface-modified nanoparticles, and solid lipid nanoparticles[6-8], have been developed and suggested for achieving these goals. As the basis for a natural encapsulation agent, gelatin is widely used in several formulations because of its biocompatibility, biodegradability, and low antigenicity. Gelatin nanoparticles have been used for delivery of different drugs, gene delivery, as carriers to deliver the drug to lungs, and recently antibody modified gelatin nanoparticles were used to target lymphocytes, leukemic cells, and primary T-lymphocytes[9&10]. The PM macro crystals are converted into nanocrystal by ball milling method, the crystal is analyzed by the XRD method for the parameter. The crystal has 23 nm as size and $IC_{50}$ value of PM nanoscale is 20.23 and used for -anti-diabetic properties. On the macro scale, the $Ic_{50}$ value is 20.26. The PM crystal has some variation of $IC_{50}$ values when compare macro to nano.

## CONCLUSION

By using ball milling method conversion of macrocrystal to nanocrystal is obtained. The PM nanocrystals are having antidiabetic properties. It is having $IC_{50}$ as 20.23value for nanoscale which is best for -anti-diabetic activity. The inhibition values are increased with an increase in concentration values. $IC_{50}$ values are well suited for -anti-diabetic analysis compared to macro-scale values.

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SP-05

# ISOLATION, PRODUCTION, CHARACTERIZATION OF PHOSPHATASE ENZYME PRODUCED BY FUNGI AND BACTERIA ISOLATED FROM IDHAYA COLLEGE

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## ABSTRACT

The enzymatic activities occurring on painted walls in a tropical environment have become critical given the rate of aesthetic biodeterioration of such walls. Qualitative and quantitative measurement was studied by phosphate solubilisation in culture medium and SDS PAGE. In this present investigation the farmers are instructed to use phosphate solubilizing organisms instead of using chemical fertilizer. The greater part of the soil p is in the form of insoluble p which can't utilize by plants, by using phosphate solubilizing microorganisms p is soluble easily.

**KEYWORDS:** Phosphate solubilizer, Pikovasaya's medium, solubilization, Nutrition, Enzymes, characterization.

## INTRODUCTION

Phosphorus (P), an essential element for plant nutrition, can only be assimilated as soluble phosphate. It is found in soil in various inorganic and organic forms. Phosphorus one of the major limiting factors for crop production on many tropical and subtropical soils as a result of high phosphorus fixation. A large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants.[1] Pikovasaya's medium incorporated with tricalcium phosphate used for the enrichment of phosphate solubilizing bacteria. The samples were serially diluted, plated on the medium and incubated at 30 ± $2^0$C for for 4 days. The colonies showing solubilization were packed up and purified by streaking on the surface of soil extract agar medium. The purified colonies were preserved on Pikovaskaya's agar slants.

## PHOSPHATE SOLUBILIZING MICROORGANISMS (PSMS)

Among the bacterial genera with this capability are *Pseudomonase, Azospirillum*, *Bacillus, Serratia, Enterbacteria, Acinetobacter, Flavobacterium and Erwinia*[2]. Among the algal some has the capacity to solubilize phosphate which were Cyanobacterium, Pythium and Phoma. Seed or soil inoculation with PSMs is known to improve solubilization of fixed soil phosphorus and appilied phosphates resulting in higher crop yields[3]. PSMs are a low-cost solution that enriches the soil giving a thrust to economic development without disturbing ecological balance.

## MATERIALS AND METHOD

### Site of collection

The soil sample was collected from garden soil at Idhaya College, Kumbakonam, Tamil Nadu.

**THE EFFECT OF PH ON PHOSPHATASE ENZYME**

The pikovaskaya's broth was prepared. The phosphate solubilising organism was inoculated into a pikovaskaya's medium and incubated at room temperature for 4 days (fungi) and 48 hours (bacteria). After incubation the culture filtrate was taken to examine the effect of pH on phosphatase enzyme of soil of microorganism. For analyzing acid phosphate assay 1ml of citrate buffer off pH 3, 4, 5 and 6 were taken in separate test tubes. 1ml of culture filtrate obtained was added to this buffer and vortexed for 5 minutes. Then add 1 ml of p-nitro phenol phosphate was added to each test tube. Then the tubes were incubated at room temperature for 30 minutes, to this add 4ml of O.I.N Sodium hydroxide was added and O.D was noted at 405nm. For analyzing Alkaline Phosphate assay 1ml of sodium carbonate and bicarbonate buffer of Ph 8, 8, 5 and 10 were taken in separate test tubes, 1ml of p- nitrophenol phosphate was added to each test tubes. Then the tubes were incubated at room temperature for 30 minutes, to this add 4 ml of 0.1N Sodium hydroxide was added and O.D was noted at 405nm.

**THE EFFECT OF TEMPERATURE ON PHOSPHATE ENZYME**

The phosphate solubilizing organism was inoculated into a pikovsaya's medium and incubated at room temperature for 4 days (fungi) and for 48hrs (bacteria). After incubation the culture filtrate was taken to examine the effect of temperature on phosphatase enzyme of soil microorganism. For analyzing acid phosphatase assay 1 ml of citrate buffer of pH 5 for *Aspergillus niger*, *Pencillium Chrysogenum*, and Aerobic spore former were taken in separate test tubes, 1 ml of culture filtrate obtained was added to this buffer and vortexed for 5 minutes 1ml of p-nitro phenol phosphate was added to each test tubes. Then the tubes were incubated at different temperature i.e.$10^0$ C, 30 $^0$ C, $40^0$ C and $60^0$ C for 30 minutes. To this add 4ml of 0.1N Sodium Hydroxide was added and O.D was noted at 405nm.

**QUALITATIVE AND QUANTITATIVE MEASUREMENT OF PHOSPHATE SOLUBILISATION IN CULTURE MEDIUM**

Quantitative measurement by Vanadium Molybdate Method.

**PREPARATION OF STANDARD CURVE**

Dissolve 0.2195g potassium dihydrogen phosphate in distilled water and make the volume to 1 litre (1ml= 59ppm phosphrous). Take aliquots of 2,4,6,8,10,12,14,16,18,20,22,24,26,28 and 30ml of the 50ppm stock solution in 50ml volumetric flasks, add 2.5ml of Barton's reagent and water to make the final volume to 50ml. After 10 minutes intervals, measure OD in a colorimeter and plot a graph between OD and concentration of phosphorus, and calculate values of P from experimental samples.

**RESULT**

**CHACTERIZATION OF PHOSPHATE SOLUBILIZING MICROORGANISMS**

Transparent zones of clearing around the colonies of microorganism in the pikovaskaya's agar plates were obtained. Among many organisms isolated, two fungal and two bacterial species formed transparent zone of clearance around them in pikovaskaya's agar plate, which indicate them as a phosphate solubilizing organism. Based on the morphology, the fungal culture and bacterial culture obtained were identified as *Aspergillus niger, Penicillium Chrysogenum, Bacillus Polymyxa* and Aerobic Spore former.

**THE EFFECT OF PH ON PHOSPHATASE ENZYME**

In our present investigation, Table 1 shows the acid Phosphatase *of Aspergillus niger, Penicillium Chrysogenum, Bacillus Polymyx*a and Aerobic Spore former shows the maximum activity at pH 5. But acid Phosphatase of *Bacillus Polymyxa* shows the maximum activity pH 4. Graph (1) Alkaline Phosphatase of *Penicillium Chrysogenum*, and aerobic spore former shows the maximum activity at 8.5 pH. but Alkaline Phosphatase of *Aspergillus niger* and *Bacillus Polymyxa* shows the maximum activity at pH 9.

**THE EFFECT OF TEMPERATURE ON PHOSPHATASE ENZYME**

In our study, Table .2 shows the acid phosphatase *Aspergillus niger, Penicillium Chrysogenum* shows the maximum activity of temperature $30^0$C. But acid phosphatase of Aerobic Spore former and Bacillus Polymyxa shows the maximum activity at temperature $40^0$C. Graph (2) Alkaline Phosphatase of Penicillium Chrysogenum, and Bacillus Polymyxa shows the maximum activity at temperature $30^0$ C. But alkaline phosphatase of Aerobic Spore former shows the maximum activity at temperature $40^0$C.

## THE EFFECT OF VARIOUS SUBSTRATES ON PHOSPHATASE ENZYME

In our present study Table. 3A, 3B shows there was no growth observed in minimal medium having p-nitro phenol phosphate as substrate. Growth observed in minimal medium having Tri Calcium Phosphate and Monopotassium Phosphate As Substrate, Graph(3) the pikovaskaya's liquid medium with calcium phosphate as substrate which is used for the estimation of phosphate solubilization showed a greater value when compared to pikovaskaya's liquid medium with mono potassium phosphate as substrates.

## QUANTITATIVE MEASUREMENT OF PHOSPHATE SOLUBILIZATION IN CULTURE MEDIUM

In the study, Table.4 shows the acid phosphatase of *Aspergillus niger* showed a highest solubilization when compared to *Penicillium chrysogenum*, Aerobic Spore former and *Bacillus Polymyxa*. The alkaline phosphatase of *Penicillium chrysogenum*, showed a highest solubilization when compared to Aspergillus niger, Aerobic Spore former and *Bacillus Polymyxa*. Graph (4).

## QUANTITATIVE MEASUREMENT ON PHOSPHATE SOLUBILIZATION IN CULTURE MEDIUM

A very good zone of clearance around the enzyme extracted from *Aspergillus niger*, *Penicillium Chrysogenum, Bacillus Polymyxa* and Aerobic Spore former are seen. Even the 1µ of enzyme extracted showed the zone of clearance.

## EXTRACTION AND PURIFICATION OF PHOSPHATASE ENZYME

In our study, the phosphatase enzyme was extracted successfully from *Aspergillus niger, Penicillium Chrysogenum, Aerobic Spore former and Bacillus Polymyxa.* The enzyme was purified by 60% of ammonium sulphate salt by dialysis method.

## ESTIMATION OF PHOSPHATASE ENZYME ACTIVITY

IN our present investigation, Table. 5 shows the alkaline phosphatase enzyme showed the highest activity when compared to acid phosphatase enzyme. The enzyme extracted from fungi showed the better activity than bacteria. The acid phophatase of *Aspergillus niger* showed a highest activity when compared to *Penicillium chrysogenum, Aerobic Spore former and Bacillus Polymyxa*. The alkaline phosphatase *Penicillium Chrysogenum*, showed a highest activity when compared to *Aspergillus niger.*

## DETERMINATION OF MOLECULAR WEIGHT BY SDS PAGE

The bands were observed and the molecular weight was found to be 60 kilo Daltons.

## EFFECT OF PH ON PHOSPHATASE ENZYME

| **ENZYME PHOSPHATASE** | **ACID PHOSPHATASE** | | | | | | **ALKALINE** | |
|---|---|---|---|---|---|---|---|---|
| ***ORGANISM / Ph*** | **3** | **4** | **5** | **6** | **8** | **8.5** | **9** | **10** |
| ***Penicillium chrysogenum*** | 0.05 | 0.08 | **0.10** | 0.07 | 0.08 | **0.11** | 0.07 | 0.06 |
| ***Aspergillus niger*** | 0.06 | 0.08 | **0.12** | 0.09 | 0.08 | 0.11 | **0.13** | 0.09 |
| ***Aerobic Spore former*** | 0.04 | 0.04 | **0.06** | 0.05 | 0.04 | **0.06** | 0.03 | 0.03 |
| ***Bacillus Polymyxa*** | 0.04 | **0.05** | 0.03 | 0.02 | 0.03 | 0.04 | **0.05** | 0.04 |

## EFFECT OFTEMPERATURE ON PHOSPHATASE ENZYME

| **ENZYME PHOSPHATASE** | **ACID PHOSPHATASE** | | | | | | **ALKALINE** | |
|---|---|---|---|---|---|---|---|---|
| **ORGANISM / TEMP ($^0$c)** | **10** | **30** | **40** | **60** | **10** | **30** | **40** | **60** |
| ***Penicillium chrysogenum*** | 0.07 | **0.13** | 0.08 | 0.06 | 0.09 | **0.15** | 0.10 | 0.08 |
| ***Aspergillus niger*** | 0.12 | **0.16** | 0.13 | 0.10 | 0.11 | **0.18** | 0.15 | 0.12 |
| ***Aerobic Spore former*** | 0.07 | 0.08 | **0.10** | 0.07 | 0.07 | 0.09 | **0.11** | 0.07 |
| ***Bacillus Polymyxa*** | 0.06 | **0.07** | **0.09** | 0.04 | 0.08 | **0.10** | 0.09 | 0.05 |

**THE EFFECT OF VARIOUS SUBSTRATES ON PHOSPHATASE ENZYME**

| 59ppm Stock (ml) | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| O.D | 0.04 | 0.07 | 0.10 | 0.13 | 0.15 | 0.18 | 0.21 | 0.23 | 0.25 | 0.27 | 0.31 | 0.34 | 0.37 | 0.41 | 0.43 |
| Conc (µg) | 59 | 118 | 177 | 236 | 296 | 354 | 413 | 472 | 531 | 590 | 649 | 708 | 767 | 826 | 885 |

**ESTIMATION OF PHOSPHATE SOLUBILISED BY ACID AND ALKALINE PHOPHATASE**

| ORGANISM | ACID PHOSPHATASE | ALKALINE PHOSPHATASE |
|---|---|---|
| ***Penicillium chrysogenum*** | 668 | 649 |
| ***Aspergillus niger*** | 708 | 628 |
| ***Aerobic Spore former*** | 531 | 590 |
| ***Bacillus Polymyxa*** | 472 | 531 |

**ESTIMATION OF PHOSPHATASE ENZYME ACTIVITY**

| ORGANISM | ACID PHOSPHATASE | ALKALINE PHOSPHATASE |
|---|---|---|
| ***Penicillium chrysogenum*** | 0.18 | 0.20 |
| ***Aspergillus niger*** | 0.16 | 0.21 |
| ***Aerobic Spore former*** | 0.12 | 0.13 |
| ***Bacillus Polymyxa*** | 0.09 | 0.11 |

## DISCUSSION

Optimum temperature range of 28 °C−30 °C for fungi and temperature of 25 °C for some bacteria.[4] Furthermore, Optimum phosphatase production at temperature of 37 °C in *Mycococcus*. The fact that phosphatases are produced optimally at such temperature range shows that phosphatase activity would decrease as the temperature increases above 37 °C. On the other hand, an earlier study [5,6] showed that the optimum temperature for the hydrolysis of pNPP by alkaline phosphatase was 47 °C. In our study optimum temperature was discussed with previous observation for the production of phosphatase enzyme.

## SUMMARY

- ✓ In our present work the soil was collected from garden at Idhaya College for women. The organisms like *Aspergillus Niger , Penicillium Chrysogenum , Aerobic Spore former and Bacillus Polymyxa* .
- ✓ For testing pH by adding acid buffer *to Aspergillus Niger , Penicillium Chrysogenum , Aerobic Spore former* shows maximum activity at pH 5 but Bacillus Polymyxa shows at pH 4. By adding alkaline buffer Penicillium Chrysogenum, Aerobic Spore former at pH 8.5, Aspergillus Niger , Bacillus Polymyxa at pH 9.
- ✓ For testing temperature, Acid phosphate *of Aspergillus niger , Penicillium Chrysogenum* shows maximum activity at $30^0$C, but Aerobic Spore former, Bacillus Polymyxa at $40^0$ C . In alkaline phosphate of *Aspergillus Niger, Penicillium Chrysogenum, Bacillus Polymyxa* highest activity shows at $30^0$C, but Aerobic Spore former at $40^0$ C.
- ✓ In our present investigation, the pikovskaya's liquid medium with tricalcium phosphate solubilize phosphate highly than Monopotassium phosphate.
- ✓ In our experiment Acid Phosphate of *Aspergillus niger* shows highest solubilizing capacity then other. Alkaline phosphate of *Penicillium Chrysogenum* shows highest solubilizing capacity than others.
- ✓ The phosphate enzyme is extracted, purified and the activity of the enzyme is estimated.The molecular weight of the enzyme is identified using SDS PAGE.
- ✓ In our present investigation the farmers are instructed to use phosphate solubilizing organisms instead of using chemical fertilizer. The greater part of the soil P is in the form of insoluble p

which can't utilize by plants, by using phosphate solubilizing microorganisms P is soluble easily.

- ✓ For the commercial production of more p solubilizing microorganisms by using Pikovskaya's agar medium.

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SP-06

# THE EFFECTS OF WATER POLLUTION BY USING HISTOPATHOLOGICAL STUDY OF *Tilapia Mossambica* IN POLLUTED POND

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## ABSTRACT

The pollution of the pond leads to the spoilage of the fishes and causes diseases to the fish population. By eating the polluted pond fishes, indirectly it causes some of the serious diseases to the human population. The polluted pond water and the experimental fish *Tilapia mossambica* is used to find out the pollution of the pond and its serious damage to the living organisms. The water sample and the experimental fish *Tilapia mossambica* are collected from the contamination pond. The water quality is finding out by the $P^H$ test, electrical conductivity test, biological oxygen demand test, chemical oxygen demand test, salinity test. Each test is carried out 13 times in 3 months at 7 days interval for each time, to avoid the error. The pathogens are isolated from the pond water and the experimental fish *Tilapia mossambica*. The pathogens *Corynebacterium Michigan's, Micrococcus roseus, Proteus mirabilis are* isolated from the pond water. The bacterial pathogens isolated from the fish *Tilapia mossambica* are *Corynebacterium michiganese, Micrococcus roseus , Proteus mirabilis,* and the isolated fungi pathogens are *Fusarium solani,Trichoderma harzianum, Penicillium digitatum*. The isolated bacterial and fungi pathogens from the polluted pond are the same as the pathogens isolated from the experimental fish *Tilapia mossambica*. The fish *Tilapia mossambica* has the same pathogens of the polluted pond because of living in the polluted pond. In this present study, the effect of water pollution was determined by studying the histopathology of *Tilapia Mossambica* in a polluted pond.

**KEYWORDS:** *Tilapia Mossambica*, Pollution, *Corynebacterium michiganese, Micrococcus roseus, Proteus mirabilis*

## INTRODUCTION

Water is an essential component of inorganic mixtures from which chemists, in their quest to understand the origin of life, attempt to produce complex organic molecules.[1] Among natural materials, the water probably possesses the highest heat capacity and very high latent heats of melting and evaporation. Water is an extraordinary geological agent, shaping the earth's surface by denudation and deposition governing the evolution of the earth's crust[2]. water needed to sustain the growth cycle of plants is referred to as consumptive water. Water on the earth is so intimately tied up with life that a discussion of the hydrological cycle is incomplete without a look at nutrient cycles. In many parts of the country, groundwater levels continue to decline due to over. Assured clean water supplies are lacking in urban centers and rural villages[3]. The natural feed is derived from phytoplankton and zooplankton. Production of these planktons is done by manuring which is rich in the nitrogenous matter or chemical or green manure or less organic carbon provided by natural means in the freshwater. But the fish is polluted due to the numerous microorganisms in the freshwater that affect human health directly. Polluted drinking water is the major source of illness and death throughout the world[4].

## MATERIALS & METHODS

## CHARACTERIZATION OF THE POLLUTED POND WATER

### $P^H$ MEASUREMENT

$P^H$ measurements of the sample were measured by using the $P^H$ meter. The $P^H$ meter bulb was washed in the clean distilled water. Then, $P^H$ blub was dipped into the sample, and readings were noted for 13 times in 3 months at 7 days interval for each.

### ELECTRICAL CONDUCTIVITY TEST

The electrical conductivity value of the sample was detected by using an electrical conductivity meter. The sample was taken in a sterilized beaker. The electrical conductivity bridge was switched on and waited for 10 minutes. The electrodes were washed carefully and immersed in the sample. The readings were noted for 13 times in 3 months at 7 days interval for each time.

### BIOLOGICAL OXYGEN DEMAND TEST

200 ml of the effluent was collected in 6 B.O.D bottles respectively. 1 ml of allyl thiourea was added to each of the bottles. From 6 B.O.D bottles containing the sample, 3 B.O.D bottles were taken and added with 2ml of $mg_2so_4$ with alkaline iodine solution. The brown color precipitate appeared and 2 ml of concentrated sulphuric acid was added. From the above mixture, 50 ml of the solution was taken and titrate against the thiosulphate; the endpoint was noted as the color change from brown to pale straw. 2 drops of thiosulphate solution was added. It was titrated again with a thiosulphate solution until the blue color disappeared. The mean value titrant of the unincubated 3 B.O.D bottles was taken as DI. The remaining 3 B.O.D bottles were incubated. After incubation, the same procedure was done. The mean value of the 3 incubated bottles was noted as D2. The readings were noted for 13 times in 3 months at 7 days interval for each time.

### CALCULATION

B.O.D=D1-D2

$$D=\frac{8 \times 1000 \times N \times A}{V}$$

N=Normality of the titrant
V=Volume of the sample taken
8=is the constant.
A=Volume of the titrant used.

### ENUMERATION OF BACTERIAL PATHOGENS FROM THE POLLUTED POND WATER

Enumeration was carried out using the serial dilution technique. Biochemical analysis performed to Indole, Methyl red, Voges-Proskauer, Citrate utilization, Catalase, Urease, Hydrogen sulfide & Carbohydrate fermentation test. The incubated colony of bacteria is mixed with $H_2O_2$ in the slide. the production bubble indicates a positive result. The isolated colonies were detected by using the different selected media.

### ENUMERATION OF THE BACTERIAL AND FUNGAL PATHOGENS FROM THE POLLUTED POND CONTAINING FISH *Tilapia mossambica*

1ml of the homogenized supernatant of the tissues was taken as the inoculum. The sample was inoculated by the spread plate method both in the nutrients as well as in the potato dextrose agar. The inoculated nutrient agar and PDA plates were incubated for 24 hours at $37^0$C and 48 hours at $28^0$C respectively. After the incubation, the colonies were counted and expressed in CFU/ml. The isolation and identification of bacteria and fungi were done as per in the Enumeration of pathogens from the polluted pond water.

## RESULTS

### CHARACTERIZATION OF THE POLLUTED POND WATER

The water sample was collected from the polluted pond in the Nellikuppam area at Cuddalore district.

### $P^H$ MEASUREMENT

The $P^H$ was measured in the pond 13 times in 3 months at 7 days interval for each time. It was varied from 6.2 to 6.5 with an average of 6.3. It showed that the pond water was acidic nature.

## ELECTRICAL CONDUCTIVITY TEST

The electrical conductivity test was measured for 13 times in 3 months at 7 days interval for each time. It was varied from 1.422 to 1.756 with an average of 1.563. It showed that more amount of soluble salts present in the pond. The results were tabulated.

## BIOLOGICAL OXYGEN DEMAND TEST

The biological oxygen demand was measured in the polluted pond water for 13 intervals in 3 months at 7 days interval. The biological oxygen demand was varied from 1009 to 1016 with an average of 1012. The biological results showed that the maximum amount of organic pollution in the pond. The results were tabulated.

## CHEMICAL OXYGEN DEMAND TEST

The chemical oxygen demand was measured in the pond water for 13 intervals in 3 months at 7 days interval. The chemical oxygen demand differed from 600 to 620 with an average of 614. The results of chemical oxygen demand showed that the presence of oxidizing agents. The results are tabulated.

## SALINITY TEST

The salinity was varied from 0.179 to 0.812 with an average of 0.553. It showed that more amount of chloride ions is present in the pond. The results are tabulated.

## ENUMERATION OF THE BACTERIAL AND FUNGAL PATHOGENS FROM THE POLLUTED WATER

The pond water was serially diluted up to $10^{-7}$. One ml of the water from $10^{-4}$ was inoculated in nutrient agar and incubated for 24 hours. After the incubation period, *Micrococcus Roseus, Corynebacterium Michiganese, Proteus Mirabilis* are isolated from the pond water. The results were tabulated. *Micrococcus roseus* gives positive to the Gram's staining test, Indole test, Citrate test, Catalase test, Maltose fermentation test, Lactose fermentation test, sucrose fermentation test, and negative to the Motility test, methyl red test, Voges Proskauer test, Oxidase test, Hydrogen sulfide test, round regular pink colour colonies to the Tryptophan soya bean agar media. *Corynebacterium michiganese* gives positive to Gram's staining test, , Indole test, Citrate test, Oxidase test, Catalase test, Maltose fermentation test, Lactose fermentation test, sucrose fermentation test, and negative to the Motility test, methyl red test, Voges Proskauer test, Urease test, Hydrogen sulfide test, black colour concave colonies to the tellurite blood agar media. *Proteus mirabilis* gives positive to the Gram's staining test, methyl red test, Citrate test, catalase test, Urease test, Hydrogen sulfide test, maltose fermentation test, Lactose fermentation test, Sucrose fermentation test, and negative to the Motility test, Indole test, Oxidase test, white mucoid colonies to the XLD media[5]. The $10^{-2}$ dilution was inoculation in the PDA agar plate and incubated for 48 hours at $28^0$C. After incubation, the isolated fungi are *Fusarium solani* with micro and macroconidia, *Penicillium digitatum* with conidiophores containing sterigmata, *Trichoderma harzianum* with groups arranged conidia[6].

## ENUMERATION OF BACTERIAL AND FUNGAL PATHOGENS FROM THE EXPERIMENTAL FISH *Tilapia Mossambica*

The fish Tilapia Mossambica was collected from the same polluted pond. After sterilization, the tissues of the fish were taken and homogenized. By using the homogenized fish the bacteria isolated are *Micrococcus roseus,Corynebacterium michiganese, Proteus mirabilis,* and the isolated fungi pathogens are *Fusarium solani, Penicillium digitatum, Trichoderma harzianum.* The isolated bacteria and fungi pathogens from experimental fish Tilapia Mossambica are the same as the pathogens isolated from the polluted pond.

**Table:1. Characterization of the polluted pond water**

| INTERVALS | B.O.D | C.O.D | EC | $P^H$ | SALINITY |
|---|---|---|---|---|---|
| 03/02/10 | 1010 | 615 | 1.528 | 6.2 | 0.256 |
| 10/02/10 | 1015 | 610 | 1.756 | 6.5 | 0.567 |
| 17/02/10 | 1009 | 600 | 1.422 | 6.5 | 0.179 |
| 24/02/10 | 1016 | 620 | 1.546 | 6.3 | 0.812 |
| 03/03/10 | 1015 | 612 | 1.548 | 6.4 | 0.421 |
| 10/03/10 | 1010 | 615 | 1.576 | 6.4 | 0.518 |

| | | | | | |
|---|---|---|---|---|---|
| 17/03/10 | 1010 | 615 | 1.478 | 6.4 | 0.417 |
| 24/03/10 | 1013 | 618 | 1.492 | 6.3 | 0.412 |
| 31/03/10 | 1014 | 610 | 1.312 | 6.2 | 0.412 |
| 07/04/10 | 1012 | 615 | 1.562 | 6.2 | 0.718 |
| 14/04/10 | 1014 | 615 | 1.618 | 6.5 | 0.815 |
| 21/04/10 | 1013 | 620 | 1.742 | 6.5 | 0.816 |
| 28/04/10 | 1016 | 620 | 1.751 | 6.5 | 0.816 |

## DISCUSSION

Water is an extraordinary natural phenomenon. The hydrological nutrient and erosional cycles on which all living communities depend are delicately interlinked. Undue human intervention with these cycles can force them towards new equilibria that may seriously stress on otherwise endanger existing communities. Among the water resource pond is one of the aquatic resources. Due to human activities, the ponds are polluted. The pollution of the pond leads to the spoilage of the fishes and causes diseases to the fish population. By eating the polluted pond fishes, indirectly it causes some of the serious diseases to the human population[7]. In the present study, the polluted pond water and the experimental fish *Tilapia mossambica* is used to find out the pollution of the pond and its serious damage to the living organisms. The water sample and the experimental fish *Tilapia mossambica* are collected from the contamination pond. The water quality is finding out by the $P^H$ test, electrical conductivity test, biological oxygen demand test, chemical oxygen demand test, salinity test. Each test is carried out 13 times in 3 months at 7 days interval for each time,to avoid the error. The hydrogen ion concentration is the important hydrobiological parameters which influence the growth and metabolic function of aquatic organisms. In the present study, the $P^H$ value of the pollution pond is acidic acidic with an average value of 6.3. It is not suitable for some of the living organisms. The $P^H$ of the pollution pond is mostly acidic. Research conducted by Edith Fanta in the histopathological of fish pollution with sublethal levels of organophosphorus in water and food is similar to the present study. It resulted that the gill respiratory lamellae, hyperplasia, edema, and detachment occurred, diminishing sooner after contamination by food than after contamination through the water.

## SUMMARY

The location of research is the polluted pond in the Nellikuppam area, at Cuddalore district. The experimental fish Tilapia mossmbica and the pond water are collected from that polluted pond. By using the pollution pond water the $P^H$, Biological oxygen demand test, chemical oxygen demand test, Electrical conductivity test, and the salinity test are done. The $P^H$ test shows that the pond is in the acidic nature. The electrical conductivity test results show that the soluble salts are present in a large amount. The biological oxygen demand test result tells that the pond containing a higher amount of organic pollution. The chemical oxygen demand test refers that the presence of chemical oxidizing agents is more in the pond water. The salinity test shows that the presence of the chloride ions in the polluted pond. The bacterial pathogens isolated from the pond are *Corynebacterium michiganese*, *Micrococcus users, Proteus mirabilis.* Fungal pathogens isolated from the pond are *Penicillium digitatum, Fusarium solani, Trichoderma harzianum.* The pathogens present in the fish are also the same which is present in the polluted pond water.

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SP-07

# EVALUATION OF PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL EFFICACY OF *Syzygium cumini* ON DIABETIC WOUND PATHOGENIC BACTERIA

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## ABSTRACT

*Syzygium cumini* is one of the widely used medicinal plants in the treatment of various diseases. The phytochemical investigation was carried out on the Ethanol, Aqueous, Acetone extract of *Syzygium cumini*. Phytochemical studies revealed the presence of flavonoids, alkaloids phenols, saponins tannin, terpenoids, and steroids as the chemical class present in the extract. Antibacterial activity against *Klebsiella sp.,*, *Staphylococcus aureus Pseudomonas,* and inhibitory effect of ethanol, aqueous and acetone extraction of *Syzygium cumini* was investigated in-vitro. All these strains were observed with moderate to good antibacterial activity.

**KEYWORDS**: *Syzygium cumini,* phytochemistry, antibacterial and medicinal uses.

## INTRODUCTION

### DIABETIC WOUND INFECTION

Wounds are generally classified as wounds without tissue loss (e.g. in surgery) and wound with tissue loss, such as burn, wound caused as a result of trauma, abrasions or as secondary events in chronic ailments when diabetic patients develop an ulcer, they are exposed to high risk for major complications including infection and amputation. People with diabetes are also at risk of developing a diabetic foot ulcer. Research estimates that the lifetime incidence of foot ulcers within the diabetic community is around 15% and may become as high as 25%. Plants which have been selected for medicinal use over thousands of years constitute the most obvious starting point for new therapeutically effective drugs such as anticancer drugs and antimicrobial drugs.[10]

### MEDICINAL PLANT

In recent years the antimicrobial properties of the medicinal plants are being increasingly reported from different parts of the world [12]

### Syzygium cumini PLANT

The barks, leaves, and seeds extract of *S.cumin*i have been reported to possess anti-inflammatory [3] and antidiarrheal effects [6]

### PHYTOCHEMICAL COMPOUNDS

Medicinal properties of plants need to evaluating scientifically, for the treatment of infectious disease produced by the pathogen. The medicinal values of *Syzygium cumini* have been recognized in different traditional medication systems, for the treatment of various diseases of human beings. The fruits and seeds of *Syzygium cumin* have been studied mainly for its anti-diabetic properties. Multidrug resistance in clinical bacteria like *S.aureus* is responsible for nosocomial infection. [8]Leaves have been used in traditional medicine as a remedy for diabetes mellitus in many countries [11.] The leaves are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy, constipation, and to inhibit blood discharge in the feces[2]. The screening of plant extracts for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents. [1] According to the World Health Organization plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world drugs are of natural product origin[7].

## MATERIALS AND METHODS

### Collection of wound samples

In, the present study diabetics wound samples were collected from diabetics patients at Anbu hospital, Kumbakonam.

### Isolation and identification

Isolation and identification process was performed

### Preparation of pure culture

The pure culture was made from the isolated pathogens like *Staphylococcus sp., and Klebsiella sp., and Pseudomonas sp.,* from Diabetic wound sample by inoculating into McConkey agar, mannitol salt agar media incubate at 37-degree celsius for 24hours.

### COLLECTION OF PLANT

*The Syzygium cumini* plant leaves were collected from the local area in Anniyur, Kudavasal taluk, nearby Kumbakonam, Thiruvarur district.

### PREPARATION OF PLANT EXTRACT

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant (leaves*). Syzygium cumini* plant leaves were washed with distilled water and kept in an incubator at 37ºC for 3-4days and grinned into a fine powder. Now, the plant material was dissolved in 70%ethanol and 80% Acetone, and distilled water 1g samples should be dissolved in 10ml of solvent. Mixtures were kept in the dark for 3days at room temperature in sterilized beakers.

### TEST FOR PHYTOCHEMICALS ANALYSIS

The extracts were analyzed for the presence of alkaloids, terpenoids, reducing sugar, saponins, tannins, flavonoids, and steroids.

### Test for Alkaloids

Weight about 0.2g of plant extract in a separate test tube and warmed with sulphuric acids for 2 minutes. It was filtered in a separate test tube and few drops of dragendrouff`s reagent were added and observe for the presence of orange and red precipitates for the presence of alkaloids.

### Test for Terpenoids

Weight about 0.5ml of plant extracts in a separate test tube with 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1ml of concentrated tetraoxo sulfate acid. And observe for brown ring formation at the ring interface.

### Test for Reducing sugar

Take a test tube and add 2ml of crude plant extract and add 5ml of distilled water and then heat to boil. Observe for orange-red precipitate which indicates the presence of reducing sugars.

### Test for saponins

Weight about 0.2ml of plant extract in the test tube and 5ml of distilled water and then heat to boil. Observe the occurrence of frothing (appearance of a creamy mass of small bubble) which indicates the presence of saponins.

### Test for Tannin

The small quantity of plant extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. Observe dark green solutions that indicate the presence of tannin.

### Test for steroids

The plant extract was mixed with 2ml of acetic anhydride and add 0.5gm of ethanolic extract of each ample with 2mlof sulphuric acid. Observe for the color change from violet to blue or green in sampling indicating

the presence of steroids.

## DETERMINATION OF ANTIBACTERIAL EFFECT OF *Syzygium cumini* BY ANTIBACTERIAL ASSAY

### Agar well diffusion method

The agar well diffusion method as adopted earlier was used.0.1ml of diluted inoculums ($10^5$CFU/ml) of the test organism *(Klebsiella sp., Staphylococcus sp.,& Pseudomonas sp.,)* was spread on Muller-Hinton agar plates.

### Agar disc diffusion

A disc diffusion method was used for the antibacterial assay. Sterile Muller Hinton agar plates were prepared for bacterial strains *(Klebsiella sp., Staphylococcus sp., & Pseudomonas sp.,)* and inoculated by a spread plate method under aseptic condition. The antibacterial activity of each extract was expressed in terms of the means of the diameter of the zone of inhibition (in mm).

## RESULT

### PATHOGENIC BACTERIA

The pathogenic bacteria were isolated from the diabetic wound infection by using the swab technique and isolated in nutrient agar medium (Fig.1). Then the isolated bacteria were identified through a gram staining method and number of various biochemical tests (2A, B, C).

### PHYTOCHEMICAL ANALYSIS

Phytochemical screening of the plant extract was analyzed the secondary metabolites like Alkaloids, Saponin, Tannin, Flavonoid&Terpenoids, and Steroids were found in leaf extract of *Syzygiumcumini*

### EFFECT OF ANTIBACTERIAL POTENTIAL OF *Syzygium cumini* ON *Klebsiella, Pseudomonas and staphylococcus* BY AGAR WELL DIFFUSION METHOD

70% Ethanolic extract, 80% Acetone extract & Aqueous extract of *Syzygium cumini* showed (Fig.3A) the inhibition activity of *Klebsiella*.

70% Ethanolic extract,80% Acetone extract & Aqueous extract of *Syzygium cumini* showed (Fig.3B) the inhibition activity of *Pseudomonas*.

70% Ethanolic extract,80% Acetone extract & Aqueous extract of *Syzygium cumini* showed (Fig3C) the inhibition activity of *staphylococcus.*

### EFFECT OF ANTIBACTERIAL POTENTIAL OF *Syzygium cumini* ON *Klebsiella, Pseudomonas* and *Staphylococcus* BY DISC DIFFUSION METHOD

70% Ethanolic extract,80% Acetone extract & Aqueous extract of *Syzygium cumini* showed (Fig.4A) the inhibition activity of *Klebsiella.*

70% Ethanolic extract,80% Acetone extract & Aqueous extract of *Syzygium cumini* showed (Fig.4B) the inhibition activity of *pseudomonas.*

70% Ethanolic extract,80% Acetone extract & Aqueous extract of *Syzygium cumini* showed (Fig.4C) the inhibition activity of *Staphylococcus.*

### ANTIBIOTIC SENSITIVITY TEST

For the comparative study, the synthetic drug erythromycin is used for the antibacterial assay.


**FIGURE 1. BIOCHEMICAL TESTS**

**FIGURE:2A Biochemical test for *Pseudomonas sp.,***

**FIGURE: 2B** Biochemical test for *Staphylococcus aureus*

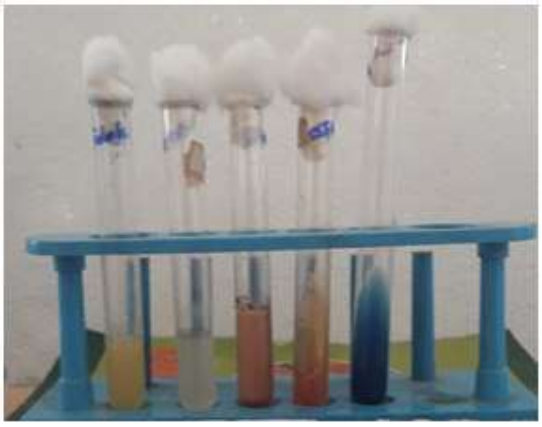

**FIGURE:2C** Biochemical test *Klebsiella sp.,*

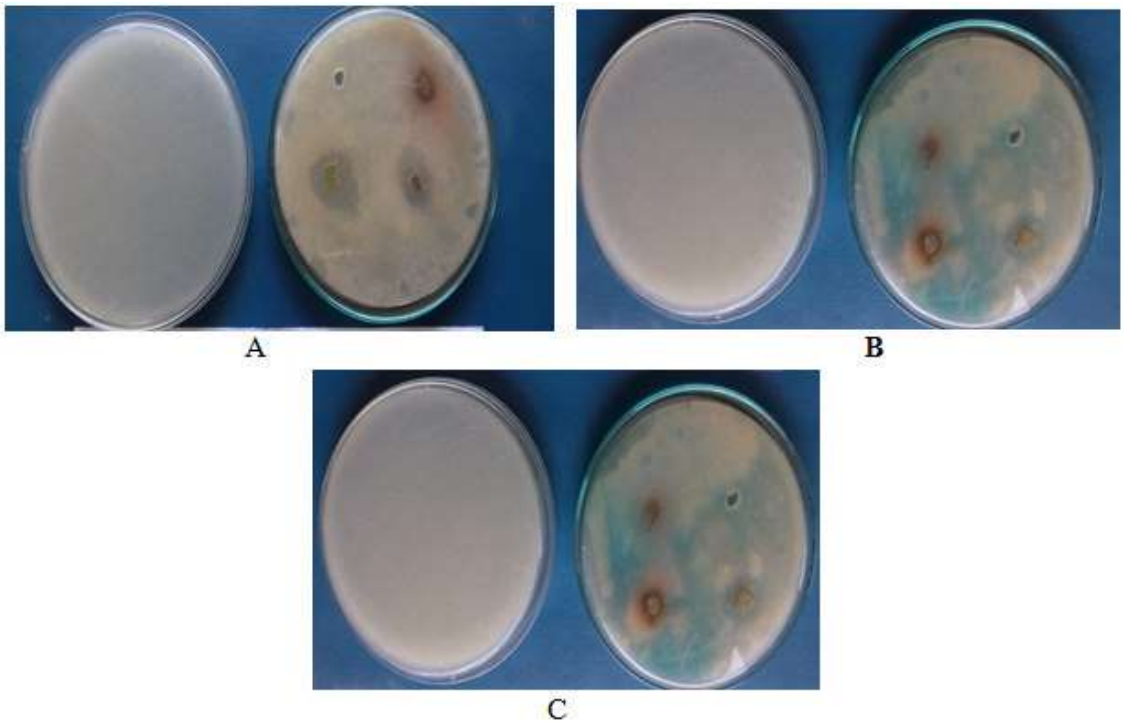


**FIGURE 3 ANTIBACTERIAL EFFECT OF *Syzygium cumini* ON *Klebsiella sp., Staphylococcus sp., Pseudomonas sp.,* USING WELL DIFFUSION METHOD**

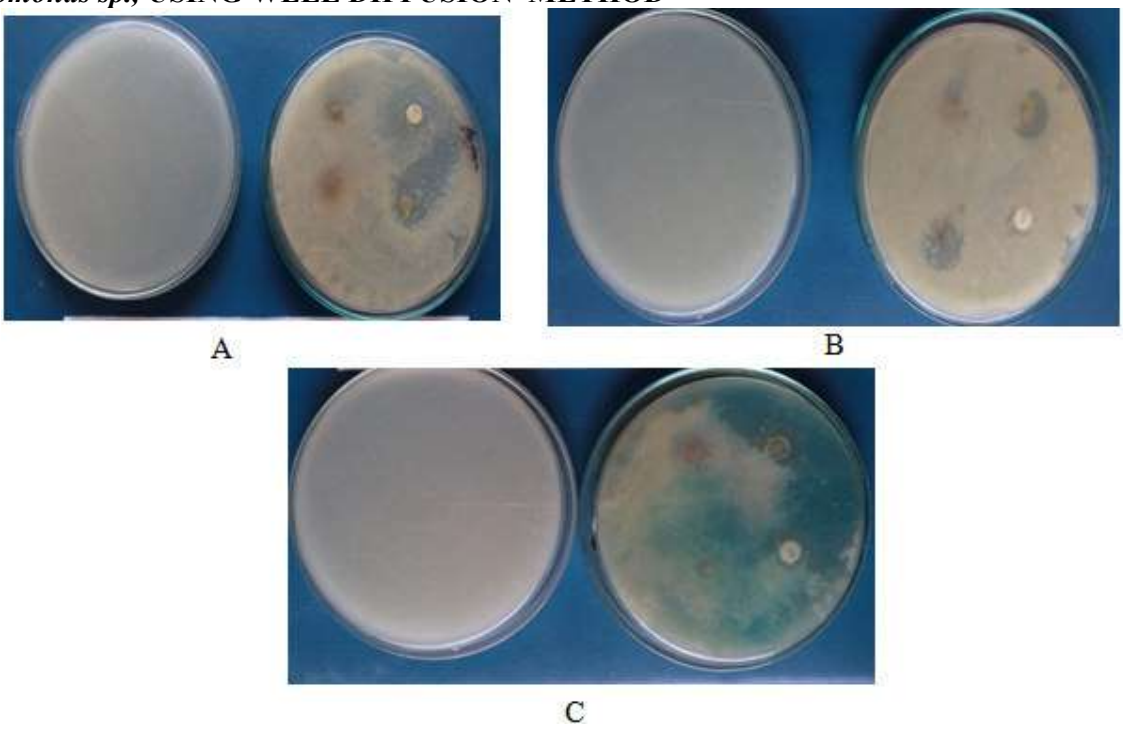


**FIG4. ANTIBACTERIAL EFFECT OF *Syzygium cumini* ON *Klebsiella sp., Staphylococcus sp., and Pseudomonas sp.,* - DISC DIFFUSION METHOD**

## DISCUSSION

Diabetic wound patients is quite susceptible to a bacterial infection which contributes to express morbidity. In this study among diabetic wound infection patients. In this study among diabetic wound infection cause the predominant organism isolated was three bacterial species were used in the course of this study. This was coagulase positive *Staphylococcus aureus,* coagulase-negative *Pseudomonas aeroginosa, Klebsiella pneumonia.* This study was to find out the antibacterial activity of *Syzygium cumini* leaf extract against some selected strains of multidrug-resistant *S.aureus and E.coli* strains. The extracts of *the S.cumini* leaf

showed significance, antimicrobial activity against all tested bacterial strains. Antibacterial activity of leaf extracts can be attributed due to the presence of these phytochemicals [4], The results of this study support the use of this plant for human diseases and explore the ethnobotanical importance of plant as a potential source of bioactive substances. The study focuses on the antibacterial activity of a variety of solvent extracts of *S.cumini.*The present research also observes the sensitivity pattern of selected pathogens towards the extract of *S.cumini* as well as standard antibiotics. In our study, the *S.aureus and E.coli* strains were observed multidrug-resistant against the common antibiotics used.[9] *S.cumini* extract showed the ofsignificant antibacterial activity against almost all bacterial strains tested. Antimicrobial activity of *S.cumini* was also previously reported by other workers Combined therapy has been justified to decrease bacterial resistance and produce a desirable significant synergistic effect.[5]Antibiotic synergism with bioactive plant extract in useful in treating infectious diseases.

## CONCLUSION

Our study certifies that the extract of Syzygium cumini has a significant potential antimicrobial activity against the multidrug-resistant bacteria and also serves important data regarding the valuable research in treating infectious diseases. It also reveals that the petroleum ether may be an effective solvent in the future for antimicrobial studies of Syzygium cumini. The research data are also comparable with the common antibiotics used against S. aureus and Escherichia coli. A considerable synergism was also obtained between the Syzygium cumini and antibiotics used against *S. aureus* and *Escherichia coli.* so *Syzygium cumini* may be an effective alternative of antibiotics in the treatment of infectious diseases

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SP-08

# A STUDY ON EVALUATION OF ANTIBACTERIAL EFFECT OF CITRUS FRUITS EXTRACTS AGAINST WOUND PATHOGENS

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## ABSTRACT

Medicinal plants are the natural resources in the development of new drugs. The antibacterial activity of natural products from the medicinal plant is applicable for the treatment of bacterial, fungal, and viral diseases and these are recent technical advancements taken place in this area. In my present study focused on isolation, identification of pathogenic bacteria, and antibacterial activity of citrus fruits. Normal wound patients are quite susceptible to bacterial infections which contribute to excess morbidity. In this study among wound infection patients. In the present work of the citrus fruits showed antibacterial activity against the normal wound pathogenic bacteria *Escherichia coli, Staphylococcus aureus.* Citrus fruits lemon (*Citrus limon),* Sweet orange *(Citrus sinensis),* bitter orange *(Citrus aurantium)* juice is effective than the antibiotics against the pathogens. This finding can form the basis for further studies to prepare an optimized preparation of the herbal extracts as a medical product.

**KEYWORDS:** Citrus fruit, pathogenic strains, antibacterial effect

## INTRODUCTION

The citrus fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetics, and folk medicine [17]. In addition to large scale consumption as fresh fruits, the fruits are mainly processed to produce juice. The waste of the Citrus processing industry left after juice extraction, such as peels, seeds, and pulps, corresponding to about 50% of the raw processed fruit, can be used as a potential source of valuable by-products [5]. A skin forms part of the non-specific host defenses and functions as a mechanical barrier to the surrounding environment and against microbial invasion[10]. Injuries to the skin (punctures, burns, bites, etc..) provide an entry route for pathogens to infect the skin and underlying tissues. The severity of skin damage is related to the degree of invasion into the epidermis and dermis layers [22]. Skin damage may originate from physical injuries such as burns, microbial agents(bacterial, viral, fungal, parasitic) or a combination of the two such as burn wound infections. Infection not only delays healing but may also lead to tissue necrosis in wounds. The presence of necrotic tissue and large amounts of exudates in wounds encourage microbial proliferation and infection [7]. In most of developing countries, traditional medicines and medicinal plants have been used as therapeutic agents for the maintenance of good health that has been widely observed. Medicinal plants are considered as clinically effective and safer alternatives to synthetic antibiotics. Bacterial resistance has appeared for every major class of antibiotics[8.] Screening of bacterial strains for susceptibility to *C. Limonum* fruit juice extract was by a modification of agar well diffusion technique [14]. The phenolic compounds in citrus peels are responsible for antimicrobial activity[4]. One study found the use of different concentrations of Citrus juice extracts had an effective antibacterial activity against *Staphylococcus aureus*, *Proteus Vulgaris,* and *Pseudomonas aeruginosa*[1].In medicinal plants, the different parts of plants were used and extracts were subjected to antimicrobial assays[16]. Plants are an important source of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant derived ingredient[15]. Antibacterial effects of various citrus peels have been demonstrated in the literature [9]. Potent antibacterial activity (against *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermis, Shigella flexineri, Bacillus subtilis, and Escherichia coli).*

## MATERIALS AND METHODS

### COLLECTION OF WOUND SAMPLE

The clinical sample (Normal wound) collected aseptically was from the Government Hospital from Kumbakonam, Tanjore Dt, (Tamilnadu).The sampled swab was taken immediately to the laboratory for processing of the sample.

### ISOLATION OF PATHOGENIC ORGANISMS FROM CLINICAL SAMPLES

Isolation of clinical samples was performed by serial dilution technique using nutrient agar media. Slants containing pure culture were stored at $4^0$c for further examination.

### MORPHOLOGICAL AND PHYSIOLOGICAL TEST

Cultural characteristics of pure isolates in nutrient agar media were recorded after the incubation period for $30^0$c of 48 hrs. Morphological methods consist of macroscopic and microscopic methods. Motility and Gram staining tests were performed.

### INOCULATION INTO SELECTIVE MEDIUM

Based on the colony morphology and the grams staining and motility, culture was inoculated in selective media like *Macconkey agar, Blood agar, and Eosin methylene blue agar, cetrimide agar.* It was incubated at $37^0$c for 24hrs.

### BIOCHEMICAL TEST

The various be test was performed.

### PREPARATION OF CITRUS FRUIT JUICES AT VARIOUS CONCENTRATION:

The preparation of Lemon juice *(Citrus limon)*, Sweet orange juice *(Citrus sinensis)*, Bitter orange juice *(Citrus aurantium)* in various concentrations like 100%, 75%, 50% & 25% by the following manner. In 100% consist of pure lemon juice without diluted, 75% consists of 7.5ml of lemon juice diluted with 2.5ml of distilled water, 25% consist of 2.5ml of lemon juice diluted with 7.5ml of distilled water.

### DETERMINATION OF ANTIBACTERIAL EFFECT OF CITRUS FRUITS

### PREPARATION OF LEMON JUICE, SWEET ORANGE JUICE & BITTER ORANGE JUICE EXTRACT DISC

Whatman no.1, 6mm filtered paper disc was prepared and sterilized by autoclaving. These discs were plated and each disc was impregnated with 15l diffusion concentration of juices and dried overnight at $31^0$C. This was carried out under sterile conditions inside a laminar flow.

* Muller-Hinton agar plate was inoculated with the standard inoculums like *(S. aureus & E. coli)*
* The citrus fruits Lemon juice, Sweet orange juice & Bitter orange juice extract under the concentration of 25%, 50% & 75%, & 100% was dipped into the disc.
* The fruit extract disc was placed over the inoculated agar with pathogens *(S. aureus & E. coli).*
* After 24 hours of incubation at $37^0$C, Zone of inhibition was measured and recorded. A triplicated plate was also carried out.

### ANTIBIOTIC SENSITIVITY TEST

To compare the activity of the test material, synthetic antibiotics such as Gentamicin (10mg), Streptomycin (10mg), Kanamycin (10mg) and Amikacin (10mg) discs were used.

## RESULT

### PATHOGENIC BACTERIA

The pathogenic bacteria were isolated from the wound infection by using the swab technique and isolated in nutrient agar medium. Then the isolated bacteria were identified through a gram staining method (Table-1) and several various biochemical tests (Table-2).

## COLLECTION OF SAMPLES

The collection of citrus fruits Lemon *(Citrus limon)*, Sweet orange*(Citrus sinensis)*, Bitter orange *(Citrus aurantium).*The preparation of citrus fruit juice from various concentration (25%, 50%, 75% & 100%) of fruit samples.

## ANTIBACTERIAL ACTIVITY

The antibacterial activity of citrus fruit juices of lemon *(Citrus limon)*, Sweet orange *(Citrus sinensis)*, Bitter orange *(Citrus aurantium).* All the juices exhibited different degrees of antibacterial activity. Various concentrations of citrus fruit juices showed the activity against the two tested organisms.

## ANTIBACTERIAL ACTIVITY OF CITRUS FRUIT JUICES ON *Staphylococcus aureus*

Antibacterial activity of citrus fruit juices on *Staphylococcus aureus* was determined and shown in (Table-4).

## ANTIBIOTIC SENSITIVITY TEST

The antibacterial activity of citrus fruit juices of Lemon, Sweet orange, and Bitter orange compared with the synthetic antibiotics such as Amikacin, Gentamycin, Kanamycin, Streptomycin.

**Table: 1. MORPHOLOGY IDENTIFICATION TESTS FOR ISOLATED ORGANISMS**

| Tests | *S.aureus* | *E.coli* |
|---|---|---|
| **COLONY MORPHOLOGY** | Round smooth raised and glistening, usually from gray to deep golden yellow colour. | Circular, convex and smooth colonies with distinct edges grayish-white colour colonies. |
| **GRAM REACTION** | Gram-positive cocci and appears as grape-like cluster. | Gram-negative Rods. |
| **MOTILITY** | Nonmotile. | Motile and Non Motile. |

**Table: 2. BIOCHEMICAL IDENTIFICATION TESTS FOR ISOLATED ORGANISMS**

| Biochemical Test | *S.aureus* | *E.coli* |
|---|---|---|
| INDOLE | - | + |
| METHYL RED | + | + |
| VOGES PROSUKAUER | - | - |
| CITRATE | - | - |
| TSI TEST | + | - |
| UREASE | - | - |
| OXIDASE | - | - |
| CHO TEST | A | A |
| CATALASE | + | + |
| SUCROSE | A | A |
| LACTOSE | A | A&G |
| GLUCOSE | A | A&G |

**Table: 3. ANTIBIOTICS SENSITIVITY TEST ON *S.aureus***

| NAME OF THE ANTIBIOTICS | ZONE OF INHIBITION |
|---|---|
| Amikacin | 16mm |
| Gentamycin | 14mm |
| Kanamycin | 10mm |
| Streptomycin | 6mm |

**Table: 4. ZONE OF INHIBITION (MM) AT VARIOUS DILUTION OF CITRUS FRUITS ON *Staphylococcus aureus***

**Table: 4(a)**

| LEMON FRUIT JUICE | ZONE OF INHIBITION | CONTROL |
|---|---|---|
| **25%** | 9mm | - |
| **50%** | 11mm | - |
| **75%** | 13mm | - |
| **100%** | 18mm | - |

**Table: 4(b)**

| SWEET ORANGE JUICE | ZONE OF INHIBITION | CONTROL |
|---|---|---|
| **25%** | 4mm | - |
| **50%** | 6mm | - |
| **75%** | 10mm | - |
| **100%** | 13mm | - |

**Table: 4(c)**

| BITTER ORANGE FRUIT JUICE | ZONE OF INHIBITION | CONTROL |
|---|---|---|
| **25%** | 8mm | - |
| **50%** | 11mm | - |
| **75%** | 14mm | - |
| **100%** | 17mm | - |

## DISCUSSION

In this study among normal wound infection case the predominant organisms isolated was *Staphylococcus aureus, Escherichia coli.* Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, and Staphylococcus aureus were isolated from patients with wound infections in the work carried out by [21].Similar pathogenic organisms have been reported[2,3,11,13,18,19,20,23.]In my present investigation two organisms namely E.coli,&Staphylococcus were found. The juice of citrus fruit shows its actions as cytotoxic [24] and an antimicrobial against upper respiratory tract bacterial pathogens. Ethanolic extracts of all the fruits used exhibited a higher antibacterial effect than aqueous extracts[12,21]. The water solvent was ineffective in extracting the components of the fruit[12] which contradicts the present study where orange peel aqueous extract was ranked second highest. In research where citrus juice, peel, and leaves were used, results showed that citrus juices had the highest antibacterial activity against most of the studied bacterial isolates, moderate activity produced by citrus peel while citrus leaves showed the lowest activity produced [6.] The present study higher activity of citrus fruit juices against *Staphylococcus aureus and Escherichia coli.* Several medicinal fruits have been screened for antibacterial activity against the pathogenic organisms. In my present investigation lemon, sweet orange, bitter orange juices of citrus fruit was tested on several human pathogenic microorganisms. The antibacterial activity of citrus fruit juices was tested on pathogenic microorganisms by the disc diffusion method.

## CONCLUSION

This project intends to detect the antimicrobial activity of juice extract from citrus fruit. Due to increasing antibiotic resistance among bacteria, the results of the present study gives substantial evidence that natural citrus fruit juice extracts have the capacity of possessing effective antibacterial agent against. The antibacterial activity of citrus fruits juices show a higher inhibition compared with synthetic antibiotics. Finally, I concluded that citrus fruits generally good medicine for all wound infections.

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SP-09

# TISSUE CULTURE, PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES ON *Withania somnifera* (Ashwagandha)

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## ABSTRACT

Plants have been an important source of medicine for thousands of years. Even today, World health organization estimates that uptime 80% of people still rely mainly on traditional remedies such as herbs for their medicines. Ashwagandha is a small, woody shrub in the Solanaceae family that has chemopreventive properties make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. Tissue explants from different parts of important Indian medicinal plants. Withania somnifera conifers Linn. Were cultured invitro and their morphogenetic potential was elucidated in the present investigation. Explants from leaf, stem, and node were cultured on MS medium supplement with different concentrations and combinations of plant hormones like IAA, NAA, 2,4, D, IBA, and BAP. Qualitative phytochemical efficacy of Withania somnifera and its antibacterial study was determined.

**KEYWORDS**: Withania somnifera, phytochemical efficacy

## INTRODUCTION

Ashwagandha is also considered to be an adaptogen, facilitating the ability to withstand stressors, and has antioxidant properties as well. Other studies have shown ashwagandha to have an immunostimulatory effect.The activity of the *Withania somnifera* extracts approximately equal to the activity of the Panax ginseng extract. *Withania somnifera* , however, has an advantage over Panax ginseng in that it does not appear to result in ginseng-abuse syndrome, a condition characterized by high blood pressure, water retention, muscle tension, and insomnia.[6,7,8] Ayurveda is a type of healing craft practiced in India but not unknown in the United States. Ayurvedic practitioners rely on plant extracts, bot "pure" single-plant preparations, and mixed formulations. The preparations have lyrical names, such as Ashwagandha (*Withania somnifer* root)[10], Cauvery 100 (a mixture), and Livo-vet. These preparations are used to treat animals as well as humans. In addition to their antimicrobial activities, they have been found to have antidiarrhoeal.[17] A series of animal studies show ashwagandha to have profound effects on the hematopoietic system, acting as an immunoregulator and a chemprotectiveagent[15] In a mouse study, the administration of a powdered root extract form ashwagandha as found to enhance total white blood cell count. Also, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group.[9] Studies show Aswhagandha to be effective in the treatment of osteoarthritis[16] inflammation,[2] strokes,[6.] Studies also reveal Ashwagandha to be a potential antimicrobial agent, with antifungal activity[7] and moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*[1] Recent research suggests a possible mechanism behind the increased cytotoxic effect of macrophages exposed to *W. Somnifera* extracts[13] Nitric oxide has been determined to have a significant effect on macrophage cytotoxicity against microorganism and tumor cells. Ashwagandha exhibited stimulatory effects, both in vitro and in vivo, on the generation of cytotoxic T lymphocyte's, and demonstrated the potential to reduce tumor growth[15] In an animal study assessing the anxiolytic and antidepressive actions of ashwagandha compared to commonly prescribed pharmaceuticals, an extract of the root was administered orally to rats once daily for five days. The results were compared to a group-administered the benzodiazepine lorazepam for anxiolytic activity. Methicillin-resistant *S. aureus* as well as 12 other gram-negative and gram-positive bacteria. The antibacterial activity of *Withania somnifera* L., an Indian traditional medicinal plant against pathogenic bacteria. Both aqueous wells as alcoholic extracts of the plant (root as well as leaves) were found to possess strong antibacterial activity against a range of bacteria, as revealed by in vitro Agar Well Diffusion Method. Moreover, in contrast to the synthetic antibiotic (viz. chloramphenicol), these extracts did not induce lysis on incubation

with human erythrocytes, advocating their safety to the living cells. Finally, the antibacterial efficacy of the extracts isolated from the plant (both root and leaves)was determined against experimental salmonellosis in Balb/C mice.

## MATERIALS AND METHODS

### Collection of plant materials

Selected medicinal plant *Withania somnifera*(Linn).Wild was collected from the herbal garden of Tamil University, Thanjavur. The botanical identity of the specimens was confirmed by using Floras of the presidency of Madras and Flora of Tamil Nadu, Carnatic and standard reference**.** The voucher specimens were kept in Tamil university Herbarium.

### PREPARATION OF EXPLANTS

Leaf explants and shoot apices were used for the present study. The first fully expanded leaves in the shoot apex were collected from the garden grown plant. The explants were excised with the help of sterile forceps and blade. The nodes were cut into 0.5-1.0 cm sized segments and was taken that each explant included the mid rip portion. Apical shoot buds measuring 10-15mm in length with 2-3 leaf primordial attached were also used.

### SURFACE STERILIZATION OF THE EXPLANT

Surface sterilization was done by using mercuric chloride and alcohol. The explants were treated with 0.01 percent mercuric chloride for 1 minute and washed twice with sterile distilled water. Then the materials were rinsed in 70 percent alcohol for 2 to 3 minutes. Then the explants were thoroughly washed twice with sterile distilled water.

### PREPARATION OF MEDIUM

(MS) medium[18] was used throughout the study.

### COMPOSITION OF MS BASAL MEDIUM

| COMPONENTS | CONCENTRATION (mg/l) |
|---|---|
| **Major Elements:** | |
| $NH_4NO_3$ | 1650.0 |
| $KNO_3$ | 1900.0 |
| $CaCL_2.2H_2O$ | 440.0 |
| $Mg\ So_4.7H_2O$ | 370.0 |
| $KH_2PO_4$ | 170.0 |
| $Na_2$ EDTA | 37.0 |
| $FeSO_4.7H_2$ | 27.0 |
| **Mineral elements** | |
| $H_3BO_3$ | 6.2 |
| $MnSO_4.4H_2O$ | 22.3 |
| $ZnSO4.4H_2O$ | 8.6 |
| KI | 0.83 |
| $Na_2\ M_oO_4.2H_2O$ | 0.25 |
| $CuSO_4.5H_2O$ | 0.25 |
| $CaCl_2.6H_2O$ | 0.025 |
| **GROWTH REGULATORS** | |
| IAA | 0.5-5mg/l |
| BAP | 0.5-5mg/l |
| 2,4-D | 0.5-5mg/l |
| NAA | 0.5-5mg/l |
| IBA | 0.5-5mg/l |
| PH | 5.5 |

All the stock solution was stored in glass bottles under refrigeration.

**Extract Preparation**

The callus of the plants was dried under shade. The shade dried callus was separately subjected to pulverization to get a coarse powder. Extract with alcohol by continuous hot percolation process using the Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. Black residue was obtained. The extract was then stored in a refrigerator at $4^0$C.

**Qualitative Phytochemical analysis**

Qualitative Phytochemical analysiswas done using the procedure[14]Alkaloids, Carbohydrates, tannins, and phenols, flavonoids, gums, and mucilages fixed oils and fats, sponinsphytosterols, volatile oils, protein, and free amino acids were qualitatively analyzed.

**Alkaloids**

The extracts were dissolved in dilute hydrochloric acid and filtered. The filtrate was titrated with Mayer's shaggy's and Wagner's reagent separately. The appearance of a cream, yellow, and reddish-brown precipitates indicate the presence of alkaloids.

**Carbohydrates**

300 mg of alcoholic extracts were dissolved in water and filtered. The filtrate was boiled with Fehling's and with Benedict's solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non-reducing sugars respectively.

**Tannins and phenols**

A small quantity of 50 percent alcoholic extract was dissolved water in ferric chloride solution (5%) or gelatin solution (1%) or lead acetate solution (10%) was added. The appearance of blue colour with ferric chloride of precipitation indicated the presence of tannins and phenols.

**Flavonoids**

**a)** The extract mixed with a few ml of alcohol was heated with magnesium and then conc. HCL was added undercooling. The appearance of pink colour indicates the presence of flavonoids.

**b)** The extract treated with few ml of aqueous NaOH, appearance of yellow colour, and changes to colorless with HCL, indicate the presence of flavonoids.

**Gums and mucilages**

About 10ml of the extract was slowly added to 25 ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gums and mucilages.

**Fixed oils and fats**

A drop of the concentrated extract was pressed in between two filter papers and kept undisturbed. Oils stains on the paper indicated the presence of oils and fats.

**Saponins**

About 1ml of the extract was dissolved in 20ml of water shaken in a graduated cylinder for 15 minutes. The formation of one cm layer of foam indicates the presence of saponins.

**Phytosterols**

Alcoholic extracts were mixed with alcoholic KOH till complete saponification takes place. It was diluted with distilled water. Either extract was evaporated and the residue as subjected to LB (**LibermannBurchard's**) test. The appearance of orange to red colour indicates the presence of Phytosterols.

**Volatile oils**

50g of powdered material is taken in a volatile oil estimation apparatus and subject it to hydrodistillation, for the detection of volatile oil. Collect the distillate in the graduated tube of the assembly in which the aqueous portion is automatically separated from the volatile oil, if it is present in the drug, and returned to the distillation flask.

**Proteins and free amino acids**
Dissolve small quantities of alcoholic extract in a few ml of water and subject the solution to Biuret tests, Prink purple colour indicates the presence of proteins.

**Anti-Bacterial study**
The alcoholic extract (100 µg/ml) of *Withania somnifera*were tested for their ant-Bacterial activity. The experiments on ant-Bacterial activity were demonstrated/determined by well diffusion assay as well as disc diffusion assay.[5] The bacteria tested against the alcoholic extract of *Withania somnifera* were.

**Bacteria**

*Escherichia coli*
*Staphylococcus aureus*
*Staphylococcus epidermidis*
*Streptococcus pyogenes*

**Sensitivity test for antibiotics**
Commercially available antibiotics discs viz., Ampicillin 10 µg, Ciprofloxacin (CL) 5 µg, Cephalexin (CP) 30 µg, Norfloxacin (NF) 10 µ, Ofloxacin (OF) 5µg, were used in the study for all the test microorganisms and considered as standard controls. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by the National Committee of Clinical Laboratory Standards (**NCCLS**).

**Well, diffusion technique**
Few minutes after inoculation, 3 wells of 5 min size were made. Two wells were loaded with 0.1 ml of extract. The other well was loaded with the only solvent and treated as control.

**Disc diffusion technique**
Sterile whatmann No. 1 filter paper discs of 5 mm diameter were loaded with these extracts and were completely dried off the solvent under laminar flow. The plates were incubated for 48 hrs in $37\pm2^0$C for bacteria. These plates were observed after 48 hrs for clearing zone around the disc. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

**RESULT**

**Phytochemical studies**
Alcoholic extracts of callus were subjected to evaluate the phytochemical constituents. The results were presented in Tables 1 and 2.

**Determination of optimal concentration of Hormones**
Of the different concentrations of IAA (0.5, 1.2, and 5 mg/1) used, 5 mg/1 was found to be enormously promoting growth from the node explants. The other concentration of this hormone was also induced maximum callus (Table-1).

**Effect of a factorial combination of IAA and BAP**
Factorial combination of different concentrations and combinations of IAA and BAP was tried to ellicitmorphologenetic potential of node explants. The shoot grew only 1 cm but has not elongated. The minimum amount of calluses were induced on the media containing combinations of 2 and 5 mg/ 1 of IAA with all concentration of BAP (0.5, 1.2 and 5 mg/1).(Table-3)

**Effect of factorial combinant of NAA and BAP**
NAA 0.5 mg and 1 mg combined with BAP induced from the explants. The Shoot grew only 1cm but has not elongated.The minimum amount of callus was induced on the media containing a combination of 2&5mg /1 of IAA with all concentration of BAP90.5,1,2 & 5MG/1) (Table-2)

**Callus induction**
The minimum amount of callus were from the node on the media containing IAA, among all

concentrations, 2 and 5 mg/ 1 of this hormone vas induced callus from the cut ends of the explants which white and most firable nature.

**Shoot induction**

Multiple shoots were formed on nodal explants of *Withania somnifera*. After 3 to 4 weeks of the culture period. MS medium supplements individually with BAP induced shoot from the node explants of *Withania somnifera*. Shoot formed on the media containing 1mg/ 1 of BAP. Which length was 5cm, which media also induced any form of callus? All shoot formations were obtained from callusing (Fig).

**Root induction**

Any individual hormone should not initiate roots from the explants. Higher concentrations of NAA with a lower concentration of BAP induced roots directly from the explants, some times roots have developed the callus.

**Anti – Microbial study**

Anti-microbial efficacy of alcoholic extract of *Withania somnifera* was tested against some bacterial strains by good diffusion and disc diffusion method. The antibiotic standard was performed for all the microbial strains and the results were recorded inTable 4

**DISCUSSION**

*Withania somnifera* is widely used in the Indian and African traditional medicine. It is generally used as an abortifacient, amoebicide, bactericide, and contraceptive[3] The plant is reported to have anti-stress, anti-inflammatory, anti-arthritic and anti-tumor properties. Anxiolytic-anti-depressant activity of Withania somnifera glycowithanolids: an experimental study. [6,7,8] Protocols for in vitro regeneration in *Withania somnifera* were developed by (3)Explants were taken from the leaves, hypocotyls, roots, and cotyledonary leaf segments. Callus was induced in MS medium supplemented with various concentrations and combinations of 2,4-D and kinetic. Maximum callusing (100%) was obtained from the root and cotyledonary leaf segments grown on the medium supplemented with a combination of 2 mg 1-1 2,4-D and 0.2 mg 1-1 kinetin. Maximum shoot multiplication was observed after 60 days of the second subculture on the medium containing 2 mg 1-1 BA. These shoots were rooted best on the medium containing 2 mg 1-1 (IBA). The plantlets were transferred to the field after acclimatization and showed 60% survival. Structure and antimicrobial activity of diterpenes from the roots of *Plectranthus ushereroensis*[4].Animicrobialstudies were determined in their investigation studies.

**Table: 1. QUALITATIVE PHYTOCHEMICAL SCREENING OF CALLUS OF *Withania somnifera***

| S. No | Compounds tested | Test applied Reagent used | Alcoholic extract |
|---|---|---|---|
| 1. | Alkaloids | a) Mayer's<br>b) Wagner's<br>c) Hanger's | +++-<br>+++<br>+++ |
| 2. | Carbohydrate | a) Fehling's<br>b) Benedict's | +++<br>+++ |
| 3. | Tannins and phenol | a) 10% lead acetate<br>b) FeCl2 | ++<br>++ |
| 4. | Flavonoids | NAOH/HCL | +++ |
| 5. | Gums and mucilages | Alcoholic precipitation | ++- |
| 6. | Fixed oils and fats | Spot test | +- |
| 7. | Saponins | Foam Test | ++- |
| 8. | Phytosterols | LB Test | ++ |
| 9. | Volatile oil | Hydro distillation method | ++ |
| 10. | Protein and free amino acids | Biuret Test | ++- |

**++:Appreciable amount, ++:Moderate amount, +:Small amount**

**Table: 2. EFFECT OF NAA ON THE NODAL EXPLANTS *Withania somnifera***

| S.No | BAP concentration (mg/l) | Formation of callus (%) | Weight at harvest (mg) | |
|---|---|---|---|---|
| | | | Fresh wt. | Dry wt. |
| 1. | 0.5 | 78 | 194.4±2.5 | 117.3±0.7 |
| 2. | 1.0 | 76 | 1284.6±0.3 | 83.2±0.3 |
| 3. | 2.0 | 58 | 939.8±2.5 | 56.0±0.5 |
| 4. | 5.0 | 55 | 484.6±2.3 | 28.0±0.4 |

Incubation period : 6 weeks
Initial Fresh weight : 19.6±3.2mg
Initial Dry weight : 3.9±0.9 mg

**TABLE: 3. EFFECT OF AUXINS AND CYTOKININ ON THE NODAL EXPLANTS *Withania somnifera***

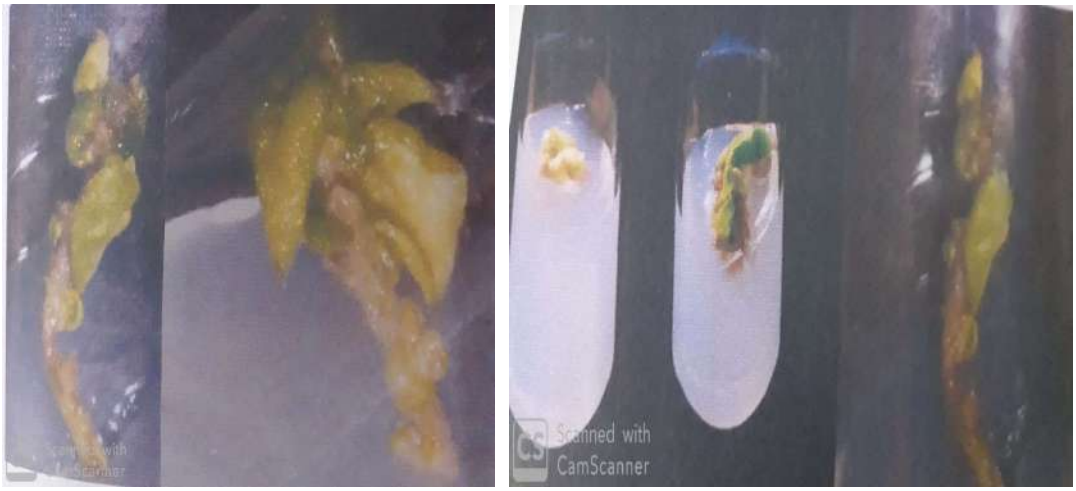
| Sl.No. | Hormones concentration NAA + BAP (mg/l) | Formation of callus (%) | Formulation of Shoot | Formulation of Root (%) | Fresh Wt. (mg) | Dry Wt. (mg) |
|---|---|---|---|---|---|---|
| 1. | 0.5+ 05 | 56 | - | - | 625±4.9 | 45.1±1.2 |
| 2. | 0.5+1.0 | 64 | - | - | 805±3.2 | 56.6±.5 |
| 3. | 0.5+ 2.0 | 45 | - | - | 414±3.3 | 32.8±1.5 |
| 4. | 0.5+0.5 | 39 | - | - | 351±4.2 | 46.2±1.3 |
| 5. | 1.0+ 0.5 | 27 | - | - | 233±2.3 | 52.7±1.9 |
| 6. | 1.0+ 1.0 | 56 | - | - | 632±3.2 | 36.2±0.2 |
| 7. | 1.0+2.0 | 88 | - | - | 1222±3.3 | 65.2±1.4 |
| 8. | 1.0+5.0 | 25 | 28 | - | 395±3.8 | 61.4±1.2 |
| 9. | 2.0+0.5 | 30 | - | - | 993±3.4 | 56.7±1.1 |
| 10. | 2.0+1.0 | 19 | 12 | - | 125±2.7 | 84.2±2.6 |
| 11. | 2.0+2.0 | 14 | 31 | - | 214±4.5 | 114.0±2.3 |
| 12. | 2.0+5.0 | 10 | 39 | - | 2512±2.8 | 133.0±2.6 |
| 13. | 5.0+5.0 | 11 | - | - | 512±0.1 | 33.2±0.4 |
| 14. | 5.0+1.0 | 49 | 29 | - | 118.9±0.2 | 55.5±o.4 |
| 15. | 5.0+2.0 | 23 | - | 65 | 88.5±0.9 | 43.2±0.5 |
| 16. | 5.0+5.0 | 42 | - | 17 | 55.0±0.8 | 23.1±0.3 |

Incubation period : 6 weeks
Initial Fresh weight : 21.9±3.4 mg
Initial Dry weight : 4.1±0.9 mg

**Table:4. ZONE OF INHIBITION (in mm)OF WITHANIA SOMNIFERA AGAINST BACTERIA**

| S.NO | NAME OF THE ORGANISMS | ZONE OF INHIBITION |
|---|---|---|
| 1 | *Escherichia coli* | 11 |
| 2 | *Staphylococcus aureus* | 21.2 |
| 3 | *Staphylococcus epidermidis* | 22.4 |
| 4 | *Streptococcus pyogenes* | 21.2 |

**FIGURE: CALLUS INDUCTION OF *Withania somnifera***




## SUMMARY

Tissue explants from different parts of an important Indian medicinal plant. *Withania somnifera Linn.* It was observed that nodal explants have maximum morphogenetic potential than there explants of *Withania somnifera Linn*.The optimum concentration of individual hormones for the grown of the nodal explants was determined. It was found to 5 mg/I for 2, 4 D, and 2mg/1 for IAA and 1mg/1 for BAP. But there are no responses in that all concentrations of IBA. Factorial combination of different concentrations of the combination of IAA and BAP was tried to select morphogenetic potential of node explants. Compact a delight brown colour callus was observed on the all combination of IAA and BAP. 0.5mg/1 of BAP combinations gave high callulogentic responses. Which was 1:10? 0.5 mg/1 of IAA with all concentrations of BAP (0.5, 1.2, 5mg/1) showed the only callus. *Withania somnifera* mediated transferred callus containing compounds were higher than hormone-induced callus. Quantitatively estimated the highest amounts of total phenol and alkaloid were 312.6 µg / g and 286.5 µg / g respectively. Finally, the anti-Bacterial effect of *Withania somnifera* was studied.

## CONCLUSION

*Withania somnifera* is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The different extracts of the root of WS contained many bioactive chemical constituents including, alkaloids, glycosides, steroids, terpenoids, saponins, tannins, and reducing sugars. The anti-inflammatory, antispasmodic, anti analgesic and diuretic effects can be attributed to the high steroids, tannins, terpenoids, saponins and glycosides present in *Withania somnifera* Although the results from this review are quite promising for the use of this plant as a multi-purpose medicinal agent. The present study can be used in the future for the economical formulation of the active chemical ingredients in natural drugs against a variety of neurological and inflammatory diseases. Novel methods are to be developed to increase the phytochemical content of the plant.

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SP-10

# STUDIES ON BIOREMEDIATION POTENTIAL OF OIL DEGRADING BACTERIA ISOLATED FROM CONTAMINATED SOIL

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## ABSTRACT

*Bioremediation* is a process used to treat contaminated media, including water, soil, and subsurface material, by altering environmental conditions to stimulate the growth of microorganisms and degrade the target pollutants. Bioremediation provides an effective and efficient strategy to speed up the clean-up processes. One major environmental concern of soil contaminated with crude oil or petroleum product is an increase in organic carbon of the soil with an associated decrease in soil nitrate and phosphorous, thus imposing a condition that impaired oil degradation in the oil. The work carried out biosurfactant production is detected by various techniques using drop collapsing technique, oil displacement test, and emulsification index tests. To demonstrate the oil-degrading activity of the five isolates (*S.aureus, E.coli, Pseudomonas aerogenosa, Bacillus cereus, and Micrococcus*) against the petroleum hydrocarbon using shake flask method, Agar well diffusion method, spread plate method. To determine the effect of petroleum on the crop plant were analyzed in seed Germination experiment. Seed Germination tests were carried out in the comparative study of treated and untreated soil samples, *Vigna mango* (L).was chosen for the seed germination test. This study can focus on more cost-effective applications of native bacterial strains for petrol and oil degradation at large scale in industries, where it poses an alarming problem due to its detrimental health effects on different organisms and human beings. The conclusion of the present investigation demonstrates the feasibility of adopting a sustainable andeco-friendly approach to minimize hydrocarbon pollutants.

**KEYWORDS:** Biosurfactants, Bioremediation, Emulsification, Degradation.

## INTRODUCTION

### Bioremediation

Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation deals with the methods of solving environmental problems. It also plays a vital role in cleaning the environment from pollutants and contaminants by using the microorganisms, Bacteria are the most important microbes used in this process because they break the dead materials into organic matter and nutrients[13]**.** Hydrocarbon is highly toxic and posed great danger especially to plant nearby communities. Therefore, there is a need for effective treatment before discharge. The natural process of employing microorganisms is considered to be a very effective and environmentally friendly method of decontamination[14.] Now a day's pollution is considered as one of the major problems of the world which could be either organic or inorganic. Oil spills have been a major issue across decades. One of the famous oil spills which are also ongoing is in Taylor Energy Well in the Gulf of Mexico, U.S.A caused due to Hurricane. (Sept 16, 2004, till present date) and almost 0.03 – 0.05 tons of oil per day is estimated to leak. Another recent oil spill is in Mumbai (India) and caused due to the leakage in Mumbai- Uran pipeline dated January 21 2011 and about 55 tons of oil was leaked in the Arabian Sea. Various such accidents occur throughout the years and it causes damage to over surrounding ecosystem.[9]

### Biodegradation

Biodegradation is the chemical dissolution of materials by bacteria or other biological means. Although often conflated, biodegradable is distinct in meaning from compostable. Biodegradation is a major mechanism that removes pollutants from the environment because biodegradation by microorganisms is

more favorable than chemical[21].

**Hydrocarbons degrading bacteria**

Microorganisms degrade these compounds by using enzymes in their metabolism and can be useful in cleaning up contaminated sites. Microbial remediation of a hydrocarbon-contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in the soil. These microorganisms can degrade a wide range of target constituents present in oily sludge [4,8.19]

**Biosurfactant**

Biosurfactants are amphiphilic compounds produced in living surfaces, mostly on microbial cell surfaces or excreted extracellular hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively[11]. When microorganisms grow in an environment rich in hydrocarbon, they undergo many adaptations. One such adaptation is biosurfactant production which is a frequent chance upon a feature in hydrocarbon-degrading bacteria or sometimes even a condition for growth on hydrocarbons.

**Effect of hydrocarbon**

Which contaminated environment was hazardous to humans and animals and decreases the agricultural productivity of the soil[6]. Prolonged exposure of BTX may cause the lung, heart, liver and kidney disease, bone marrow damage, and benzene to cause cancer. Spent engine oil causes great damage to soil and soil microflora. It creates an unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients, and lowering of soil pH [26]. Benzene, Toluene, and Xylene (BTX) are major aromatic hydrocarbon in many petroleum products[3].

## MATERIALS AND METHODS

**Study site**

Study sites were mechanic workshops in Kumbakonam, Tamilnadu.

**Sample collection**

Soil samples were collected from a specific location within the workshops that had heavy spoilage of petroleum oil. grasses were growing at the location and soil samples were blackish. The samples were aseptically collected using the cleaning spatula of soil 2-5cm from the ground level.

**Identification of hydrocarbon utilizing bacteria**

The identification was done by cultural (margin, color, texture, and elevation), morphological and biochemical analysis as per Bergey's Manual of Systemic Bacteriology[10].

**Identification of the bacterial isolates**

Morphological and biochemical characteristics of the all isolated strain were studied either on nutrient agar or in nutrient broth as described earlier[7].

**Biochemical tests**

Indole test, Methyl red and vogesproskeur test, Triple sugar iron agar test, Carbohydrate fermentation test, Citrate utilization test, Catalase test Oxidase test, Sub-culturing were used.

**Bioremediation studies**

**Biosurfactants production is detected by various techniques[20]**

a) Drop collapsing technique
b) Oil displacement test
c) Emulsification index

**a) Drop collapsing technique**

The isolates were grown in the NA medium with petroleum as carbon source, incubated with shaking for 48 hours at 37 C and 200 rpm. The glass slides used were rinsed with hot water, ethanol, and distilled water, and dried. The slide was coated with diesel and equilibrated for 24 hours to ensure a uniform oil

coating . 1 ml of supernatant sample was then applied to the center of the oil drops using 10 ml micropipette. The results were monitored visually after 1 hour. If the drop remained incrusted, the result was scored as negative. If the drop collapsed, the result was scored as positive.

**b) Oil spreading assay**

Oil spreading experiment was performed using the method described by Morikawa et al, 2000 [18]. If biosurfactant is present in the cell-free culture broth, the oil will be displaced with an oil free clearing zone, and the diameter of this clearing zone indicates the surfactant activity, also called oil displacement activity.

**Emulsification ability assay**

Emulsification assay is an indirect method used to screen biosurfactant production. It was presumed that if the cell-free culture broth contains biosurfactant then it would emulsify the hydrocarbons present. Here, crude oil was used as the hydrophobic substrate.[23]

$$E = \frac{\text{Height of the emulsion} \times 100}{\text{Total height of the liquid}}$$

**Oil degrading activity**

**a) Shake flask method**

Prepare nutrient broth **to** add half amount of oil (petroleum) After autoclaving cool at room temperature then inoculate bacterial culture and incubate at 37 º C in a shaker for 7 – 10 days. Day by day measurement was done and also the growth of culture was observed with the help of colorimeter taking OD at 620 nm[25]

**b) Agar well diffusion method[5]**

Prepare nutrient agar plates and spread 100µl of oil (petroleum) prepare wells and load 50 µl of bacterial culture then incubate at 37º c for overnight and observe the result, if cultures showing growth in the presence of oil it means culture have properties to degrade the oil.

**c) Spread plate method**

Prepare Nutrient agar plates and spread 100µl. of oil (petroleum) and after it spread 50 µl. of bacterial culture over it and then incubate at 37c for overnight and observe the result, if cultures showing growth in the presence of oil it means culture has properties to degrade oil[15].

**Seed Germination tests**

Seed Germination tests in treated and untreated soil samples in the present study, Vigna mungo (L) was chosen for the seed germination test. Seeds were surface sterilized by cleaning thoroughly under running tap water for 10min, washed with a solution of Tween 20 (two drops in 100 ml of water) for 1 min, and again washed with sterile distilled water. The cleaned seeds were finally treated with (0.1%) mercuric chloride (HgCl2) for four min under aseptic conditions and washed under aseptic conditions and washed five times with sterile distilled water to remove traces of HgCl2. After surface-sterilized seeds, were then sowed in Petri plates which contained treated and untreated soil samples. After a few days of watering, the germination of seeds was noted[27].

**The germination percentage can be calculated using the following formula**

$$\text{Germination percentage [\%]} = \frac{\text{Seed Germination}}{\text{Total number of seeds}} \times 100$$

## RESULTS

Soil contamination with hydrocarbons causes extensive damage ecosystems since the accumulation of pollutants in animals and plants tissues, may of local cause progeny's death or mutation[2]. Microorganisms survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche. Contaminants are often potential energy sources for microorganisms [16]

**Isolation of bacteria**

Bacterial strains were isolated from the petroleum-contaminated soil sample

**Identification of bacterial strains**

The selected five bacterial strains were named as S. *aureus,* E. *coil,*P*seudomonas aeruginosa, Bacillus cereus,* and *Micrococcus.* Were compared with Bergey's manual of systemic bacteriology classification[10].

**Grams reaction**

The microscopic visualization of colonies of strain tell that were gram-positive, coccus shape in *Staphylococcus aureus* and E. *coli* gram-negative rod shape, P. *aeruginosa* gram-negative cylindrical rod shape, and *Bacillus cereus* gram-positive, rod shape and then *Micrococcus* gram-positive cocci shape respectively.

**Test for biosurfactant producing microorganisms**

**a) Drop collapse test**

The drop of petroleum was collapsed immediately with 1 min of addition of culture both. The remaining bacterial culture broths could not collapse the drop of petroleum even after one min. The maximum level of biosurfactants was noted as *Pseudomonas aeruginous* then *Bacillus cereus, Staphylococcus cereus,* E. coil, and *Micrococcus.*

**b) Oil spreading assay**

If biosurfactant is present in the cell-free culture broth, the oil will be displaced with an oil free clearing zone, and the diameter of this clearing zone indicates the surfactant activity, also called oil displacement activity. The maximum level of biosurfactant was noted in *Pseudomonas aeruginous* (3mm) then *Bacillus cereus* (0.5 mm),E. coil(2mm),*Staphylococcus cereus*(1.6 mm),and *Micrococcus(*1.0 mm*).*

**c) Emulsification index**

The emulsification index is given as a percentage ofthe height of the emulsified layer (mm) divided by the total height of the liquid column (mm).The results for the emulsification index of biosurfactant producing microorganisms for oil. Among the *Pseudomonas aeruginous* (33%) showed the highest biosurfactant activity compared to S.*aureus*(26%), E. coli (20%), B. cereus (16%) and Micrococcus (10%).

**Oil degrading activity**

**a)Shaker flask method**

Highest growth observed in 4$^{th}$ day of incubation *Pseudomonas aeruginosa* (0.22nm). The low growth observed *Bacillus cereus* (0.18nm), E. *coli* (0.19nm), Micrococcus (0.20nm), and S. aureus (0.21nm).

**b) Agar Well Diffusion method**

The petroleum degradation ability of bacterial strains was analyzed by the plate assay method. Among this study highest zone of inhibition was noted in *Pseudomonas aeruginosa* (3.8mm in diameter) compared than E. *coli* (1.4mm in diameter), B. *cereus* (2.5 mm in diameter), *Staphylococcus aureus* (2mm in diameter), *Micrococcus* (3mm in diameter),

**C) Spread plate method**

Among the 5 bacterial isolates *Staphylococcus aureus,* E. *coli, Pseudomonas aeruginosa, Bacillus cereus,* and *Micrococcus*, were culture show growth in the presence of oil it means culture have properties to degrade the oil.

**Seed Germination Test Treated and Untreated Soil**

In the study, maximum germinating and growth ability recorded in the petroleum done experiment shows the germination percentage obtained as a result of the germination test. The germination percentage of treated soil sample (40%). This reveals that petroleum has inhibition of *Vigna mungo* seed germination potential in it.

**DISCUSSION**

Bacteria are the most important microbes in this process because they break the dead materials into organic matter and nutrient[12]. A study carried out of the samples of hydrocarbons contaminated soil supplemented

with various hydrocarbons showed the growth of *Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus,* E. *coli,*and *staphylococcus aureus*. A total of twenty positive isolates of bacterial species were identified of *Pseudomonas aeruginosa 9* followed by, *Bacillus subtilils 6,Bacillus cereus 3,* E. *coli 1*and *Staphylococcus aureus.*[24] In the study focused of biosurfactant by bacteria isolated from oil selectively*Pseudomonas aeruginosa,* which is assumed to be potent biosurfactant producer.[25] The screening of biosurfactant producing *Pseudomonas aeruginosa* by was investigated by drop collapse test, emulsification index, oil displacement test.[22] In seed germination study Vigna mungo (L) was chosen for the seed germination test. The oil-contaminated soil sample and clean surface-sterilized seeds were inoculated in two different Petri plates. The seed germination percentage (85%) was greater than that of the untreated soil. The results obtained concluded that bioremediation mediated by isolated bacterial culture has been very effective. Soil and water contamination with hydrocarbons caused extensive damage to the local system, this contamination is a crisis to plants and animals. An efficient way of remediation the oil-contaminated sites could be the employment of special microorganisms, such as bacteria, microalgae, and fungi[1] Bacteria are the most important microbes in this process because they break the dead materials into organic matter and nutrients.[17]

## CONCLUSION

The present study focused on studying the production of biosurfactant by bacteria isolated from oil contaminated soil selectively *Pseudomonas aeruginosa*(26%), E. *coli*(20%),*Bacillus cereus* (16%) and *Micrococcus* (10%) sp, which is assumed to be potent biosurfactant producer. In the conclusion of the present investigation demonstrates the feasibility of adopting a sustainable and eco-friendly approach to minimize hydrocarbon pollutants.

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SP-11

# ISOLATION AND COMPARISON OF PARASITES AND ANTIBIOTIC SUSCEPTITY PATTERNS OF COLIFORMS IN STOOL SAMPLES OF HOSPITALIZED PATIENTS.

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## ABSTRACT

The prevalence of infection with helminths parasites is approximately two billion people worldwide resulting in an estimated two million clinical episodes and sixty thousands death annually India the problem is likely to be more common and severe because of poor sanitation, limited areness, and illiteracy, misbelieve, poverty and a variety and a variety of allied factors. These were collected from patients attending the outpatient clinics and the wards in Government Hospital at Kumbakonam. The prevalence of intestinal parasites in the study group was 23.3%. The intestinal parasites detected were cycts of *Entameoba histolytica* (13.3%), ova of flora worm (6.6%) and *Trichuris trichiura* respectively. In the study of antibiotic susceptibility patterns among coliform 53.3% of the isolates were susceptible to Ampicillin, 80% were susceptible to cirprofloxacin and 100% of the isolates were susceptible to Gentamicin and 93.4% susceptible to cefazolin. In conclusion antibiotic should be used only in severely cases of diarrhoeal diseases as an over of antibiotic rapidly disseminates resistant genes in the community making the overall treatment of infection severely complicated. The judicious use of antibiotic will go a long way in limiting the current problem of antibiotic resistance in the bacterial population.

**KEYWORDS**: *Entamoeba histolytica, Trichuris trichiura,* diarrhoeal diseases

## INTRODUCTION

Parasitic infections have a worldwide distribution and constitute considerable public health problems especially in developing countries and may be considered as the problem of developing countries and may be considered as the problem of developing nations. Most children living in developing countries regardless of social status have been infected with worms at some time during their lives. Intestinal helminths affect over 1,800 million clinical episodes 60,000 death annually. Children and women of reproductive age bear the greatest disease burden. Worm infestation is a major problem in children from developing countries due to bad hygienic conditions. It is not unusual to find up to 90% of school age children in poorer communities harboring one or more intestinal helminths and these children are 5-7 times more likely to be under weight. Infection with *Entamoeba histolytica* is worldwide but is more common in tropics and subtropics. It is a leading cause of morbidity and mortality in developing countries. It is the causative agent of an estimated 40 to 50 million cases of amoebic colitis and liver abscess and is responsible for up to 100,000 deaths worldwide. Each year it has been generally observed that a majority of individuals infected with the *Entamoeba histolytica* do not develop symptomatic disease[1]. In the acute phase of the infection they exist as trophozoites in the intestine. The trophozoties range in size from 18 to 14mm. The tropzoites exhibit active unidirectional movement achieved with the help of borad hyaline, finger like pseudopodia. The cysts 0f Entamoeba histrolytica range in size from 8to 22mm and are spherical. Depending upon the maturity of the cyst, they may contain one, two or four nuclei. The immature cyst have a single nucleus, diffuse glycogen mass and sausage shaped chromatid bodies.Eggs are oval and measure 60-40mm. They are colourless [non bil-stained] with a thin tranceparent hyaline shell membaren. The ovum present inside is usually segment with four blastomeres. Ancylostoma duodenale

causes ancylostomiasis. Most hookworm infections are asymptomatic. Symptomatic hookworm infections are due to either larvae or adult hookworms. They produce epigastric pain, diarrhea and vomiting during the early phase of infection. These symptoms are also common in heavy infections. The most important manifestation of hookworm infection is microcytic hypochromic anaemia. The diagnosis is established by finding the characteristics egg in the faeces[2]. The eggs are barrel shaped and measure 50mm-250mm. Projecting mucous plugs are present at both poles. The eggs are brown [bile –stained] Diagnosis is by demonstration of the characteristics, barrel or football shaped eggs in the faeces[2.] It affects children more frequently than adults although all ages. May exhibit symptoms ranging from mild diarrheoa, flatulence, anorexia, crampy abdominal pains and epigastic tenderness to diarrhea and full blown malabsorption syndrome. In acute giardiasis, trophozoite show the typical "falling –leaf" motility in wet mount examination of faeces[3]. Hymenoleplis nana the dwarf tapworm of human exists in two forms an adult and larval form and both the forms live in human intestine. The adult worm lives in the small intestine. It is 2 to 3 cm long and has about 200 to 300 proglottids. Infected people may develop anorexia, headache, abdominal pain, diarrhea, restless. Eggs are 30 to 45 mm in diameter and are not bile –stained. The egg has two distinct coverings. The outer covering is thin and colorless while the inner covering is the embryophore. The space between the two coverings is filled with yolk granules and 4 to 8polar filment arising from little knobs at two poles of the embryophore. The oncospher has two hook lets[2]. The pinworm *Enterobius vermicularis* is by all odds the most common helminthic parasite of temperate regions. Diagnosis is established by demonstration of eggs. The eggs are colourless and have a clear shell. They are Plano convex and measure 55mm-30mm. Eggs contain a fully formed larva. Adults live in the appendix and adjacent parts of ascending colon. Diagnosis of pinworm infection is made by the recovery of the characteristic eggs[4].

## MATERIALS AND METHODS

### Collection of samples

Thirty stool samples were collected from patients attending the outpatient clinic and those admitted to the wards of Government Hospital, Kumabakonam at Thanjavur-Dt. Clean containers were given the previous day to the patients and they were instructed to collect the stool samples preferably in the morning. The sample were transported to the department of clinical microbiology within 2 hours and was subjected to macroscopic and microscopic examination. In macroscopic examination the consistency, color and presence of segments of worm were noted.

### Microscopic examination

A clean glass slide was taken and drop of saline was added on one side of the slide and iodine on the other side. With the help of an applicator stick a small portion of the stool sample was taken and emulsified first in saline and then in iodine. After emulsification a cover slip was put and slide was observed under the Microscope first in 10x objective and later in 40x objective. The presence of ova, cysts and larvae etc. were noted. This examination was done both under alkaline and iodine mount.

### Concentration technique

If the number of cysts, eggs or larvae are low in faeces, direct examination may not reveal them, hence the faeces was concentrated and the microsopy was repeated. The concentration technique done for this study was formal ether sedimentation method.

### BIOCHIMICAL TEST

Indole Test, Triple sugar Iron agar (TIS) test, Citrate utilization test, Urease test, Mannitol agar test was performed.

## DIFFUSION METHOD

The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. It is based on the use of an antimicrobial impregnated filter paper disk. The impregnated disk is placed on an agar surface, resulting in diffusion of the antimicrobial into the surrounding medium. Effectiveness of the antimicrobial can be shown by measuring the zone of inhibition for a pure culture of an organism[5]. Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganism.

## RESULT

This study was conducted in the Laboratory in Idhaya College for women, Kumbakonam at Thanjavur (Dt). On 30 stool samples collected from paitents coming to the out paitent clinics and those admitted to the wards in Government Hospital, Kumbakonam, Thanjavur. The samples were examined macrospically for the presence of mucus and blood and microscopically for the presence of cysts of Protozoan parasites and the ova of helminthic parasites. The samples were examined both by the direct method and by the formal ether sedimentation method. This study shows a prevalence of 23.3% intestinal parasites in the study population.

**Table: 1 Shows the percentage of parasite presence**

| Age | Parasite |
|---|---|
| <23 year | Nil |
| 23-43 | 17% |
| >50 | 17% |

1. In the present study the parasite population Entamoeba histolytica occur 13.3% , Hook worms occurs 6.6% Trichuris trichura occurs 3.3% in the Microscopic observation of stool sample.
2. In our present investigation the prevalence of intestinal parasites in different age groups most other studies their study did not found any practice in children. Highest prevalence was seen in >50 age group. None of the coccidian parasites were observed in any of the samples.
3. The general antibiotic susceptibility patterns of normal coliforms to a few commonly used antibiotics were also studied in these patients.
4. This study showed that among the 30 isolates none were multidrug resistant. The result of Kirby Bauer's disc diffusion method shows that 100% were susceptibile to Gentamicin and 93.4% to cefazolin, and 80% were susceptible to ciprofloxacin,53.3% of all isolates were susceptible to Ampicillin.
5. This showed that among the 19 isolates of Escherichia 47.6% were resistant to Ampicillin, 20% showed resistance to Ciprofloxacin, 6.6% showed resistance to cefazolin, and none of the isolates were resistant to Gentamicin.
6. Among 7 strasins of Klebsiella all (100%) were resistant to Ampicillin, and sensitive to all other antibioties.
7. The single proteus mirabilis that was included in the study was susceptible to all the four antibioties tested (i.e) Ampicillin, Gentamicin, Cefazolin and Ciprofloxacin.
8. Among the 3 Enterbacter sp isolated 100% susceptibility was seen to Ampicillin, Gentamicin and Ciprofloxacin 3.3% Resistance was see to cefazolin.
9. Out of the 30 isolates included in the study 3 showed the production extended spectrum lactamase.

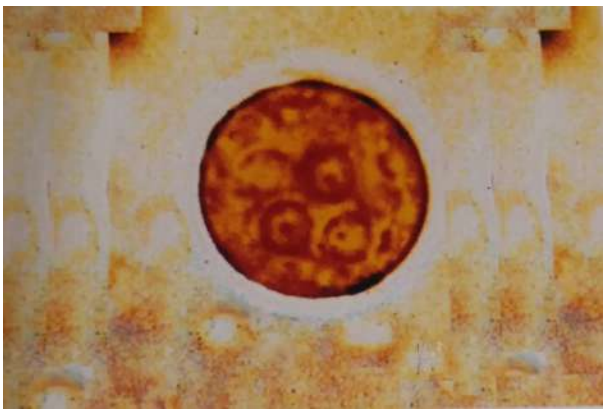


**Fig No.1 Cysts of *Entameoba histolytica***

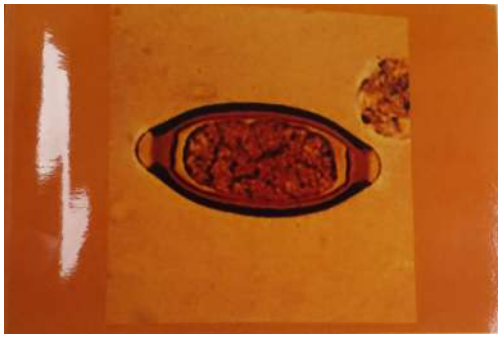
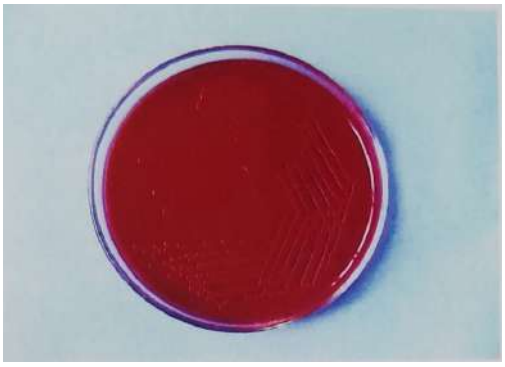


**Fig No.2 Ova of *Trichuris trichiura***



**a) *Escherichia coli***

**b) *Klebsilla sp***

**Fig No. 3. GROWTH OF THE ORGANISMS ON DIFFERENT MEDIA**

**Antibiotic Sensitivity of** *Escherichia coli*

**Fig No. 4. ANTIBIOTIC SUSCEPTIBILITY OF *Escherichia coli* BY KIRBY BAUER'S DISC DIFFUSION METHOD**

**Fig No.5 EXTENDED SPECTUM BETA LACTAMASE PRODUCTION**

## DISCUSSION

Parasitic infections have a worldwide distribution and constitute considerable public health problem especially in developing countries. Atleast one quarter of the World'S population is estimated to be chronically infected with intestinal parasites. It is not unusal to find upto 90% of school age children harboring one or more intestinal helminths. In previous studies done in Kathmandu it was found that in subjectively healthy children and adults the parasitic load was 28% and 38.8% respectively. Whereas children and adults with abdominal discomfort had a load of 62.7% and 62.8% respectively. [6] This study was conducted in the Laboratory of Idhaya College for Women, Kumbakonam at Thanjavur Dt. From Dec-2008 to June -2009 stool samples were included in the study. These were collected from patients attending the outpatient clinics and the wards in Government Hospital at Kumbakonam. The prevalence of intestinal parasites in the study group was 23.3%. The intestinal parasites dectected were cysts of *Entameoba histolyica* (13.3%), ova of flora worm (6.6%) and Trichuris trichirura respectively. In the study of antibiotic susceptibility patterns among *coliform* 53.3% of the isolates were susceptible to Ampicillin, 80% were susceptible to ciprofloxacin and 100% of the isolates were susceptible to Gentamicin and 93.4% susceptible to cefazolin.

## CONCLUSION

In conclusion antibiotic should be used only in severe cases of diarrhoeal diseases as an over of antibiotic rapidly disseminates resistant genes in the community making the overall treatment of infection severely complicated. The judicious use of antibiotic will go a long way in limiting the current problem of antibiotic resistance in the bacterial population.

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SP-12

# PROTECTIVE EFFECTS OF GANODERMA LUCIDUM (CURT.: FR.) P. KARST. ON MAMMARY CELLS OF DMBA INDUCED SPRAGUE DAWLEY RATS

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## ABSTRACT

To study the protective effects of carboxy methyl cellulose extracts of *Ganoderma lucidum* on mammary cells of DMBA induced Sprague dawley rats. Group I noted as control and totally 36 animals were used with six groups. Group II as DMBA induced alone, and Group III was Tamoxifen as positive control respectively while groups VI and V were the treatment groups which were pretreated with 250-500mg/kg bodyweight per day of *G.lucidum* extract one month before each dose of the DMBA. Group VI as *G.lucidum* extract alone for checking hypersensitivity. Finally animal serum, liver, kidney was separated and used for calculating the parameters, chemical enzymes. Mammary carcinoma was confirmed by palpation and histopathological examination was studied. Results: Oral administration of CMCGL (250, 500mg/kg body weight) showed beneficial effects on mammary carcinoma. G.*lucidum* possesses anticancer activity and probably by its antioxidant properties on mammary cells. Significant value was calculated with ($p<0.05$). These indicate that *G.lucidum* showed significant tumour reducing activity against DMBA induced mammary carcinoma.

**KEYWORDS:** *Ganoderma lucidum*, Mammary carcinoma, Tamoxifen, CMCGLPP

## 1. INTRODUCTION

Breast cancer is one of the most frequent malignancies among women and the incidence is increasing at an alarming rate. It is the major cause of cancer deaths in women worldwide, both in developed and developing countries. [1,2]Hope for treating cancer lies in four modules, surgery, radiation, chemotheraphy and a combination of all the three[3]. The role of polycyclic aromatic hydrocarbons (PAH) is clearly implicated in the process of carcinogenesis especially 7-12-dimethylbenz (*a*) anthracene (DMBA), which is one of the most potent breast carcinogens known. Mushrooms have been valued throughout the world as both food and medicine for thousands of years. They represent a major and as yet largely untapped source of potent pharmaceutical products. In Chinese folkfore, fruiting bodies of *Ganoderma lucidum* (Fr.) P.Karst., a highly ranked oriental traditional medicine have been regarded as a panacea for all types of diseases[4]. *G.lucidum* occurring in South India also possessed significant antioxidant, anti-inflammatory and antinociceptive properties[5]. In this study, we examined the protective effects of Carboxy methyl cellulose *G.lucidum* extract against mammary carcinoma. The results of the investigations were reported in this communication.

## 2. MATERIALS AND METHODS

### 2.1. Fungal material

The fresh and matured fruit body of *G.lucidum* was collected from the substrate of *Cocos nucifera* of Aadudhurai, Mayiladudhurai district. The fungal organism was identified and confirmed by standard manuals. [6-11]

### 2.2. Preparation of CMC *G.lucidum* extract

The fresh fruit body was air-dried for 2 weeks at room temperature. Then it was chopped into small pieces and ground into coarse powder with a mechanical grinder and stored in airtight container. Dried 200g of powder was dissolved in 750ml of 1% Carboxy methyl cellulose solvent in screw cap bottles for 72 hrs. After the dissolved extracts from the bottles were transferred to centrifugal tubes and centrifuged at 3000 rpm for 10 min. The centrifuged extracts (Supernatant) were again re-centrifuged and filtered with Millipore filter[12]. Brownish colour extract (18g. yield 7.5%w/w) was preserved at 4°C until further use.

### 2.3. Chemicals

Chemicals & DMBA was purchased from Sigma chemicals, Mumbai. Tamoxifen citrate used as vehicle. Estimation was calculated for biochemical enzymes such as ALP, SGOT, SGPT, creatinine and protein.

### 2.4. Experimental animal

Female Sprague-dawley rats were purchased from Shri Venkatesh Animal House, Banglore, India having age of 21 days and 70 gm body weight was used throughout the study. All animal procedures performed after getting the permission from IAEC (Institution of animal ethical committee CPCSEA/685.

### 2.5. Drug administration

Thirty days before the induction of tumour, CMCGL extract was administered by single gastric intubation at the following doses of 250, 500mg/kg. At the end of the 30$^{th}$ day Tumour was induced by administrating DMBA (25 mg/ kg) was induced by single gastric intubation in 1ml olive oil and then the treatment was continued up to a period of 120 days.

### 2.6. Animal allotment

In each model of mammary cancer, thirty six female Sprague dawley rats were evenly divided into six groups.

- Group I : Normal saline
- Group II : Inducer (DMBA (25 mg/ kg)
- Group III : Inducer (DMBA (25 mg/ kg) + Cancer control (Tamoxifen Citrate)
- Group IV : Inducer (DMBA (25 mg/ kg) + CMCGL Extract 250 mg/kg
- Group V : Inducer (DMBA (25 mg/ kg) + CMCGL Extract 500 mg/kg
- Group VI : Extract alone 500 mg/kg

### 2.7. Experimental procedure

Finally, biochemical parameters were studied. After treatment the time of latency period (the number of days between the NMU injection and the appearance of the first Tumour in each rat) was noted. Mammary carcinoma was confirmed by palpation and histopathological examination was studied with haematoxylin and eosin method of staining.

### Determination of biochemical parameters

For assessment of liver function, blood samples were collected from the animals by puncturing the retro-orbital plexus and centrifuged. The serum collected after centrifugation was analyzed for various biochemical parameters like SGOT, SGPT, ALP, Urea, Uric acid, TB and total protein (TP)[13]. Serum transaminase, ALP and serum bilirubin was measured by chemical methods[14,15].

### 2.8. Statistical analysis

| S.No. | Body weight(g) | Groups | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | Only DMBA (25 mg/ kg bw) | DMBA + Tamoxifen Citrate (10mg/kg bw) | DMBA + CMCGL (250mg/kg bw) | DMBA + CMCGL (500mg/kg bw) | ONLY CMCGL (500mg/kg bw) |
| 1. | 1$^{st}$ week | 137.25±54.926 | 181.00±17.790 | 109.00±40.951 | 128.75±17.500 | 176.00±40.258 | 170.20±44.229 |
| 2. | 2$^{nd}$ week | 164.25±44.079 | 205.00±22.836 | 146.67±31.470 | 157.25±12.842 | 199.00±39.758 | 197.80±40.923 |
| 3. | 3$^{rd}$ week | 157.75±38.699 | 204.20±29.567 | 141.67±41.041 | 173.75±8.884 | 192.50±35.464 | 202.60±36.143 |
| 4. | 4$^{th}$ week | 167.50±21.063 | 217.60±23.287 | 167.67±24.420 | 177.25±3.403 | 199.50±42.775 | 210.20±40.245 |
| 5. | 5$^{th}$ week | 183.75±27.391 | 218.20±30.768 | 181.00±17.436 | 183.00±3.367 | 212.25±46.821 | 213.00±43.324 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6. | 6th week | 186.50±30.480 | 224.40±31.746 | 183.00±17.692 | 185.00±4.690 | 214.00±34.186 | 214.60±32.647 |
| 7. | 7th week | 182.00±24.993 | 227.60±26.633 | 181.00±14.107 | 184.75±5.909 | 217.75±44.545 | 220.00±42.042 |
| 8. | 8th week | 185.00±14.491 | 234.00±23.527 | 189.33±23.180 | 191.75±2.872 | 220.25±47.836 | 227.40±40.741 |
| 9. | 9th week | 196.00±24.111 | 240.00±25.407 | 196.33±18.037 | 202.25±6.238 | 229.50±44.321 | 238.20±42.482 |
| 10 | 10th week | 198.00±20.211 | 241.00±25.993 | 198.24±20.076 | 204.56±7.336 | 230.45±45.761 | 239.34±45.543 |
| 11 | 11th week | 200.00±24.651 | 243.98±20.543 | 200.32±23.120 | 208.43±8.221 | 231.90±37.452 | 240.12±40.543 |
| 12 | 12th week | 205.00±26.770 | 245.12±20.901 | 205.12±25.124 | 210.55±8.102 | 234.81±40.231 | 241.22±38.704 |
| 13 | 13th week | 207.25±29.949 | 246.80±22.830 | 210.33±27.755 | 211.00±10.677 | 235.75±38.793 | 242.40±32.868 |
| 14 | 14th week | 209.75±28.194 | 243.60±21.836 | 207.00±29.614 | 211.50±6.807 | 237.25±45.945 | 237.80±38.983 |
| 15 | 15th week | 218.25±33.886 | 251.60±20.959 | 220.67±27.934 | 218.00±10.456 | 242.25±50.042 | 244.20±39.840 |
| 16 | 16th week | 221.25±33.260 | 254.60±19.578 | 222.33±25.580 | 220.00±9.129 | 244.50±49.749 | 244.80±39.802 |
| 17 | 17th week | 225.34±34.379 | 255.35±22.145 | 225.12±27.453 | 221.00±6.980 | 245.68±28.987 | 245.12±45.370 |

Statistics was studied with S.E.M and ANOVA followed by Student t test. Significant value was $P<0.01$

## 3. RESULTS AND DISCUSSION

### 3.1. Body weight

Body weight increased significantly in all animals. All animals ingested normal amounts of food and water during the study period (Table 1.)

**Table.1 Effect of CMCGL extract on the changes of body weight on mammary carcinoma of SD rats observed for 120 days**

| S.No. | Parameters | Control | Only DMBA (25 mg/ kg bw) | DMBA + Tamoxifen Citrate (10mg/kg bw) | DMBA + CMCGL (250mg/kg bw) | DMBA + CMCGL (500mg/kg bw) | ONLY CMCGL (500mg/kg bw) |
|---|---|---|---|---|---|---|---|
| 1. | Tumour burden | .±. | 7.67±1.528 | 5.00±2.000 | 4.33±2.517 | 5.33±1.528 | .±. |
| 2. | Tumour incidence | .±. | 6.67±0.577 | 3.33±2.309 | 3.00±1.528 | 3.33±1.528 | .±. |
| 3. | Tumour volume | .±. | 0.84±0.045 | $0.41\pm0.038^{c}$ | $0.27\pm0.059^{c}$ | $0.55\pm0.154^{a}$ | .±. |
| 4. | Tumour weight | .±. | 47.36±6.251 | $33.14\pm8.729^{a}$ | $5.11\pm1.449^{c}$ | $5.08\pm1.382^{c}$ | .±. |

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P < 0.01$,$^{a}P < 0.05$ calculated by comparing treated group with Induced group

### 3.2. Determination of tumour parameters

In the present study, tumour parameters such as, tumour burden, incidence, volume, weight was observed and calculated. (Table: 2)

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P < 0.01$,$^{a}P < 0.05$ calculated by comparing treated group with Induced group

### 3.3. Determination of biochemical parameters

**Table:3. Effect of CMCGL extract on the biochemical parameters on mammary carcinoma of SD rats**

| S.No. | Parameters | Control | Only DMBA (25 mg/ kg bw) | DMBA + Tamoxifen Citrate (10mg/kg bw) | DMBA + CMCGL (250mg/kg bw) | DMBA + CMCGL (500mg/kg bw) | ONLY CMCGL (500mg/kg bw) |
|---|---|---|---|---|---|---|---|
| 1. | Serum Glutamate Oxaloacetate Transaminase(u/l) | 61.67±7.095 | 71.67±6.658 | 60.67±2.082 | **49.67±11.060[a]** | **70.00±7.000** | 60.00±7.937 |
| 2. | Serum Glutamate Pyruvate Transaminase (u/l) | 76.33±10.214[c] | 108.00±3.606 | 87.67±3.512[b] | **80.33±6.028[c]** | **75.67±2.887[c]** | 46.33±5.686[c] |
| 3. | Serum Alkaline Phosphatase (u/l) | 96.20±52.623 | 173.17±57.388 | 98.00±38.807 | **111.25±50.009** | **132.12±45.611** | 106.33±50.536 |
| 4. | Serum Bilurubin(u/l) | 0.08±0.045 | 0.13±0.121 | 0.13±0.126 | **0.10±0.000** | **0.17±0.142** | 0.10±0.000 |
| 5. | Total Protein | 40.67±43.595 | 83.62±91.317 | 55.32±60.426 | **77.27±83.824** | **377.21±421.276** | 285.44±313.631 |
| 6. | Urea (u/l) | 51.60±9.725[c] | 91.20±7.662 | 47.97±3.356[c] | **72.37±11.811** | **47.03±9.943[c]** | 37.80±4.484[c] |
| 7. | Uric acid (u/l) | 3.67±0.252 | 3.93±0.252 | 3.50±0.608 | **3.20±0.608** | **2.97±0.551** | 2.90±0.436 |

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's [c]P< 0.001, [b]P < 0.01,[a]P < 0.05 calculated by comparing treated group with Induced group

### 3.4. Histopathological investigation

Histopathology of breast tissue (Fig.2) I) DMBA alone, II) DMBA with standard Tamoxifen Citrate, III) DMBA with CMCGL Extract 250 mg/kg, IV) DMBA with CMCGL Extract 500 mg/kg. The tumour was soft, rubbery and as it grew, grey white became irregular and lobulated. Tumours adhered more to the skin than the body wall. Section from breast shows an infiltrating neoplasm composed of cells arranged glands. Individual cells are round to oval with moderate eosinophilic cytoplasm and round oval vasicular nuclei with some showing prominent nucleoli, 2-3 mitosis/hpf was noted. Surrounding areas of necrosis and scattered lymphocytic infiltrates. The lesion size of I was 5.2×4.6×4.2cms, II was 2.8×2.0×1.8cms, III was 3.0×2.4×2.2cms and IV was 3.0×2.2×2.0cms.

Tumour markers are most useful for monitoring response to therapy and early detection of cancer. The result of this study showed elevated serum ALP, SGOT, SGPT, Bilirubin, Urea, Uric acid concentration in the tumour-bearing rats. One study showed that serum ALP concentration increased significantly in cancer patients with metastasis[16]. In our rats, there was no evidence of metastasis, suggesting that the increased serum ALP may in fact be due to the primary tumour. In contrast, there are studies[17] that reported serum GGT significant increase in women with breast malignant neoplasm, while others reported no significant increase in GGT levels in cancer patients without metastasis[18]. It is possible that either this enzyme is not related to mammary tumour development or maintenance or four weeks of tumour development were not sufficient to cause changes in these serum parameters. Longer studies are recommended to determine the importance of these enzymes in the prognosis of mammary gland and breast cancers. Our results are also consistent with the above reports. Other advantages of this tumour induction method are the ease of inoculation method, continuity and reproducibility of tumour growth, safe and economical. The model may be used for breast cancer studies, to include determination of the processes of cancer and testing of new chemotherapy agents. Another important advantage of this model is that the tumour rarely developed metastasis or expressed cell surface molecules which are different from the cells of the origin tissue[19]. In our study, the body weights of the rats did not show significant difference between normal and tumour-bearing rats, suggesting that this method did not produce side-effects that could cause weight loss, while in the study of[20,21], although initially, there was no significant change in the body weight of the control and experimental rats, but finally, there was a significant ($p < 0.001$) decrease in the body weight of the DMBA induced tumour in female SD rats. The histopathological feature of our experimental tumour model was similar to human breast cancers in characteristics, particularly in morphological feature or cellular components. Therefore, this animal model is suitable for application in the study of breast cancers.

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SP-13

# MICROBIAL ANALYSIS OF PROBIOTICS AND PATHOGENIC MICROBES ON CHOCOLATES

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## ABSTRACT

Chocolate are consumed entirely for pleasure. Food product with added supplements tends to increase industrial and social interest in the contemporary society which can be an added value to probiotics. Theobromacacao seeds are roasted and grounded with added flavours to prepare chocolate. The unique taste and flavour of chocolate is very much appealing. The liquid form pastes or solidified block possesses unique texture and thus it has become a part of our daily consumable product. In our findings *Lactobacillus sp.,* isolated all the dairy products in which candies are most commonly used probiotics are from the genera *Bifidobacteriumsp., Lactobacillus sp., Streptococcus sp., thermophiles* and non - pathogenic strains of *Enterococcus sp., Bacillus sp., E.coli* and *Saccharomyces cerevisiae* for example*, Saccharomyces boulardii*. In the observation Snickers, Eclairs, Dairymilk silk, and Dairymilk, Lolly pop consist of *Lactobacillus sp.,* the candies are commonly used items mostly preferable by children. Thus the work focused on the formation of new variety of novel and promising approach to supplement the *Lactobacillus sp.,* to the food stuff mainly chocolate. Thus a scientific method was used for the addition of the microbes to the chocolate. As chocolate contains milk products thus act as a carrier for *Lactobacillus sp*.,The cocoa and cocoa products are important source of phyto compounds with nutritional value. The process of formation of probiotic chocolate will be willing consumed by children and can be an alternative to introduce *Lactobacillus sp.,* to the digestive system.So candies should be used on proper refrigerator, proper storage $4^0$C as a result of good hygienic condition.

**KEYWORDS:** Probiotic, chocolates, food materials, pathogenic species, contamination

## 1. INTRODUCTION

In the last two decades, predictive food microbiology has included the development of models capable of describing the growth of pathogenic bacteria. The total plate count is widely used as an indication of the microbiological quality of foods unless they are known to contain large numbers of bacteria as a natural consequences of their preparation tries include cakes and baked shells filled with custard cream, or sauces. They can be spoiled by microorganisms coming with the ingredients that are added after baking. Such as icing, nuts, toppings, and cream. Most products, because of low water activity allow only molds however, some materials used to fillings may have high water activity which allows for bacterial growth.[1] Cocoa is one of the many foods that rely on a microbial; curing process for flavour curing development. The main reason for fermentation of cocoa is to induce biochemical transformations within the beans that lead to formation of the colour, aroma, and flavour precursors of chocolate[2]. A microbial consortium: *Saccharomyces cerevisiae. Lactobacillus sp.*, acetic and spore-forming bacteria have been identified[3]. During fermentation *Saccharomyces cerevisiae* dominate for the first 24hrsas the pulp disappears acetic acid bacteria (AAB) start to dominate, temperature rises to 50°C and the heat and acid result in chemical reactions the beans known as curing[4]. Diverse traditional dairy products with health-enhancing benefits, such as improvement of nutrient absorption, inactivation of toxinsand anti-pathogenic activities, are used worldwide**.** Lactic Acid Bacteria (LABs) as a major group of gram positive, catalase negative bacteria are the most important constituent of probiotics and have numerous applications in industry[5]. According to FAO/WHO definition, probiotic "Live microorganisms which when administered in adequate amounts confer a health benefit on the host"[6]. Most commonly used probiotics are from the genera

*Bifidobacterium., Lactobacillus sp., Streptococcus sp., thermophiles* and non-pathogenic strains of *Enterococcussp.,Bacillussp., E.coli* and *Saccharomyces cerevisiae* for example *Saccharomyces boulardii***(., 2001).** Several therapeutic applications for probiotics can be cited protection against traveller's diarrhoea, prevention of urogenital diseases, reduction of hypercholesterolemia, alleviation of constipation, prevention of food allergy and protection against bladder and cancer[7].

## 2.MATERIALS AND METHODS

### 2.1.SITE OF COLLECTION

The present study carried out on the collection of samples from a Malar super market Kumbakonam, Thanjvur Dt, they carried out laboratory for finding microbes.

### 2.2 HANGING DROP METHOD

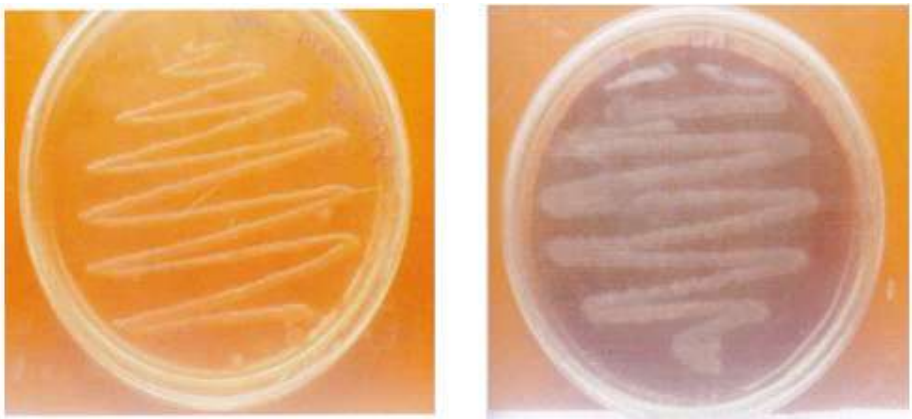
Motility of bacteria is identified using a loopful of overnight broth culture of the isolate by hanging drop method.

### 2.3 GRAM'S STAINING

A smear of suspended colony was made on a clean glass slide and heat fixed. It was flooded with cystal violet solution and allowed to remain for one minute. Then it was washed with water, flooded with iodine solution and allowed to stand for 1 minute. It was then drained and de colourised with 95% ethanol for 15-30% seconds and then washed and counter stained with safranin for one minute, then examined under microscope. The following biochemical test used to identify various types of microorganisms- Biochemical test, Indole production test, methyl red TES VP-test, Citrate utilization test, TSI agar test, gelation hydrolysis, catalase test, urease test, starch hydrolysis.

## 3. RESULT

Isolation of microbes on various chocolate like snickers, Eclairs, Dairymilk silk, Dairymilk, and Lolly pop. The microbes *staphylococcus aureus* was isolated on snickers the total microbial colony ($3x10^6$CFU/g), In Eclairs *staphylococcus epidermis* was isolated on total colony ($2.50x10^6$CFU/g), In Dairymilk silk *staphylococcus epidermidis* was isolated the total colony ($3.20x10^6$CFU/)g, In Dairymilk *shigellasp.,*was isolated on total colony ($4.50x10^6$CFU/*g), In Lolly pop shigella sp.,was isolated the total colony ($5x10^6$CFU/g).*



***Staphylococcus sp., on macconkey agarStaphylococcus sp., on blood agar***

**Fig: 1. ISOLATION OF MICROBES FROM THE SAMPLE**

**TABLE:1. ISOLATION OF MICROBES FROM VARIOUS CHOCOLATES**

| | | | |
|---|---|---|---|
| 1 | Snickers | *Staphylococcus sp., Lactobacillus* sp., | 3 x $10^6$ CFU/g |
| 2 | Eclairs | Staphylococcus *sp., Lactobacillus* sp., | 2.50 x $10^6$ CFU/g |
| 3 | Dairymilk silk | Staphylococcus *sp., Lactobacillus sp.,* | 3.20 x $10^6$CFU/g |
| 4 | Dairy milk | *Shigellasp., Lactobacillus sp.,* | 4.50 x $10^6$CFU/g |
| 5 | Lolly pop | *Shigellasp., Lactobacillus sp.,* | 5 x $10^6$CFU/g |

## DISCUSSION AND SUMMARY

In our finding the microbes *Staphylococcus sp., Shigellasp., Lactobacillus sp.,*isolated from candies microbial food safety is an increasing public health concern world wide in animal and human in our findings agreed. In the present investigation the microbes like *Shigellasp., Staphylococcus sp., Lactobacillussp.,* were isolated from different candies like Snickers, Eclairs, Dairymilk silk, Dairymilk, Lolly pop.

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SP-14

# ISOLATION AND IDENTIFICATION OF PATHOGENS FROM DIABETIC AND NON DIABETIC WOUND INFECTION AND COMPARISON OF ANTIBACTERTIAL EFFECT OF SPICES AND SYNTHETIC ANTIBIOTICS AGAINST THE ISOLATED PATHOGENS

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## ABSTRACT

The pathogens were isolated and identified from diabetic wounds such as *Staphylococcus aureus, Klebsiellapneumoniae* and from non-diabetic wound such as *E.coli, Klebsiellapneumoniae.* The antibacterial analysis was performed with spices given Ginger, Garlic, Turmeric and synthetic antibiotics Ambicillin, Erythromycin and Tetracyclin. The antibiotic Erythromycin have maximum effect on *E.coli.* Turmeric has maximum controlling effect on the pathogens.

**KEYWORDS:** Diabetic, Non-diabetic, Spices, Synthetic antibiotics*, E.coli, Klebsiella pneumoniae.*

## INTRODUCTION

Diabetic foot ulceration and infections are a major medical ,social and economic problem and a leading cause of morbidity and mortality, especially in the developing countries.[5] Once the skin is broken (typically on the plantar surface), the underlying tissues are exposed to colonization by pathogenic organisms. The resulting wound infection may begin superficially, but due to delay in treatment and impaired body defense mechanism caused by neutrophil dysfunction and vascular insufficiency, it can spread to contiguous subcutaneous tissues and even to deeper structures.[2] Patients with diabetes have impaired wound healing associated with multitude of factors, including neuropathy, vascular disease, and foot deformities. Among the 191 WHO member states, India has the highest number of people with diabetes[4]. Mostly the diabetic foot infections are mixed bacterial infection[10]. India must be the world leaders in the area of cultivation of spices. In this respect, spices such as Mustard, Garlic(antiseptic,diuretic), Ginger(digestive ,cold), Mint etc., have been reported to poses very good and antimicrobialctivity(). From ancienttime many medicinal plants represent an excellent source of antimicrobial agents.[6] In rural areas many plant materials used as a traditional medicine which are readily available and relatively cheaper than modern medicine.[7] Plant products still remain the principle source of pharmaceutical agents used in traditional medicine.[8] Turmeric(curcuma longa L.) is a therapeutic plant that belongs to family zingiberaceae. Turmeric has healthy influenceon digestive system and it also enhances the mucin secretion in the digestive. Ginger (*Zingeberofficinale*) is a medicinal plant that has been widely used all over the world.Garlic with its antibacterial properties is widely used for a number of infectious diseases (Among the Gram (-) bacteria the most commonly isolated pathogen was *E.coli* and among the Gram(+) bacteria *Staphylococcus aureus* was the most common. Wound healing is a complicated process. In diabetic foot ulcers, wound healing ability is highly impaired. The function of leucocytes is abnormal in patient with diabetes. Many of the diabetic foot ulcers involve the underlying muscles. Curcumin improves muscle regeneration and increase the rate of wound healing[9]. Curcumin may enhance wound healing through a variety of means. Curcumine was found to poses ant-bacterial activity against a variety of gram(-) and gram(+) bacteria including *staphylococcus aureus, E.coli* and *Pseudomonas* which are some of most common organisms found in diabetic foot ulcers.

## MATERIALS AND METHODS

## SAMPLE COLLECTION

Pus samples were collected from diabetic and non-diabetic patients of 40-60 age group with foot ulcers in government hospital and M.S Hospital at Kumbakonam. Wound sample were collected by using sterile cotton swab (fresh pus sample). The pus specimen was inoculated on nutrient agar plates. The inoculated plates were incubated at $37^0$C for 24 hours. Identification of isolates were done based on colony morphology. Gram staining, motility test and biochemical test.

## IDENTIFICATION OF BACTERIAL ISOLATES

## MICROSCOPIC OBSERVATION

The strains such as isolated were microscopically screened for gram reaction, spore formation and motility by hanging drop method using overnight broth culture and identified by biochemical analysis.

## BACTERIAL ISOLATES

*Escherichia coli, Staphylococcus aureus, Steptococcus pyrogens, Klebsiella pneumonia, Pseudomonas aeroginosa*, and *Proteus sp*.,

## SPICES COLLECTION

Dry powder of Turmeric and Ginger (in commercial packets) were bought from Baskaran medical shop and Garlics were bought from super market at Kumbakonam.

## PREPARATION OF SPICES EXTRACT

The spices sample was extracted for antibacterial activity by 20g of dry spice was added to 100ml of distilled water and was put in a shaker for 24 hours at 120 rpm at $37^0$C, then they were centrifuged at 3000 rpm for 10 minutes. The supernatant was filtered using whatsman filter paper and stored in a refrigerator at $4^0$C. The stock solution was then diluted to various concentration to determine the antibacterial activity. And the garlic spices were grounded with twice the weight of sterilized water in a pestle and mortar to derive the extracts. These were allowed to stand for 10 minutes and then filtered through cheese cloth. The filtrates were kept in sterilized vials.

## TEST MICROORGANISM

All bacteria used in this study were previously isolated and identified from patient wound pus sample and stored in the laboratory of the Department of microbiology of Idhaya College for women, Kumbakonam. The antibacterial activity of the spices was tested against the isolates such as *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Steptococcus pyrogens, Pseudomonas aeroginosa,* and *Proteus sp.,*

## AGAR WELL DIFFUSION METHOD

The antibacterial activity of spices (Ginger, Garlic,Turmeric) exract was determined against *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus aureus, Steptococcus pyrogenes ,Pseudomonas aeroginosa*, and *proteus sp*.,. The well diffusion method was used to screen the antimicrobial activity. The nutrient agar plates were prepared and solidified. 100 micro liters of the inoculum suspension was swabbed uniformly and allowed to dry for 5 min. each 10 microliter volumes of spices extracts were loaded into the wells using a gel punch. The plates were incubated at $37^0$C for 24 hours. The inhibition zones formed around the wells were measured.

## ANTIBIOTIC SENSITIVITY TEST

The organism isolated were subjected to antibiotic susceptibility testing on nutrient agar using Kirby-Bauer disc-agar diffusion method and interpreted as per clinical Laboratory standard institution guide lines. Three different antibiotics include Ampicillin, Erythromycin and Tetracycline.

**TABLE 1- SAMPLE COLLECTION AND ISOLATION**

| TEST ORGANISM | WOUND PATIENTS | |
|---|---|---|
| | DIABETIC | NON DIABETIC |
| *Escherichia coli* | - | + |
| *Staphylococcus aureus* | + | + |
| *Staphylococcus pyrogenes* | + | + |
| *Klebsiellapnemoniae* | + | + |

| | | |
|---|---|---|
| *Pseudomonas aeroginosa* | + | - |
| *Proteus sp.,* | + | + |

**TABLE 2-Characteristics of isolated organism (diabetic wound)**

| **CHARACTERISTICS** | ***S.aureus*** | ***S.pyrogenes*** | ***K.pnemoniae*** | ***P.aeroginosa*** | ***Proteus sp.,*** |
|---|---|---|---|---|---|
| Colonies morphology | Golden yellow | Beta-hemolytic colonies | Mucoid colonies | Circulat blue green colour colony | Bluish green |
| Gram's staining | Gram(+) cocci | Gram(+) cocci | Gram(-) rod | Gram(-) rod | Gram(-) rod |
| Spore forming | Non-spore | Non-spore | Non-spore | Non-spore | Non-spore |
| Motility | Non-motile | Non-motile | Non-motile | Motile | Motile |
| Indole | - | - | - | - | + |
| Methyl red | + | - | - | - | + |
| Vp | + | - | + | - | - |
| Citrate utilization | + | - | + | + | + |
| Urease | + | - | + | - | + |
| Oxidase | - | + | - | + | - |
| Catalase | + | - | + | + | - |
| TSI | - | - | - | - | + |
| Carbohydrate | - | - | + | - | - |

**TABLE 3- Characteristics of isolated organism (non-diabetic wound)**

| **Characteristic** | ***E.coli*** | ***S.aureus*** | ***S.pyogenes*** | ***K.pneumoniae*** | ***Proteus sp.,*** |
|---|---|---|---|---|---|
| Colony morphology | Metallic sheen colonies | Golden yellow colonies | B-hemolytic colonies | Mucoid colonies | Bluish green colonies |
| Gram's staining | Gram (-) rod | Gram(+) cocci | Gram(+) cocci | Gram(-) rod | Gram(-) rod |
| Spore forming | Non spore | Non spore | Non spore | Non spore | Non spore |
| Motility | Motile | Non motile | Non motile | Non motile | Motile |
| Indole | + | - | - | - | + |
| Methyl red | + | + | - | - | + |
| VP | - | + | - | + | - |
| Citrate utilization | - | + | - | + | + |
| Urease | - | + | - | + | + |
| Oxidase | - | - | + | - | - |
| Catalase | + | - | - | + | - |
| TSI | - | - | - | - | + |
| Carbohydrate | + | - | - | - | - |

**ANTIBACTERIAL ACTIVITY: TABLE 4**

| **Antibiotics** | **Diabetic wound organism zone of inhibition(mm)** | | | | |
|---|---|---|---|---|---|
| | ***S,aureus*** | ***S.pyogenes*** | ***K.pneumoniae*** | ***P.aeruginosa*** | ***Proteus sp.,*** |
| Ambicillin | 7mm | 9mm | - | 5mm | - |
| Erythromycin | 12mm | 11mm | 9mm | 7mm | 10mm |
| Tetracyclin | 8mm | 12mm | 13mm | 15mm | 10mm |

**TABLE 5**

| Antibiotics | Non-Diabetic wound organism zone of inhibition(mm) | | | | |
|---|---|---|---|---|---|
| | ***E.coli*** | ***S.aureus*** | ***S.pyogenes*** | ***K.pneumoniae*** | ***Proteus sp.,*** |
| Ambilicilin | 12mm | 9mm | 11mm | 9mm | 12mm |
| Erythromycin | 20mm | 13mm | 17mm | 17mm | 13mm |
| Tetracyclin | 14mm | 11mm | 15mm | 21mm | 11mm |

**TABLE 6**

| Spices extracts | Diabetic wound organism zone of inhibition(mm) | | | | |
|---|---|---|---|---|---|
| | ***S.aureus*** | ***S.pyogenes*** | ***K.pneumoniae*** | ***P.aeruginosa*** | ***Proteus sp.,*** |
| Garlic | 12mm | 13mm | 10mm | 12mm | 12mm |
| Ginger | 12mm | 12mm | 11mm | 9mm | 10mm |
| Turmeric | 14mm | 16mm | 15mm | 12mm | 13mm |

**TABLE 7**

| Spices extract | Non-diabetic wound organism zone of inhibition(mm) | | | | |
|---|---|---|---|---|---|
| | ***E.coli*** | ***S.aureus*** | ***S.pyogens*** | ***K.pneumoniae*** | ***Proteus sp.,*** |
| Garlic | 18mm | 21mm | 15mm | 17mm | 16mm |
| Ginger | 14mm | 15mm | 14mm | 12mm | 14mm |
| Turmeric | 24mm | 21mm | 18mm | 24mm | 17mm |

## RESULT AND DISCUSSION

The aim of present study focuses on isolation, identification of pathogenic bacteria and antibiogram activity from wound of diabetic and non-diabetic. The collected wound(pus) sample was plated on nutrient agar medium as swab culture. The colonies were isolated after overnight incubation of the culture plate. The typical colonies were collected and stained by gram staining procedure to identify the organism for its Gram (+) or Gram(-) characteristic, the shape of the cell structure, motility were also observed for selected typical colonies using the hanging drop method. In this study, diabetic wounds showed a significant positive culture compared to non-diabetic wounds with total organism isolated in 3 patients from diabetic wounds were 5 from cases and total organism isolated in non-diabetic wounds were about 5 from the cases (Table 1). Among bacterial isolates in on-diabetic wounds, Gram(+) and Gram(-) organisms (Table2). In this study among diabetic wound infection cases the predominant organism isolated was *Staphylococcus aureus, Streptococcus pyrogenes, Klebsiella pneumonia, Pseudomonas aeroginosa* and *Proteus* species. Among non-diabetic wound infection cases the predominant organisms isolated was *Escherichia coli, Staphylococcus aureus, Streptococcus pyoenes, Klebsiella pneumonia* and *proteus* sp (Table 4). In this study the pathogens isolated from diabetic wound, which show the antibiotic sensitivity are Staphylococcus aureus, Ampicillin-7mm, Erythromycin-12mm, Tetracyclin-8mm.*Steptococcuspyogens,* Ampicillin-9mm, Erythromycin-11mm, Tetracyclin-12mm. *lebsiellapneumonia*, Erythromycin-9mm, Tetracyclin-13mm. *Pseudomonas aeroginosa,* Ampicillin-5mm, Eruthromycin-7mm, Tetracyclin-15mm. Proteus species, Erythromycin-10mm, Tetracyclin-10mm. *Klebsiella pneumonia* and *Proteus* species are resistant against Ampicillin. The Tetracyclin shows maximum inhibition of 15mm against *Pseudomonas aeruginosa* (Table 4). The pathogen isolated from non-diabetic wound which showed the antibiotic sensitivity are Escherichia coli, Ampicillin-12mm, Erythromycin-20mm, Tetracyclin-14mm. *Staphylococcus aureus*, Ampicillin-9mm, Erythythromycin-14mm, Tetracyclin-11mm. *Sterptococcuspyogenes*, Ampicillin-11mm, Erythromycin-17mm, Tetracyclin-15mm. *Klebsiella pneumonia*, Ampicillin-9mm, Erythromycin-17mm, Tetracyclin-21mm and *Proteus* species, Ampicilli-12mm, Erythromycin-13mm,Tetracyclin-11mm. The Tetracyclin show maximum inhibition of 22mm against *Klebsiellapneumoniae* (Table 5). The strongest antibacterial activities of spices extract were noticed against diabetic wound organism with zone of inhibition being *Staphylococcus aureus*, Garlic-12mm. Ginger-12mm, Turmeric 14mm. *Streptococcus pyrogens*, Garlic-13mm. Ginger-12mm, Turmeric-16mm. *Klebsiella pneumonia*, Garlic-10mm, Ginger-11mm, Turmeric-12mm. *Proteus* species, Garlic-12mm,Ginger-10mm,Turmeric-13mm. the turmeric

shows maximum inhibition of 16mm against *Streptococcus pyogenes* (Table 6). The spices extract were noticed against non-diabetic wound organisms with zone of inhibition being Escherichia coli, Garlic-18mm, Ginger-14mm,Turmeric-24mm. *Staphgylococcusaureus*, Garlic-21mm, Ginger-15mm, Turmeric-21mm. *Streptococcus pyogenes*, Garlic-15mm, Ginger-14mm, Turmeric-18mm. *Klebsiella pneumonia*, Garlic-17mm, Ginger-12mm, Turmeric-24mm. *Proteus* species, Garlic-16mm, Ginger-14mm, Turmeric-17mm. the turmeric show maximum inhibition of 24mm against *E.coli* and *Klebsiella pneumonia* (Table 7). The antibacterial activity of the antibiotic Erythromycin shows the maximum inhibition against Escherichia coli than other antibiotics. Therefore, the antibacterial activity of species, turmeric shows high inhibition activity against the pathogens, *Escherichia coli, Streptococcus pyogenes, Klebsiella pneumonia.*

## SUMMARY AND CONCLUSION

The wound exudates cultured are *E.coli, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aerogenosa, Klebsilla pneumonia* and *proteus* species. These organisms are dangerous pathogens penetrating the wound, producing lot of pain and exudates. Some of these organisms were checked either sensitive or resistant to antibiotics like Ampicillin, Erythromycin and Tetracyclin. In the present work of the spices showed antibacterial activity against the diabetic and non-diabetic patient wound pathogenic bacteria *E.coli, Staphhylococcus aureus, Streptococcus pyogenes*, *Pseudomonas aeroginosa, Klebsiella pneumonia* and *proteus*species. The inhibition of organisms isolates was marked when compared with zone of inhibition. Turmeric and Garlic extracts is effective than the antibiotics against the pathogens.

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SP-15

# EFFICACY AND ANTIBACTERIAL ACTIVITY OF SCRUPULOUS COMMERCIAL SOAPS AGAINST BACTRIAL STRAINS

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## ABSTRACT

The efficacy and antibacterial activity of commercial soaps against bacterial strain, The human pathogens were isolated from patients with wound infections from Government hospital at Kumbakonam. The isolates were *Staphylococcus aureus, Strptococcus pyogenes* and *Pseudomonas aeruginosa.* The antibacterial activity of commercial soaps such as Medimix, Dettol and Cinthol were analyzed by Agar Diffusion method and Minimum Inhibition concentration Technique Medimix shows better inhibition in minimum concentration against *Staphylococcus aureus*.

**KEYWORDS:** *Staphylococcus aureus, Strptococcus pyogenes,* Agar well diffusion, Commercial soaps, Mannitol Salt agar, Cetrimide agar.

## INTRODUCTION

Soaps are the combination of fats and oils (of animal or vegetable origin) and Salt.[7] The aim of this work to compare the efficiency of locally available market soaps against skin infected some bacteria such as *Staphylococcus spp., Pseudomonas spp.,* and *Serratia sp.,* as well as to provide data to clinician to decide for the selection of better and protective soap against pathogenic microorganism.[10] The great majority of the studies on the effect of antibacterial soaps on the flora of human skin have been carried out on hands because of the interest in determine in surgical scrub procedures and because hands carry large numbers of microorganisms and permit the demonstration of sizable reduction in numbers.[16] Urinary tract infections were the 2$^{nd}$ in frequency to respiratory tract infections.[4] Gram negative bacteria responsible for the infection were proteins*, Enterobacter, Pseudomonas, Serratia* and *Klebsiella.*[11] Gram positive cocci are *Staphylococcus, Saprophyticus, Staphylococcus aureus, Streptococci* and *Staphylococci epidermidis*.[16] An antibacterial soap can remove 65% to 85% of bacteria from human skin.[13].In 1961 the U.S. Public Health Service recommendations directed that personnel wash their hands with soap and water for 1 to 2 minutes before and after client contact. The antibacterial soaps can remove 65 to 85% bacteria from human skin.[14] Although fats and oils are general ingredient of soaps but some detergents are added to enhance the antibacterial of soaps.[7] Use of waterless antiseptics agents was recommended only in the situations where sinks were not available. Transient bacteria are deposited on the skin surface from environmental sources and causes skin infections. Examples of such bacteria are *Pseudomonas aeruginosa*[6] and *Staphylococcus aureus*.[9] African Black soap or black soap is a natural source of vitamin A and E, and iron.[8] The soap is then allowed to cure for at least two weeks before it is ready for use .The most common organism isolated from lesions was *Staphylococus aureus.*[12] Despite widespread use of antibacterial soaps among U.S. troops serving in Vietnam, however, rampant impetigo infections contributed to lost man-days.[15]

## MATERIALS AND METHODS

### SAMPLING AREA

The samples were taken from government hospitals at Kumbakonam, where several patients with burn wound, surgical wound are coming for treatment.The sterile cotton swab was prepared to collect the wound samples from the patients .The collected samples were kept inside of a sterile screw cap tubes and transported to laboratory for further process.The isolated organisms were identified by Gram's staining and biochemical tests.

## BACTERIAL STRAINS

*Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogens.*

## COLLECTION OF SOAPS

To perform this experimental study different soaps of common use from shops were purchased and their dilution were made for testing the bactericidal activity of different organisms.[12] The soaps used were Medimix, Dettol and Cinthol.

## PERPARATION OF INOCULUM

For inoculums preparation Muller-Hilton broth was made according to manufacturer's instructions and 5 ml of broth medium was dispensed in screw capped test tubes and sterilized by autoclaving at $121^0$C for 15 minutes.[14] The test tubes were cooled the isolated strains were inoculated in an incubator overnight at $37^0$C.

## ASSAY OF ANTIBACTERIAL ACTIVITY

1. Agar Diffusion method
2. Minimum Inhibitory Concentration technique

## 1. AGAR DIFFUSION METHOD

Dilute the soaps into 10g, 20g µand 30g concentration. After solidification the wells were made and each well was incorporated with 20 to 30µl of serially diluted soaps and further incubated at $37^0$C for 12 to 24 hours.The zone of inhibition was determined by measuring the diameter in millimeters of zone to which the soap inhibited the growth of the organisms.[3]

## 2. MINIMUM INHIBITORY CONCENTRATION:

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18 – 24 hours.[6] The soaps that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. The following concentration were obtained; 10g, 20g, 30g, 40g. A tube containing broth and inoculum without soaps served as organism control.The lowest concentration of soaps which inhibited microbial growth turbidity was recorded as minimum inhibitory concentration.

## RESULT AND DISCUSSION

The table 1 represents the antibacterial activity of the three branded commercial soap at different concentration against the test organisms isolated from wound infection. The zone of inhibition against bacterial isolates was found to be a function of the relative antibacterial potency of the commercial soaps. The results show if the concentration of soaps increases the inhibition zone also increases. This indicate that the antibacterial activity of commercial soaps increases when diluted with more amount of water with the test organisms *Staphylococcus aureus, Streptococcus pyogenes and Psudomonas aeruginosa.* Medimix shows highest zone of clearance for the three organisms was obtained against the concentration of 10gm/ml, 20gm/ml and 30gm/ml. In 10gm/ml concentration of lowest efficiency against *Staphylococcus aureus* (20mm), *Streptococcus pyogenes* (13mm) and *Pseudomonas aeruginosa* (15mm). 20gm/ml concentration of three organisms obtained for the zone of inhibition against (22mm, 17mm, 15mm). 30gm/ml concentration shows highest efficiency against with a diameter of (24mm, 20mm, and 19mm). While evaluating the Dettol soap in 10gm/ml concentration shows the lowest activity against *Staphylococcus aureus* (19mm), *Streptococcus pyogenes* (14mm) and *Pseudomonas aruginosa* (14mm). 20gm/ml concentration shows the zone of inhibition against the organisms (20mm), (18mm), (16mm). 30gm/ml concentration of three organisms obtained for the highest efficiency against with a diameter of (23mm), (20mm), (21mm). Cinthol shows the lowest activity obtained for the organisms in 10gm/ml concentration against *Streptococcus pyogenes* (13mm), *Pseudomonas aruginosa* (15mm). 20gm/ml concentration of three organisms obtained for the zone of inhibition against (16mm), (14mm), (17mm). The highest zone of inhibition obtained for the three organisms respectively against 30gm/ml with a diameter of (19mm), (21mm), (20mm). Table 2 represents the Minimum inhibitory concentrations was performed with 10gm, 20gm, 30gm and 40gm of all soaps dilution to determine the minimum concentration of soap dilution which inhibits the growth of bacterial samples. While determination of Minimum Inhibitory Concentration Medimix shows better inhibition in minimum dilution of 10gm against

*Staphylococcus aureus* only 0.45nm of turbidity is recorded when measured in calorimeter. Thus medimix proved to have good bactericidal property against all the bacteria isolated from wound samples. Dettol also shows better clearance. But at highest concentration (30g). Though it shows highest activity against *Pseudomonas aruginosa.* The activity was enhanced only at highest concentration of soap dilution. Cinthol shows better inhibition at higher concentration (30g) against *Streptococcus phyogenes.* There is no activity at lowest concentration of 10 and 20g. The study suggests that antiseptic soaps w more effective gram negative and Gram positive bacteria than were plain soaps. When efficacy of antibacterial soaps was compare Medimix was found to be more effective against *Pseudomonas,* in agar diffusion method. Safeguard was found to be more effective against *Staphylococcus aureus* than all the soaps.[1] In the current investigation on efficacy of commercial soaps against the concentration 10g/ml, 20g/ml and 30g/ml of bacterial strains, Medimix shows maximum inhibition against *Staphylococcus aureus* (24mm) and lowest activity against *Pseudomonas aurginosa* (19mm). Dettol shows highest activity against *Pseudomonas aurginosa* (22mm) and lowest efficacy against *Streptococcus pyogens* (20mm) and Cinthol was revealed activity against *Streptococcus pyogens* (19mm) and lowest activity against *Staphylococcus aureus* (19mm). MIC of Sufi soap against *Staphylococcus aureus* was 10240µg/ml, and MIC of sunlite was 20480 µg/ml, where as MIC of lux was 24576 µg/ml. This showed that Sufi soap and sunlite exhibited more activity against *Staphylococcus aureus* than lux .The MIC was observed at 10g/ml concentration of Medimix against *Staphylococcus aureus,* and the lowest activity of 40gm/ml against *Pseudomonas aurginosa.* The Dettol soap was observed at 20g/ml concentration against *Pseudomonas aurginosa* and lowest inhibitory 30g/ml concentration against *Streptococcus pyogens.* The Cinthol soap was showed at 30gm/ml concentration against *Streptococcus pyogens* and the least inhibitory 40gm/ml concentration against *Staphylococcus aureus.* The result of antibacterial activity and the MIC assay showed promising evidence for the antibacterial, activity of commercial soaps against enteric pathogens.

**TABLE 1. EVALUTION OF ANTIBACTERIAL ACTIVITY OFCOMMERCIAL SOAP BY WELL MTHOD INBACTERIAL STRAINS**

| Name of the organisms | Zone of Inhibition in mm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Medimix soap | | | Dettol soap | | | Cinthol soap | | |
| | 10g | 20g | 30g | 10g | 20g | 30g | 10g | 20g | 30g |
| *Staphylococcus aureus* | 20 | 22 | 24 | 19 | 20 | 23 | 14 | 16 | 19 |
| *Streptococcus pyogens* | 15 | 17 | 20 | 15 | 18 | 20 | 13 | 14 | 21 |
| *Pseudomonas aeruginosa* | 13 | 15 | 19 | 14 | 16 | 21 | 15 | 17 | 20 |

**TABLE : II. MINUMUM INHIBITORY CONCENTRATION FOR MEDIMIX SOAP**

| Name of the organisms | Optical Density at various dilution Of Medimix Soap | | | |
|---|---|---|---|---|
| | **10g** | **20g** | **30g** | **40g** |
| *Staphylococcus aureus* | 0.45 | 0.33 | 0.27 | 0.19 |
| *Pseudomonas aeruginosa* | 0.88 | 0.70 | 0.62 | 0.45 |
| *Streptococcus pyogens* | 0.66 | 0.55 | 0.51 | 0.42 |

**TABLE : III. MINUMUM INHIBITORY CONCENTRATION FOR DETTOL SOAP**

| Name of the organisms | Optical Density at various dilution Of Dettol Soap | | | |
|---|---|---|---|---|
| | **10g** | **20g** | **30g** | **40g** |
| *Staphylococcus aureus* | 0.47 | 0.35 | 0.29 | 0.16 |
| *Pseudomonas aeruginosa* | 0.35 | 0.22 | 0.17 | 0.09 |
| *Streptococcus pyogens* | 0.50 | 0.43 | 0.31 | 0.21 |

**TABLE : IV. MINUMUM INHIBITORY CONCENTRATION FOR CINTHOL SOAP**

| Name of the organisms | Optical Density at various dilution Of Cinthol Soap | | | |
|---|---|---|---|---|
| | 10g | 20g | 30g | 40g |
| *Staphylococcus aureus* | 0.55 | 0.42 | 0.39 | 0.22 |
| *Pseudomonas aeruginosa* | 0.40 | 0.31 | 0.24 | 0.11 |
| *Streptococcus pyogens* | 0.42 | 0.30 | 0.22 | 0.18 |

## CONCLUSION

This study reveals and confirms that some claims made by the manufacture of the commercial soaps under study, Medimix and Dettol shown better activity against the test organism. Cinthol was better off but its activity was little against the microorganism used in this study except *Streptcoccus pyogenes,* contrary to the manufacture claim. Health is wealth accordingly we can advice and give awareness to the society for using standardized soaps and maintain health and hygiene.

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SP-16

# ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM DIFFERENT DAIRY PRODUCTS AND DETERMINATION OF ANTAGONISTICS ACTIVITY AGAINST FOOD BORNE PATHOGENIC BACTERIA AND ANTIBIOTIC RESISTANT ACTIVITY AGAINST FIVE SYNTHETIC ANTIBIOTICS

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## ABSTRACT

Lactic acid bacteria widespread in nature and predominant microflora of milk and its products. Lactic acid bacteria are one of the important groups of microorganisms in food fermentation. A wide variety of strains are routinely used as starter culture to manufacture dairy products such as curd,cheese,whey and yoghurt.These bacteria produce organic acid hydrogen peroxide and several enzymes during fermentation. Milk is known as one of the natural habitats of lactic acid bacteria. Lactic acid bacteria are anaerobic or facultative which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrates .Lactic acid bacteria are naturally present in milk products.LAB is generally associated with habitat rich in nutrients such as milk, cheese, meat,beverages and vegetables. Lactic acid bacteria have a long history of safe use in the production of fermented foods and beverages whereas natural,spontaneous fermentation processes are still common practice in many cases,Bacteriocins of LAB are extracellular bacteriocidal proteins that are secreted by cells. Totally 4 isolates of lactic acid bacteria *Lactobacilus,Leuconostoc, Lactococcus and Streptococcs sp* were isolated and identified from milk,youghurt and cheese sample.The antagonistic activity of the isolates against test organisms.(*Escherichia E.coli, Stapholococcus aureus,Bacillussubtilis and Shigella dysentriae).*Showed significant inhibition activity.The antibiotic resistant activity of all the isolates against tetracycline,ampicillin,erythromycin,penicillin and amphotericin was determined.It showed that most of the isolates were found be resistant to the antibiotics

**KEYWORDS:** Lactic acid Bacteria,Antagonistic Activity,Dairy products,Antibiotic resistant Activity.

## INTRODUCTION

Lactic acid bacteria were first discovered by scheele [1]) from sour milk.. Pasteur discorved in 1857 that the souring of milk was caused by the microorganisms.Lactic acid was first produced commercially by M/s Clinton processing company,USA. Lactic acid bacteria have been widely used for the fermentation of many fermented product such as cheese,sourdough,buttermilk,brined vegetables,youghut and sauerkaraut[2]**).** Lactic acid bacteria are more the most imporant bacteria in desirable food fermentation ,being responsible for the fermentation of sour dough bread, fermented foods and beverages,all fermented milks and fermented vegetables[3]**.**It plays an essential role in the prodution of all dairy products and is involved in the production of many other fermented foods and beverages,sausages,pickles and boza etc.Based on the end product of glucose fermentation lactic acid bacteria are grouped as either Homofermenters or Hetrofermenters [4]The Homofermenters produce lactic acid as the major product of fermentation of glucose.The Heteroferments produce lactic acid carbon dioxide,acetic acid,and ethanol from the fermentation of glucose[5,6] Latic acid bacteria (LAB) are a group of Gram-positive,non-sopre forming,cocci or shaped,catalase-negative and fastidious organisms,considered as 'Generally Recognized as safe' (GRAS) organisms. **).**Different antimicrobial molecules such as lactic acid,acetic acid, hydrogen peroxide,carbon dioxide and bacteriocins produced by these bacteria are widely known to inhibit food borne pathogens and spoilage microorganisms,there by extending the shelf –life and enhancing the safety of food products.Stabilization of the gut microflora [7]Bacteriocins of LAB are extracellular bacteriocidal proteins that are secreted by cells.On the basis of the protein structure,bacteriocins constitute a heterogeneous group of small peptides or high molecular weight proteins, or protein complexes **().**

Bacteriocins usually have low molecular weight (rarely over 10kDa) they undergo post translational modification and can be easily degraded by proteolytic enzymes especially by the proteases of the mammalian gastrointestinal tract, which makes them safe for human consumption8**).**Among the Gram positive (+)bacteria, the LAB have gained particular attention nowadays, due to the production of bacteriocins. These substances can be applied in the food industry as natural preservatives[9]

## MATERIALS AND METHODS

### Collection of samples

The samples (dairy products – milk, yoghurt and cheese) were collected from local marketin kumbakonam.

### Isolation of lactic acid bacteria

For isolation of lactic acid bacteria sample was homogenized with sterilized peptone water physiological saline solution (1%peptone,0.95%Nacl) for about 1-3 minutes aseptically.Appropriate serial dilution ($10^{-1}$ to $10^{-6}$) was prepared for the sample using 1 millilitres of homogenate.A volume of appropriate dilutions was spread plates on MRS agar media. Then the plates were incubated for 48 hours in anaerobic jar at $32^0$C typical LAB characteristics colonies were randomly picked up and purify by streaking two or three times on fresh MRS agar plates followed by macroscopic and microscopic examinations.The colonies displaying the general characteristics of lactic acid bacteria gram positive and catalase negative were choosen from each plate for physiological and biochemical test. The identity of the isolates were confirmed through various morphological and biochemical test.

### Identification of the Bacterial Isolates

Isolate were tested for catalase activity All Gram-staining reaction and cell morphology. All gram positive and catalase negative rods were tested for growth in MRS broth at $15^0$C and $45^0$C and production of gas from glucose Gram positive cocci were identified according to Hardie[10]). The strains were tested for production of acids from carbohydrate and related compounds. The tests were done and the results were read after incubation of strains at $37^0$C for 2 and 3 days.

### Antagonistic Activity of LAB against Pathogenic Bacteria

Isolated LAB were designated as L1,L2,L3, and L4 were maintained in sterile MRS broth.The strains of were screened for their ability to inhibit the growth of *E.coli,Staphylococcus aureus and Bacillus subtilis & Shigella dysentriae.*The antimicrobial activity was determined by disc diffusion assay. Melted nutrient agar was inoculated with 1% of an 18-24 hour old broth culture of the test organism. Eight milliliters of this seeded agar were poured into sterile petridishes and allowed to solidify. A sterile filter paper disc of 6.25mm diameter was dipped into sterile petridishes and allowed to solidify. A sterile Filter paper disc of 6.25 mm diameter was dipped into the LAB culture and disc placed on the seeded agar surface. Several discs could be placed on the plate. Each test culture was assayed in duplicate. The plates were incubated at $5^0$C for 2 hours to allow the test materials to diffuse into the agar and then incubated at $37^{0\ C}$, for 16-18 hours. After incubation the plates were examined for zone of inhibition around the various discs. Very strong inhibition (++++) was assigned to zones of 20 to 25 mm diameter , correspondingly, diameter, weak inhibition (+) 9 to 10 mm diameter , no inhibition (-) and doubtful (±) cases were also recorded.

### Determination of antibiotic resistance of the isolates

In the study, the 5 antibiotics discs were used to determine antibiotic resistance of *Lactobacilli* strains. These antibiotics discs were Tetracycline, Ampicillin, Erythromycin, Penicillin and Amphotericin. A loopful of overnight grown culture of each isolate was spread plated on MRS agar plates and the antibiotic discs were placed upside down, pressed on the top of agar plates and incubated overnight at $37^0$c.

## RESULTS

### Isolation of lactic acid bacteria in MRS medium

Totally 10 isolates were obtained from milk, yoghurt and cheese sample collected randomly and the isolates were tested for catalase negative and gram positive property, of this four are found to be lactic acid bacteria. These isolates were made pure culture in MRS agar medium.

**Identification of LAB isolates through morphological and biochemical test**

Identification of LAB isolates through morphological and biochemical test. Among the 4 isolates L1 and L3 are gram positive cocci, L2 and L4 gram positive rod and all isolates are non-motile. All the isolates were catalase negative and non-spore forming organisams.Morphological, physiological and biochemical characteristics of isolates were shown in table 1 with this the isolates were grouped into *Lactobacillus*, *Leuconostoc, Lactococcus and Streptococcus sp.*

**Determination antagonistic activity of isolates against test organisms**

The antimicrobial activity of isolates LAB against *E.coli*, *Staphylococcus aureus, Bacillus subtillis and Shigella dysentriae was* observedby disc diffusion method and the results were given in table 2. It was observed that all of the LAB isolates possessed significant antagonistic activity against test pathogens. The isolates L1, L3&L4 showed moderate inhibition (zone of inhibition 12-14mm) and L2 showed weak inhibition (zone of inhibition 9-10mm) against*E.coli*. The isolates L1, L2, &L4 showed moderate inhibition and L3 showed weak inhibition against *staphulococcus aureus*against *e.coli*.. The isolates L1, L2, &L4 showed moderate inhibition and L3 showed weak inhibition against *staphylococcus aureus*. The isolates L1&L2 showed strong inhition (zone of inhibition 12-18mm) L3&L4 showed weak inhibition against *Bacillus subtilis*. All isolates showed weak inhibition aginst *shigella dysentriae*.

**Determination of antibiotic resistant activity of isolates against synthetic antibiotics**

The antibiotic resistant activity of the isolates against ampicillin, penicillin, erythromycin, amohotericin and tetracycline was observed by disc diffusion method and the results were given in table 3. All the isolates showed resistant to most of the antibiotics. Only the isolate L1 showed sensitivity to tetracycline and erythromycin.

**Table-1. Morphological and biochemical characteristics of isolates**:

| CHARACTERISTICS | | L1 | L2 | L3 | L4 |
|---|---|---|---|---|---|
| Colonies morphology | | Circular creamy smooth&entire | Irregular creamy &smooth | Circular creamy &smooth | Circular creamy &smooth |
| Gram's staining | | Positive diplococi | Positive rod | Positive diplococci | Positive rod |
| Spore staining | | Non-spore | Non-spore | Non –spore | Non-spore |
| Motility | | _ | _ | _ | _ |
| Indole | | + | + | _ | _ |
| Methyl red | | _ | + | + | + |
| Voges prouskaur | | _ | + | + | + |
| Citrate utilization | | _ | _ | _ | _ |
| TSI | | + | + | + | + |
| Catalase | | _ | _ | _ | _ |
| Oxidase | | _ | _ | _ | _ |
| Acid Gas From action & | Glucose | + | + | + | + |
| | Lactose | + | + | + | + |
| | Sucrose | + | + | + | + |

**Table 2: Antimicrobial activity bof LAB against the test Microorganisms**

| Organisms | L1 | L2 | L3 | L4 |
|---|---|---|---|---|
| *E.coli* | 12mm | 10mm | 12mm | 13mm |
| *s.aureus* | 14mm | 12mm | 10mm | 13mm |
| *B.subtilis* | 14mm | 14mm | 10mm | 10mm |
| *S.dysentriae* | 10mm | 10mm | 10mm | 10mm |

**Table 3: Resistant activity of isolates Antibiotic**

| Spectrum of Antibiotics | L1 | L2 | L3 | L4 |
|---|---|---|---|---|
| Ampicillin | R | R | R | R |
| Penicillin | R | R | R | R |
| Tetracycline | S | R | S | R |
| Erythromycin | S | R | R | R |
| Amphotericin | R | R | R | R |

## SUMMARY AND CONCLUSION

IN the present study a total of 4 LAB isolates were isolated from different dairy products (milk, yoghurt and cheese). From the conventionalmorphological and biochemical procedures fromthe isolates were found to be the species of *Lactobacillus, Leuconostoc, Lactococcus & Streptococcus.* So the study reveals the presence of probiotics organisms in the dairy products. In the present work the antagonistic activity of the isolated probitic organisms were determined against the food-borne pathogenic bacteria. The result of the work showed that the probiotic organism have strong, moderate & some gave weak inhibition against the test pathogens. Then probiotic organisms were tested for their resistance against 5 synthetic antibiotics namely tetracycline, erythromycin, amplicillin, penicillin & amphotericin. From the result, it clearly reveals that the probiotic strains showed significant resistant activity against the antibiotics. From this it is clearl that the effect of antibiotics to gut microflora can be prevented by the use of probiotic microorganism. The usage of dairy products will be an efficient alternative to prevent food-borne infections and the use of synthetic antibiotics.

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SP-17

# FERMENTATION OF CARROT JUICE WITH PROBIOTIC ORGANISMS ISOLATED FROM MILK SAMPLE AND STUDYING ANTAGONISTIC ACTIVITY OF FERMENTED CARROT JUICE AGAINST ENTEROPATHOGENIC BACTERIA

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## ABSTRACT

Lactic acid bacteria are a group of bacteria that can preserve dairy foods by producing several organic compounds that are antagonistic to other microorganisms. Much research has focused on utilizing bacteriocins as novel food preservatives, but there is also interesting using them for the control of bacterial diseases in humans and animals. Apart from the above-mentioned medicinal importance of *Lactobacilli*, it also helps in the control of intestinal pathogens. Lactic acid bacteria exert strong antagonistic activity against many microorganisms including food spoilage organisms and pathogens. Also, some strains may contribute to the preservation of fermented foods by producing bacteriocins. Some microorganisms are also active against Gram-positive food bore pathogens such as *Listeria monocytes*, *Staphylococcus aureus, Bacillus subtilis* and spores of *Clostridium perfringens*. For this reason, they have received much attention for use as natural or so-called "Biopreservatives" in food in recent years. The lactic acid fermentation of vegetable products applied as a preservation method for the production of finished and half-finished products is considered as an important technology. Totally two isolates *(Lactobacillus* and *Leuconostoc)*were isolated and identified from raw cow milk sample and they were used to ferment the carrot juice. Determination of the antagonistic activity of unfermented carrot juice and fermented carrot juice was done by using enteropathogenic bacteria (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Shigella dysentriae*). The fermented carrot juice showed significant antagonistic activity than unfermented. The fermented carrot juice showed strong inhibition against *Shigella,* moderate inhibition against *Escherichia coli* and weak inhibition against *Staphylococcus aureus.*

**KEYWORDS:** Lactic acid bacteria, Antagonistic activity, Biopreservatives and Fermented carrot juice.

## INTRODUCTION

Lactic acid bacteria have been used for thousands of years in the food and alcoholic fermentations. Lactic acid bacteria produce various compounds such as organic acids, Diacetyl hydrogen peroxide and bacteriocins or bactericidal proteins during lactic fermentations. The antimicrobial effect of lactic acid bacteria has been appreciated by man for more than 10000 years and has enabled him to extend the shelf life of many foods through fermentation process[1] (The most important role of lactic acid bacteria is its protective role against infections and colonization of pathogenic microorganisms in the digestive tract. In most of the cases, inoculums passively transmit the gastrointestinal tract. The probiotics can influence unspecific immunity, which consists of T-lymphocytes and B-lymphocytes. The increase in the specific response corresponds with the activity of B and T- lymphocytes, which leads to an increase of interleukin and the level of circulating antibodies. [2] Lactic acid bacteria were first discovered by Scheele[3]**).** From sour milk**,** Pasteur discovered 1875, that the souring of milk was caused by the microorganism. Lactic acid bacteria have been widely used for the fermentation of many fermented products such as cheese, sourdough, buttermilk, brine, vegetables, yogurt and sauerkraut.[4] Dairy products such as curd, cheese, whey, yoghurt[5]**.** Anti-cholesterol, Anti-carcinogenic activities, and protection against other diseases[6]**).** Fermentation by starter cultures that are added into raw materials by starter culture[7]). Fermentation takes place at the temperature of about 20-30[08.] Fermentation is a traditional way to preserve vegetables nowadays it is not common to use fermented vegetables in the western diet as food preservation utilizes

pasteurization, sterilization, refrigeration, freezing and preservatives however such near sterile food does not contain microbes that may be valuable to our health. Fermented dairy products are the main source of food microbes in the western diet and dairy products are the most common food matrix for administering probiotics some populations do not consume milk products out of the principle or due to lactose intolerance. Fermentation makes a certain vegetable product.

## MATERIALS AND METHODS

### COLLECTION OF SAMPLES

Raw cow milk samples were collected from lactating cow in the surrounding area of Kumbakonam. The sample was collected using sterilized sample bottles and brought to the laboratory with a refrigerator around $4^0$C till the analysis begins.

### ISOLATION OF LACTIC ACID BACTERIA FROM MILK SAMPLE

For isolation of lactic acid bacteria was homogenized with sterilized peptone water physiological saline solution (1% peptone 0.9% NaCl) for about 1-3 minutes aseptically appropriate serial dilution ($10^{-1}$ to $10^{-6}$) was prepared for the sample using 1 milliliter of homogenate. A volume of 0.1 milliliters of appropriate dilution was spread plate was incubated for 48 hours in an anaerobic jar at $32^0$ C typical LAB characteristics colonies were randomly picked up and purify by streaking two or three times on fresh MRS agar plates followed by macroscopic and microscopic examination. The colonies displaying the general characteristics of lactic acid bacteria gram-positive and catalase-negative were chosen from each plate for physiological and biochemical. For the identification of lactic acid bacteria, overnight cultures of each isolate in MRS broth were used. The identity of isolates was confirmed through various morphological and biochemical.

### IDENTIFICATION OF BACTERIAL STRAINS

Isolates were tested for catalase activity. Gram-staining reaction and cell morphology. All Gram-positive and catalase-negative rods were tested for growth in MRS broth at $15^0$ C and $45^0$C and production of gas from glucose. Gram-positive cocci were identified according to Hardie[9]. The strains were tested for the production of acids from carbohydrates and related compounds. The tests were done and the results were read after incubation of strains at $37^0$C for 2 and 3 days.

### PRODUCING AND FERMENTING CARROT JUICE

The carrot was purchased from the local market. After peeling homogenized skin fewer slices of carrot. The fresh carrot juice was filtered through a cloth, pasteurized 30 s in a water bath in a glass bottle (500 ml) at $72^0$ C and centrifuged (16000 g, 5 minutes).LAB isolates (IS-1 AND IS-2) were precultured anaerobically at $37^0$ C overnight in MRS broth. Carrot juice was inoculated separately (1% v/v final concentration approximately $10^7$CFU/ml), with two cultures. Fermentation was performed anaerobically for 18 hours at $37^0$ C. After fermentation, the juice was divided into polypropylene tubes (50 ml) for each sampling time point and stored to dark at $4^0$ C. Viability of probiotic were studied by culturing on the MRS medium subsequently after 1,2,4,8 and 12 weeks storage. The growth of *Lactobacilli* in MRS medium was monitored.

### ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA AGAINST PATHOGENIC BACTERIA

Isolated LAB (isolated were designed as IS-1 and IS-2) were maintained in sterile MRS broth. The strains of LAB were screened for their ability to inhibit the growth of *Escherichia coli, Staphylococcus aureus, Salmonella, Bacillus subtilis, Shigella*. The antimicrobial activity was determined by the disc diffusion method. Melted nutrient agar was inoculated with 1% of an 18-24 hours old broth culture of the test organisms. Eight milliliters of this seeded agar was poured into sterile Petri dishes and allowed to solidify. A sterile filter paper disc of 6.25mm diameter was dipped into the LAB culture and disc placed on the seeded agar surface. Several discs could be placed on the plate. Each test culture was assayed in duplicate. The plates were incubated at $5^0$ C for 2 hours to allow the test material to diffuse into the agar and then incubated at $37^0$ C for 16-18 hours. After incubated the plates were examined for the zone of inhibition around the various disc. Very strong inhibition (++++) was assigned to zones of 20-25 mm diameter

correspondingly, Strong inhibition (+++) 15-18 mm diameter, Moderate inhibition (++) 12-14 mm, Weak inhibition (+) 9-10 mm diameter, No inhibition (+_) cases were also recorded.

## RESULTS

### ISOLATION OF LACTIC ACID BACTERIA IN MRS MEDIUM

Totally 8 isolates were obtained from raw cow milk samples collected randomly and the isolates were tested for catalase-negative and gram-positive property of these 2 were found to be lactic acid bacteria. These isolates were made pure culture in the MRS agar medium. This isolates shown in the isolate-1 and isolate-2.

### IDENTIFICATION OF LACTIC ACID BACTERIAL ISOLATES THROUGH MORPHOLOGICAL AND BIOCHEMICAL TEST

Identification of bacterial isolate through a morphological and biochemical test. The isolates were gram-positive rod a non-motile, both catalase show negative, Morphological, physiological and biochemical characteristics of isolates were shown in table-1 with these the isolates were grouped into *Lactobacillus* and *Leuconostoc species.*

### FERMENTATION OF CARROT JUICE WITH ISOLATED PROBIOTIC STRAIN

*Lactobacilli were* present in higher numbers in carrot juice. *Lactobacilli* fermented carrot juice well and survival in the fermented carrot juice for up to 12 weeks. Before fermentation, the pH value carrot juice was 6.7. Carrot juice fermented with *Lactobacilli* had a lower pH value (3.8-3.9).

### ANTAGONISTIC ACTIVITY OF PLAIN CARROT JUICE AND FERMENTED CARROT JUICE AGAINST THE ENTEROPATHOGEN

The antimicrobial activity of plain carrot juice and fermented carrot juice against *Escherichia coli, Bacillus subtilis, Shigella dysentriae*was observed by disc diffusion method, and the result was given in the table-1, it was observed that all of the plain carrot juice and fermented carrot juice possessed significant antagonistic activity against test pathogens. The plain carrot juice showed moderate inhibition (Zone of inhibition 12-14 mm) against *Escherichia coli.* The plain carrot juice and fermented carrot juice showed weak inhibition (zone of inhibition 10-11 mm) against *Shigella.* The fermented carrot juice showed moderate inhibition (Zone of inhibition 12mm) against *E.coli.* The fermented carrot juice showed weak inhibition (Zone of inhibition 12-14mm) against Staphylococcus *aureus*. The plain carrot juice and fermented carrot juice showed strong inhibition (Zone of inhibition 13-14mm) against *Shigella.*

**TABLE-1: ANTIMICROBIAL ACTIVITY OF DIAMETER OF GROWTH AND ZONE OF INHIBITION**

| SAMPLE | *Shigella* | *Escherichia coli* | *Staphylococcus aureus* | *Bacillus subtilis* |
|---|---|---|---|---|
| **ISOLATE-1, FRESH CARROT JUICE** | 10 mm | 12 mm | 11mm | 13 mm |
| **ISOLATE-2, FERMENTED CARROT JUICES** | 11mm | 14mm | 12mm | 14mm |

## SUMMARY AND CONCLUSION

In the present investigation, the antagonistic activity of the fermented carrot juice was determined against the enteropathogenic organisms. The fermented carrot juice showed strong moderate and weak inhibition against the test pathogen, so the study reveals that the fermented carrot juice can be given as a preventive and therapeutic measure against the enteropathogenic organisms.

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SP-18

# ISOLATION AND IDENTIFICTION OF MICROORGANISM FROM DENTAL CARIES SAMPLE AND STUDYING THE ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ACETONE EXTRACTS OF NEEM, GARLIC, CINNAMON, CLOVE BUD.

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## ABSTRACT

Dental caries is an infectious microbiological disease of the teeth that results in localized dissolution and destruction of the calcified tissues. It is the second most common cause of tooth loss and is found universally, irrespective of age, sex, caste, creed, or geographic location. It is considered to be a disease of civilized society, related to lifestyle factors, but heredity also plays a role. In the late stages, it causes severe pain, is expensive to treat and leads to loss to loss of precious man-hours. However, it is preventable to an expensive to treat and leads to loss of precious man-hours. However, it is preventable to a certain extent. The prevalence of dental caries in India is 50%-60%. The oral cavity is a dynamic environment that undergoes rapid and often substantial changes in PH, nutrient availability, carbohydrate source and oxygen process in tension. Despite the constant environmental fluctuation, it has been estimated that over 500differentbacterial species colonize old tissues, colonize old tissues, co-existing as the complex population in biofilms. Among the most abundant microorganism in the mouth, oral streptococci are primarily associated with the disease. A total of three isolates *(streptococcus mutants, Candida albicans,. Lactobacillus acidophilus)* were isolated and identified from the dental caries sample. Four medicinal substances(Neem, Garlic, Cinnamon, Clove) were used to determine the antimicrobial activity against the isolated organism from dental caries sample both aqueous and acetone extract of all the four medicinal substances showed significant antimicrobial activity against the test organism.

**KEYWORDS**: Dental caries, oral pathogens, medicinal plants, antimicrobial activity.

## INTRODUCTION

Dental caries is the predominant cause of tooth loss in children and young adults. Although the disease most commonly affects the crown of the tooth, caries of the tooth root is also prevalent in older populations. Caries of the crown of the tooth initially presents as a white spot in the enamel and dentine. As caries progresses, more extensive destruction of the enamel and dentine occurs, followed by inflammation of the pulp and peripheral tissues. Dental plaque is a thin, tenacious microbial film that forms on the tooth surfaces. Microorganism in the dental plaque ferment carbohydrate foodstuffs, especially the disaccharide sucrose, to produce acids that demineralization of inorganic substances and furnish various proteolytic enzymes to cause disintegration of the organic substances of the teeth, the processes involved in the initiation and progression of dental caries. *Streptococcus mutants* and *Streptococcus sobrinus*, two species of the *'mutant streptococci'* are the most significant in human caries [1,2,3]. There is also a strong association between *Lactobacillus spp*. And caries but little is known of the relative significance of the different species. *Candida Albicans* yeast is a common microflora in the oral cavity. Some factors that could cause the *C.albicans* into pathogenic microbes include optimum temperature($37^0$c), the presence of serum, a high PH environment and an adequate carbon source[4]. The yeast plays an important role in oral health; thus, its presence needs serious attention because the resulting infection can lead to oral thrush (oral candidiasis). Infection can be more severe when.*albicans* penetrates the deeper tissues, especially for immunocompromised patients. Superficial infection may change to invasive (invasive Fungal infections, IFI) [.5] Cloves, Neem, Garlic and Cinnamon are having the antiquity and major antimicrobial components. Plants have been a source of herbal remedies throughout the history of mankind. Plants are the primary

source of medicine. The antibacterial activity of species may differ between strains within the same species. Azadirachta indica (A.indica) A Juss (syn. Melia Azadirachta) belonging to the Meliaceae family commonly known as neem is oneof the most versatile medicinal plants having a wide spectrum of biological activity. Garlic (Allium sativum) is one of the most extensively researched medicinal plants and typical order and antibacterial activity depend on allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyse) on allin after crushing or cutting garlic clove. Cinnamon has been reported to be eugenol and cinnamaldehyde, respectively which have been given special attention to finding their antibacterial activity against foodborne pathogens[6].

## MATERIALS AND METHODS

### COLLECTION OF SAMPLE

The samples were collected from various cavities of affected teeth by scraping soft caries using sterile cotton swabs from four human volunteers. After collection, the cotton was dropped into 20ml of peptone broth which was used as transport media.

### ISOLATION OF BACTERIA AND FUNGI FROM SAMPLE

Nutrient agar medium and blood agar medium were prepared, sterilized and inoculated with the sample. After incubation Bacterial colonies were observed. Selected colonies of two isolates (IS-1,IS-2) were separated. Their colony morphology was studied and pure culture was made.Seaboard Dextrose agar was prepared, sterilized and inoculated with sample. After incubation bacterial colonies were observed. Selected colonies of one isolate (IS-3) were separated. The colony morphology was studied and pure culture was made

### IDENTIFICATION OF BACTERIAL ISOLATES

Preliminary identification was performed using gram staining, spore staining, motility test(hanging drop technique). Biochemical tests: Indole test, methyl red and vogesproskeur test, triple sugar iron agar test, carbohydrate fermentation test, citrate utilization test,catalase test, oxidase, test, urease test. Further identification was made by culturing in selective medium blood agar medium was prepared and IS-1 was inoculated. The plates were incubated at $37^0$c for 24-48 hours and observed for the presence or absence of hemolysis. MRS agar medium was prepared & IS-2 was inoculated. The plates were incubated at $37^0$c for 24-48 hours. Colony morphology was observed. The colonies formed in the selective medium were further confirmed by standard morphological and biochemical methods.

### IDENTIFICATION OF FUNGAL ISOLATE

The fungal colony (IS-3) picked up from the isolation procedure was screened. The colony morphology of IS-3 revealed the presence of *candida albicans* in the dental carried sample. It was confirmed by the standard confirmatory test (ie germ tube method) for *candida albicans* (Reynolds-Brude phenomenon).

### GERM TUBE METHOD

12/75mm test tube was labeled. 3 drops of fresh pooled human serum were dispensed into test tubes using sterile Pasteur pipet. The colony of IS-3 was lightly touched using a sterile wooden applicator stick & placed into the serum. The culture was suspended in the serum. The tube was incubated at $35^0$C for 2 1/2 -3 hours. After incubation, the drop of the suspension was placed on a clean microscopic slide. A clean cover glass was placed over the suspension. Then the slide was examined under a microscope for the presence or absence of a germ tube.

### PREPARATION AQUEOUS AND ACETONE EXTRACTS

The medicinal plant parts including clove(Syzygium aromaticum), twigs of neem(Azadirachta indica), Garlic(Allium sativum), cinnamon(Cinnamon zeylanicum) were purchased from a local market. They were washed with distilled water thoroughly. Garlic/(100gm) was washed first by distilled water and then by 95% ethanol. Garlic was homogenized using sterile motor and pestle. And then sieved through a double layer of sterile fine mesh cloth to make 100% extract. Others clove, twigs of neem, cinnamon (100gm each) were crushed and sieved through mesh cloth to get the fine powder. These powders were soaked in 200ml of distilled water and were kept at room temperature for 24 hours, then were filtered using Whatman no.1 filter paper. The filtrate was heated at 40-$50^0$C using a water bath until the thick paste was formed.

The thick paste was considered as 100% concentration of extract. These extracts were stored at $4^0$C in the refrigerator. The Acetone extract was prepared following the same procedure except solvent which was 95% Acetone instead of sterilized distilled water.

**ANTIMICROBIAL SENSITIVITY USING DISC DIFFUSION METHOD**

Filter disc of 5mm diameter using Whatman no.1 filter paper was prepared and sterilized. The isolates and fungal isolates microorganisms were transferred from the medium to sterile Muller Hinton agar plates with the help of sterile cotton swabs. Using an Ethanol dipped and flamed forceps the discs were aseptically placed over the Muller Hinton agar plates seeded with the microorganism. 10micro1 of the various extracts i.e Acetone extracts and aqueous extract were aseptically transferred to each disc that was made in triplicate. For bacterial isolates, 10 micro1 of 95% Chlorhexidine was added in sterile filter paper disc as [positive control. 10 micro 1 of 95% Acetone was added in sterile filter paper disc as a negative control. For fungal isolates 10 micro1 of 95% Penicillin was added in sterile filter paper disc as a positive control. 10 micro1 of 95% Acetone was added in sterile filter paper disc as a negative control. Triplicate sample of each dilution was tested. After 24 hours the diameter of the zone of inhibition were measured in mm and results were recorded.

**RESULT**

Various morphological and biochemical characteristics of isolated bacteria and fungi the dental caries sample were given in (Table:1) were classified as three strains such as (*streptococcus mutants, Candida albicans , Lactobacillus acidophilus).* The colony morphology of IS -1 was gram-positive. Cells are spherical or ovoid 0.5-2πm. It occurring in pairs or chains. They are sometimes lancet in shape. Nonmotile, nonsporing facultative anaerobes, chemoorganotrophs. The colony of the IS-2 strain was white in color, big in size, concave, and circular. IS-3 was sabourauds dextrose agar colonies are white to cream-colored, smooth, glabrous, and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like or blastoconida ,2.0-7.0$\times$ 3.0 – 8.5$\pi m$. Antibacterial properties of clove have already been reported), but in the present study, we tested the antibacterial as well as the antifungal activity of clove and its oil against dental caries microorganism. The results of antimicrobial activities of acetone and aqueous extracts of clove buds and as well as the positive control chlorhexidine (for bacteria ) and penicillin (for fungi) are presented in table 1 and the MIC of the five extracts as well as clove oil against the test pathogens are presented in table 2. The antimicrobial activity of clove bud extracts on the agar plates varied greatly in different solvents. Both the positive control produced significantly sized inhibition zones against the test bacteria (chlorhexidine0 and yeasts (Penicillin). However the negative control produced no observable *streptococcus mutants* inhibitory effect. Of the two extracts screened for antimicrobial activity, both showed antibacterial activity against *S.mutants*. Showing the highest zone of inhibition (22.65mm) followed by the cold aqueous (20.32mm), acetonic extract (14.65mm). The aqueous extract of clove but showed 20.65+0.57mm zone of inhibition against *L.acdiophilus* and for yeast 0 mm. The acetonic extract showed 14.65+0.57mm zone of inhibition against *L.acdiophilus* and for yeast 0mm. The aqueous extract of neem showed a 20.65+0.57mm zone of inhibition against *L.acidophilus* and for yeast 17+0.52mm. the acetonic extract showed 10mm zone of inhibition against *L.acidophilus* and for yeast 0mm. the aqueous extract of neem inhibition the growth of both bacterial and fungal isolates. The maximum activity was noted against *Klebsiella pneumonia* (8mm), *Bacillus* (7mm), *Escherichia coli*, and (6mm*)*and minimum antibacterial activity against *Bacillus cereus, Escherichia coli, Salmonella typhi by methanal.* The maximum activity was noted against *lactobacillus acidophilus* (10mm)followed by. *Streptococcus mutants* (10mm) and *Candida albicans* (5mm).

The aqueous extract of garlic inhibition the growth of both bacterial and fungal isolates. The maximum activity was noted against *Klebsiella pneumonia* (8mm), *Bacillus* (7mm), *Escherichia coli, Salmonella typhi* by minimum antibacterial activity against *Bacillus cereus, Escherichia coli*, *Salmonella Typhi* by methanal. The maximum activity was noted against *Lactobacillus acidophilus* (18$\pm$0.38mm) followed by *Streptococcus mutans* (20.32$\pm$0.57mm) and *Candida albicans* (5mm). The acetone extract of garlic exhibited a zone of 22$\pm$0.38mm towards *streptococcus mutants 24$\pm$0.52mm Lactobacillus acidophilus and* 24$\pm$0.38mm towards *Candida albican (6mm).* The aqueous extract of cinnamon showed 16+0.45mm zone of inhibition against *L. acidophilus* and for yeast 0mm. The acetonic extract showed a 17+ 0.52mm zone of inhibition against *L.acdiophilus* and for yeast 0mm. The aqueous extract of cinnamon inhibition

the growth of both bacterial and fungal isolates. The maximum activity was noted against *Klebsiella pneumonia* (8mm), *Bacillus* (7mm), *Escherichia coli* (6mm) and minimum antibacterial activity against *Bacillus cereus, Escherichia coli.*), *Salmonella typhi* by methanal. The maximum activity 16+0.45mm) and *candida albicans* (5mm) were noted against *Lactobacillus acid bacillus* (17+0.52) followed by *Streptococcus mutants*.

**TABLE 1: ANTIMICROBIAL ACTIVITY OF CLOVE AND CLOVE OIL AGAINST THE TEST MICROOGANISM**

| S.NO | DIAMETER GROWTH OF INHIBITION ZONES (mm) | | | |
|---|---|---|---|---|
| | Streptococcus mutants | | Lactobacillus acidophilus | Candida albicans |
| CLOVE | Acetone | 14.65[a]±0.57[b] | - | 20+0 |
| | Aqueous | 20.32±0.57 | - | - |
| NEEM | Acetone | 10 | 10 | 6 |
| | Aqueous | 6 | 6 | 10 |
| GARLIC | Acetone | 26±0.45 | 14±0.42 | 9.02 |
| | Aqueous | 24±0.52 | 16±0.45 | 7 |
| CINNAMON | Acetone | 14±0.42 | 10±0.36 | 5 |
| | Aqueous | 16± ±0.45 | 17±0.52 | 10 |

## SUMMARY AND DISCUSSION

This study demonstrates the antimicrobial activity of aqueous, acetone of clove neem, garlic, cinnamon the antimicrobial activity of aqueous, acetone of clove, neem, garlic, cinnamon the most causative agents of dental caries; *Steptococci mutants* and *lactobacilli* growth on dental caries samples from normal adults population. Our principal findings are: (Dhinahar S et al., 2011). All plant candidates have excellent antimicrobial activity on the growth of both bacteria types but their effects are more on *Lactobacilli* than*mutantstreptococci*. Current study demonstrated the comparison between four plants on the major causative pathogens off dental caries. This is an additional advantage in using these medicinal plants in preventive dentistry, because, in the search for these novel antimicrobial compounds, traditional plants have been proved to a better source. The advantage of traditional medicine is that it is less likely to form allergies and side effects. Nowadays because of their high antimicrobial, anti-inflammatory, anti-oxidant and biocompatible properties their use in dentistry is becoming more popular.

## CONCLUSION

From the present study, it has been concluded that the acetone and aqueous extracts of all the four medicinal plants showed significant antimicrobial activity against the dental carries causing organisms. Hence these plants can be used as novel antimicrobial compounds in preventive dentistry.

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# ISOLATION, CHARACTERIZATION AND EFFECTS OF ANTIBIOTICS AND DISINFECTANTS ON VARIOUS WOUND SAMPLES

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## ABSTRACT

The surgical wound may occur within 30 days after surgical operation. A surgical wound may get infected by the exogenous bacterial which may be present in the environmental air of operation theatre or by the endogenous flora. The rate of infection of surgical wounds is influenced by the duration of preoperative hospitalization, administration of prophylactic antibiotics, duration of surgery, whether it is emergency or elective. The organisms most frequently involved in surgical infections change from time to time. The present study shows the emergence of gram-negative *bacilli* as the principal offenders responsible for surgical wound infections. *Streptococcus pyogenes* is a dangerous pathogen penetrating the wound, producing lot of pain and exudates. Some of these organisms were checked either sensitive or resistant to antibiotics like ciprofloxacin, Amikacin, Gentamycin, Chloramphenicol, Norfloxacin, Tobramycin, Cotrimoxazole, Vancomycin, Lincomycin, Cefotaxime.

**KEYWORDS:** antibiotics, *Streptococcus pyogenes*, beta-hemolytic, predominant**,** microbial flora

## INTRODCTION

The wound is a break in tissues of the body. Wounds are mainly classified into types. They are open wounds and closed wounds. The breakage which causes in the external part of the body is termed as an open wound and the breakage which causes in the internal part of the body is termed as closed wounds. *Streptococcus pyogenes* is a Beta hemolytic streptococcus and it is the Lancefield group A most clinically important species. *Pseudomonas aeruginosa* produces a water-soluble fluorescent pigment, pyoverdin. The organism is mainly a soil and water saprophyte but it is also frequently an opportunistic pathogen and can often be isolated from wound and burn infections. Polymicrobial flora was observed in one case in which *Clostridium perfringens* was associated with *Klebsiella pneumonia*. In many cases, the common isolates were *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus* in various contaminations. Hence it is important to known the etiological agents institute specific therapy, to reduce morbidity and mortality[1]. *Pseudomonas aeroginosa* is an opportunistic pathogen that not only posses a threat to burn patients but also causes significant mortality and morbidity in cystic fibrosis and immunocompromised patients. A factor that contributes to the persistence of methicillin-resistant *staphylococcus aureus* colonization or infection includes the type of surgery, length of hospital stays and admission of infected patients from other wards or hospitals[2]. *Klebsiella pneumonia* is not an intracellular pathogen and hence iron is not readily accessible to this organism in the body. To circumvent their invivo iron restriction *klebsiella pneumonia* produces siderophores i.e enterochelin and aerobactin, which are capable of removing iron from the host iron-binding proteins. Siderophores are the soluble, low molecular high-affinity iron-chelating compounds produced by organism[3]. Isolates of *Escherchia coli* obtained from pus were serum sensitive and were subjected to the sensitivity of the complement-mediated lysis by serum using the plaque technique. *Pseudomonas aeruginosa* was found to be the commonest isolate from various samples with a pyogenic infection like oral swab samples, umbilical swabs, axillary swabs, drainage toe, burn tissue swabs and wound swabs. *Staphylococcus aureus* is the leading cause of nosocomial infections. More than 50% of these infections are caused by methicillin-resistant *Staphylococcus aureus*. *Staphylococcus pyogenes* and *Clostridial infections* within two days, while *Staphylococcal* infections

typically take four or five days and gram-negative bacillary infections take six or seven days to appear. Nonsurgical wound infections cut downs, umbilical stumps, ulcers, burns. *Pseudomonas aeruginosa* is the most important cause of infection in burns.

## MATERIALS AND METHODS

### SAMPLING AREA

The samples for bacteriological screening were taken from Government Hospital at Kumbakonam, where several patients with burn wounds, surgical wounds, pressure sores, and accidental wounds are coming for treatment.

### SAMPLE COLLECTION

Wound samples were collected from various sites of different patients. The wound site of the patients should be decontaminated with surgical soap and 70% ethyl or isopropyl alcohol to prevent the contamination from the environment[4].

### BACTERIOLOGICAL ANALYSIS

### HANGING DROP METHOD

Motility of bacteria is identified using a loopful of overnight broth culture by hanging drop method.

### GRAM STAINING

Gram staining was done with a standard procedure manual.

### BIOCHEMICAL TEST

**A biochemical test involves**

Indole production test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple sugar iron agar test, Gelatin hydrolysis, starch hydrolysis, Catalase test, Urease test, Lipid hydrolysis, Coagulation test, Bile Esculin test.

**TABLE-1. TYPES OF WOUNDS AND PERCENTAGE OF SAMPLE C0LLECTION**

| Type of wound | Total | Male(35) | | Female (35) | |
|---|---|---|---|---|---|
| | | No | Percentage | No | Percentage |
| Accidental | 40 | 20 | 50 | 20 | 50 |
| Surgical | 26 | 13 | 50 | 13 | 50 |
| Burns | 2 | 1 | 50 | 1 | 50 |
| Pressure sore | 2 | 1 | 50 | 1 | 50 |

**TABLE-2. PREVALENCE OF BACTERIAL FLORA IN WOUND**

| Type of wound | Total | Male(35) | | Female (35) | |
|---|---|---|---|---|---|
| | | No | Percentage | No | Percentage |
| Accidental | 40 | 20 | 50 | 20 | 50 |
| Surgical | 26 | 13 | 50 | 13 | 50 |
| Burns | 2 | 1 | 50 | 1 | 50 |
| Pressure sore | 2 | 1 | 50 | 1 | 50 |

**TABLE-3. Percentage of the presence of bacterial population.**

| S. NO | Bacterial flora | No | Percentage |
|---|---|---|---|
| 1 | *Staphylococcus aureus* | 36 | 51.3 |
| 2 | *Streptococcus pyogenes* | 18 | 25.7 |
| 3 | *Pseudomonas aeruginosa* | 23 | 32.8 |
| 4 | *Escherichia coli* | 9 | 12.7 |
| 5 | *Klebsiella pneumonaie* | 39 | 55.1 |

## DISCUSSION

In the present study out of 70 samples, 32.8% is *pseudomonas*. *Klebsiella pneumonia* was the most frequent isolate causing surgical wound infection, followed by *staphylococcus aureus* and *pseudomonas aeruginosa*. All the isolates were resistant to the commonly used antimicrobial agents.[5] In the present study, *Klebsiella pneumonia* predominates with 55% and *Staphylococcus aureus* comes next with 51.3%. *Pseudomonas aeruginosa* has been isolated from 23 cases with 32.8%., *Streptococcus pyogenes* isolated from 18 cases with 25.7% and *Escherichia coli* shows less percentage with 12.7%. On examining phenol co-efficient, Dettol has very good antimicrobial activity. Salvon is good and Lysol is less applicable. Cefotaxime inhibits transpeptidation enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall peptidoglycan, and activates cell wall lytic enzymes. Gentamycin binds with the 30s subunit of the bacterial ribosomes to inhibit protein synthesis and cause misreading of mRNA. Chloramphenicol binds to the ribosomal subunit and blocks peptidyl transferase. Kanamycin is an antibiotic derived from *Streptomyces,* with activity against a very broad spectrum of bacteria, including gram-positive and gram-negative Cocci and many other gram-negative rods. Most strains of *Enterobacter* are sensitive to kanamycin. Lincomycin is sensitive to gram-positive organisms and resistant to gram-negative organisms. Ciprofloxacin is effective as all organisms are sensitive to these antibiotics. Amikacin is resistant to gram-positive organisms and sensitive to gram-negative organisms. Norfloxacin is resistant to positive organisms and resistant to negative organisms. Cotrimoxazole and tobramycin are resistant to positive organisms and sensitive to negative organisms. Vancomycin is sensitive to positive organisms and resistant to negative organisms.

## SUMMARY AND CONCLUSION

Wound infections being a hindrance to patients in their daily life it is necessary to investigate. Serious complications may occur to neglected wounds and, at times, to even the best cared-for wounds. If we notice any of the signs when examining wounds of injuries, we should see a doctor immediately. The signs are redness, excessive swelling, tenderness, or increased warmth of the skin around the wound. From 39 culture-positive cases, which did not yield growth of any anaerobe, the common isolates were 14 *klebsiella pneumonia,* 15 *Escherichia coli* and 5 *staphylococcus aureaus*[6&7]. In the present study from 70 samples 36 *Staphylococcus aureus*, 39 *Klebsiella pneumoniae* and 9 *Escherichia coli.* All these three isolates were susceptible to metronidazole, 39 penicillin chloramphenicol, erythromycin, and tetracyclin[6]. In the present study, *Escherichia coli* is resistant to vancomycin, chloramphenicol Lincomycin, cefotaxime and sensitive to the remaining antibiotics that are used. *Klebsiella pneumonia* is resistant to Lincomycin, chloramphenicol, vancomycin and cefotaxime.

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SP-20

# THE CALLUS INDUCTION AND ANTIBACTERIAL ACTIVITY STUDIES ON *Centella Asiatica*

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## ABSTRACT

The present study shows the phytochemical analysis and antibacterial activity of leaf and callus of *Centella Asiatica*. Leaf explants of *C. Asiatica* were cultured on MS medium supplemented with different concentrations of plant growth regulators for callus initiation. In the preliminary phytochemical screening, alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins, and reducing sugars were present in most of the tested extracts of leaf and in vitro grown callus of C. Asiatica. Methanol, acetone, chloroform and water extracts of leaf and callus were evaluated for in vitro antibacterial activity against Bacillus cereus, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa by agar plate well diffusion method. All the extracts from leaf and callus of *C. Asiatica* showed significant antibacterial activity against the tested organisms.

**KEYWORDS:** *Centella Asiatica*, callus, antimicrobial activity, phytochemicals, diffusion technique.

## INTRODUCTION

*Centella* can also be propagated by seeds set in damp soil. The plant enjoys a considerable reputation in the Indian system of medicines as a brain tonic. It is also used for the treatment of asthma, bronchitis, dropsy, elephantiasis, gastric catarrh, kidney troubles, leucorrhoea, skin disease and urethritis[1] with antibacterial, anti-feeding, ant-filarial, anti-stress, anti-tuberculosis activities and wound healing properties[2,3 & 4]. The leaves are commonly employed, but the use of the whole plant is recommended[5]. In vitro, culture techniques offer a viable tool for mass propagation of plants and conservation of rare, threatened, and endangered germplasm (Rao, 2004). In vitro, culture studies on Centella have reported high incidences of microbial contamination, which drastically reduced explant survival[6]. Micropropagation of *C. asiatica* through leaf and nodal explants has been reported earlier [6&7], and there is one report of somatic embryogenesis[8]. Plant regeneration through shoot morphogenesis is considered to have a multicellular origin that may result in the formation of genetically variable, chimeric plants. In contrast, somatic embryos are considered to arise either directly or indirectly from single cells and can largely to genetically stable nonchimeric plants [9&10.]

## MATERIALS AND METHODS

### Site of collection

For callus induction, explants were collected from the wildly growing plant population of *Centella asiatica* in Idhaya College Herbal Garden at Kumbakonam, Thanjavur District. The wound infection sample was collected by Government Hospital at Kumbakonam, Thanjavur District.

### Sterilization of explants

Sterilization was carried out with standard chemicals with procedures.

**Induction of callus**

For the induction of callus, thee nodal explants were used. These explants were inoculated in different media with different concentrations of growth hormones. The callus induction was tested on various concentrations and combinations of BAP and NAA (0.5 to 3 mg/1) each. The induced callus was a subculture in intervals of 30 days on the MS medium containing different concentration of NAA (0.5 to 3 mg/1) and BAP (1.0 mg/1)

**Antibacterial study**

**Plant collection**

Plant materials were collected from Idhaya College Herbal Garden at Kumbakonam, Thanjavur District. The freshly collected leaf, stem, and root materials were dried in shaped at 30oC for 10 days, ground to a fine powder and stored in airtight bottles at 4oC.

**Preparation of extract**

The shade dried and powdered plant materials were extracted with methanol and chloroform using a soxhlet apparatus, while water extract was centrifuged at 5000 rpm and the supernatant was taken.

**Microscopic examination**[11]

The microbes motility was determined by the hanging drop method and by gram staining method determine the staining nature of isolated microbes.

**Hanging drop method**

The motility of bacteria is identified using a loopful of an overnight broth culture of the isolate by hanging drop method.

**BIOCHEMICAL TEST**

Biochemical test such as Indole Production Test, Methyl Red Test, VP-Test, Citrate Utilization Test, TSI Agar test, Gelatin hydrolysis, Catalase test, Urease Test, Starch Hydrolysis, Inoculum preparation, Antibacterial sensitivity test were performed.

## 4. RESULT

**Callus induction**

**Initiation and maintenance of aseptic culture**

Culture of explants from field-grown plants encountered with contamination, phenolic secretion and poor regeneration response. To overcome this problem, different sterilizing agents and the addition of charcoal powder (1.0%) to the culture medium were carried out. The addition of charcoal powder to the primary culture medium reduced the secretion of phenol from the explants. Callus response of explants collected from widely growing plants was found very low. Hence, the callus obtained from wild explants subculture on medium containing NAA and they give Bulky friable callus and it was used for the antibacterial study.

**Effect of hormones**

When the BAP and NAA were added in the culture medium in increasing concentration from 0.5 to 3.0 mg/l, the explants showed growth range from compact to bulky callus was formed. BAP and NAA at lower concentrations the nodal explants shown less response of callus formation. The optimum concentration of cytokinins with auxins, which initiated callus with a high percentage of responses were selected for this antimicrobial study. The use of different concentrations of BAP and IBA to produce callus was not encouraging as they produced a negligible amount of callus. The percentage of callus induction was very low in the higher concentration of NAA and BAP. The highest response (80%) in callus formation was observed in MS medium supplemented with 1.5 mg/l of NAA and 1 mg/l of BAP (plate 1). The growth of callus increased significantly with the increase in the incubation perio up to 30 days (table 1).

**TABLE-1. ANTIBACTERIAL SCREENING OF THREE DIFFERENT CRUDE PLANT EXTRACT OF C.*asiatica***

| ORGANISMS | METHANOL EXTRACT | CHLOROFORM EXTRACT | AQUEOUS EXTRACT | CONTROL |
|---|---|---|---|---|
| ***Streptococcus mutans*** | 21 | 11 | 11 | - |
| ***Staphylococcus aureus*** | 23 | 17 | 22 | - |
| ***Escherichia coli*** | 15 | 23 | 11 | - |
| ***Proteus mirabilis*** | 9 | 5 | 19 | - |
| ***Klebsiella pneumoniae*** | 23 | 22 | 21 | - |

**Table: 2. CALLUSING RESPONSE OF NODAL EXPLANTS ON THE MEDIA CONTAINING VARIOUS CONCENTRATION OF BAP AND NAA.**

| AUXIN (mg/l) NAA | CYTOKININS (mg/l) BAP | CULTURE SHOWING RESPONSE (1%) | NATURE OF CALLUS |
|---|---|---|---|
| 0.5 | 1.0 | 24 | Compact very slow growth |
| 1.0 | 1.0 | 59 | Compact, slow growth |
| 1.5 | 1.0 | 89 | Friable bulky, chlorophyllous |
| 2.0 | 1.0 | 49 | Compact, pale green |
| 2.5 | 1.0 | 39 | Compact, slow growth |
| 3.0 | 1.0 | 19 | Poor growth |

**Table 3. ANTIBACTERIAL SCREENING OF THREE DIFFERENT CALLUS EXTRACT OF *Centella Asiatica.***

| ORGANISMS | METHANOL EXTRACT | CHLOROFORM EXTRACT | AQUEOUS EXTRACT | CONTROL |
|---|---|---|---|---|
| *Streptococcus mutans* | 9 | 12 | 4 | - |
| *Staphylococcus aureus* | 19 | 21 | 21 | - |
| *Escherichia coli* | 23 | 15 | 19 | - |
| *Proteus mirabilis* | 17 | 9 | ¤ 9 | - |
| *Klebsiella pneumoniae* | 15 | 13 | 10 | - |

**ISOLATION OF MICROBES FROM WOUND INFECTION**

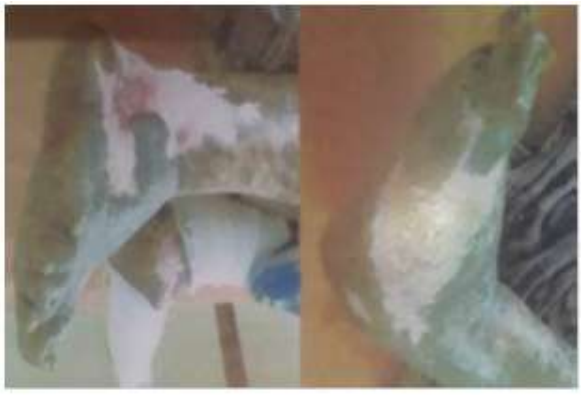

**Fig. 1. Patient legs with wound infection**

## 5. DISCUSSION AND SUMMARY

### CALLUS INDUCTION

The importance of this part of the study was the preparation of contamination free cultures by using explants from one-year-old wildly growing plants. For sterilization, the explants were soaked in detergent solution (Teepol 5%) for 10 minutes and thoroughly washed in tap water with double distilled water for 5 times. The explants were surface sterilized with 0.1 percent mercuric chloride (HgCl2) for 5 minutes. This treatment favored the maximum proliferation of explants and minimum contamination[12.]

### Effect of hormones

In nodal explants culture of *C.asiatica, a* low combination of cytokinins with auxin was found ideal for callus production.[13] Whereas in *Artemisia pallens* callus had been observed on the media conditioning individual auxin or cytokinins or combination of auxin and cytokinins. A combination of BAP and NAA induced the maximum amount of callus (Usha and Swamy, 1994). In *Sorghum bicolor,* Kn along with 2,4-D or NAA was found to be best suited for the production of the green compact and nodular callus. In the same experiment pale yellow callus was induced by individual 2,4-D or NAA was found. To be best suited for the production of the green compact and nodular callus. In the same experiment pale yellow callus was induced by individual 2,4-D.

## 6. SUMMARY AND CONCLUSION

(i) MS-medium with B5 vitamins were used for callus culture initiations.
(ii) 1.5 mg/l NAA and 1.0 mg/l BAP induce maximum callus induction and they were friable bulky and chlorophyllous.
(iii) The high concentration of auxins with low concentration of cytokinins of favored callus formation.
(iv) The antibacterial activity of the callus and wild plant crude extract was determined against six bacterial pathogens such as *S. mutans, S. aureus, E. coli, P. mirabilis,* and *K. pneumoniae*. (v) An organic solvent such as methanol, chloroform and aqueous solution were used

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# ISOLATION AND IDENTIFICATION OF MICROBES FROM NAIL SAMPLE AND ANTIDERMATOPHYTIC ACTIVITIES ON *Lawsonia inermis* (HENNA PLANT), DETTOL SOLUTION AND ALOE VERA (*Aloe barbadensis*) HERBAL SOAP

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## ABSTRACT

This study was conducted to isolate and identify bacteria contaminants of under nails (long nails. Bacterial pathogens isolated from the under nails of students include *Staphylococcus aureus Bacillus cereus*, *Acinetobacter, Bacillus, Streptococcus, Pseudomonas aeruginosa, Klebsiella.* The world is endowed with a rich wealth of medicinal plants. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones. Medicinal plants have been used to cure some diseases. The ancient plant *Lawsonia inermis* or henna is used as a medicinal plant because of its attributed strong fungicidal, anti-inflammatory, analgesic, antibacterial, virucidal, antiparasitic, antiamoebiasis, astringent, antihemorrhagic, hypotensive, sedative, anticancer effect and possible anti-sweating properties. In this study, we isolate, identify the pathogens from the nail sample and antidermatophytic activity of *Lawsonia inermis* and *Aloe barbadensis* was studied.

**KEYWORDS:** *Lawsonia inermis*, Antidermatophytic activity, *Aloe barbadensis*, Pathogens, Medicinal plants

## INTRODUCTION

Onychomycosis is a term derived from the Greek word "onyx" that means a nail and "makes, means a fungus. It is a fungal infection of nails and is amongst the most common causes of distorted nails, and accounts for almost 50% of all nail disease[1]. It represents about 30% of all mycotic cutaneous infections[2]. Increasingly onychomycosis is being viewed as more than a more ornamental problem. Despite better personal sanitation and living environment, onychomycosis continues to spread. The prevalence rate of onychomycosis is determined by age, predisposing factors, social condition, profession, climate, and living environment[3]. Fungal infections have become a significant health problem affecting all age groups. Correct diagnosis is important not only for initiating appropriate and timely treatment but also for the epidemiological record. The recognition of the pathogen helps the treatment approach and potential implementation of control measures. The prevalence of Dermatophytes, non-dermatophytes, and yeasts tends to vary with different times of the year as well as based on geographic location[4]. British society for medical mycology proposed standards for patients with invasive fungal infections. Dermatophytes are fungi capable of invading keratinized regions such as nails of human beings causing diseases known as dermatophytosis or dermatomycoses[5]. dermatomycoses due to, T.ruburum, Microsporum gypseum and Epidermophyton floccosum Trichophyton mentagrophytes occur common skin infections in the North East Region of India. Unlike other superficial fungal infections, the incidence of dermatophytoses, commonly called ringworm or tinea, has increased considerably due to several reasons and the situation has worsened with the increase in the number of immune-compromised host[6]. Although several synthetic antimycotic drugs are available in the market, at present the use of these drugs has minimized because of some factors which includes low potency, poor solubility, development of resistant strains, drug toxicity, and side effects, like gastrointestinal disturbance, cutaneous reaction, hepatoxicity, Leucopenia. Fungal infections constitute a significant health problem in our country.

## MATERIALS AND METHODS

### SITE OF COLLECTION

The present study was carried out in school children in the age group of 3-6 years age and 10-15 age groups. A total number of 20 samples were collected from Banadurai Higher Secondary School students at Kumbakonam. Thanjavur district, Tamilnadu during the study period between Dec.2017 to March 2018. The nail clippings were collected for present work collected from nails white yellow discoloration. After giving therapy for after treatment with Herbal soap Aleo vera, herbal lotion, and Dettol. Isolation of fungi was carried out according to standard Operating procedure for Medical Mycology[7].

### TRANSPORTATION OF SAMPLE

The samples were collected were kept in polythene bags for further studies.

### MEDIUM FOR ISOLATION

SDA and Dermatophyte medium was used for the isolation of fungal species

### ISOLATION OF FUNGI FROM NAILS

Nail sample was dipped into sterile water for 15 min for the softening of nails. Then small pieces were cut and used for incubation on medium for further study.

### MICROSCOPIC EXAMINATION OF SAMPLES

A microscopic examination is done using the KOH mount method.

### IDENTIFICATION OF FUNGI

Identification of isolated fungi was carried out according to the following literature[8 & 9], while the identification of *Rhodotorula mucilaginous and Prototheca wickerhamii* was done by APL 20c system (BioMerieux Vitek, Inc., France). Isolated fungi were sub-cultured and maintained on Sabouraud Dextrose Agar slants in tissue culture bottles.

### DIRECT MICROSCOPIC OBSERVATION BY KOH MOUNT

A drop of 40% (in case of nail clipping) was kept on a clean, grease-free glass slide. Then the sample (nail) was mixed gently with the KOH drop and the slide passed through a burner flame. It was covered by a glass coverslip. Then it was observed under high power object.

### ANTIDERMATOPHYTIC ACTIVITY OF Lawsonia inermis (HENNAPLANT) COLLECTION OF HENNA PLANT (*Lawsonia inermis*)

The leaf portion of the henna plant was collected and dried in shade.

### PREPARATION OF SOLVENT EXTRACTION OF HENNA PLANT (Lawsonia inermis)

29g of the henna leaf (Lawsonia inermis) was suspended in 50 ml of distilled water in 100 ml flask for 24 hrs at room temperature. The filtrates were then mixed with the solvents (chloroform, petroleum ether) in separating funnel and the mixer was shaking well until separation was observed in form of two layers; the water and the solvent extract.

### PREPARATION FOR HENNA PLANT (*Lawsonia inermis*) EXTRACT DISC

The sterile discs were loaded with 50 ml of extracts dissolved in Dimethylsulfoxide (DMSO) and were left to dry for 6 to 10 hours in $37^0$c in a sterile condition. Prepared discs were stored at $4^0$c in the refrigeration till use. To avoid any condensations the discs were kept at room temperature for 1 hour before use.

### AGAR DISC DIFFUSION ASSAY

Sabouraud's dextrose agar was prepared and sterilized. Then 0.1 ml of spore suspensions of each clinical isolates were aseptically transferred into sterile Petri plates and 20ml of the cooled molten sabouraud's dextrose agar was poured to each plate and the plates were rotated clockwise and anti-clockwise for uniform mixing of fungal spore suspensions. After solidification, the discs impregnated with henna plant (Lawsonia inermis) extract were placed on inoculated SDA plate using sterile forceps. Antifungal antibiotics such as nystatin and amphotericin B act as a control.

**BIOCHEMICAL TEST**

Indole Production Test, Methyl Red Test, VP-Test, Citrate Utilization Test, TSI Agar Test, Gelatin Hydrolysis, Catalase Test, Urease Test, Starch Hydrolysis.

**Table-1. Isolation of microbes on Nail sample of the age group of 3-6 and 10-15**

| S.No | Bacterial isolates | Fungal isolates |
|---|---|---|
| | 3-6 age | |
| 1. | E.coli | *Alternaria sp. Aspergillus sp* |
| 2. | Staphylococcus sp/ Lactobacillus sp. | *Penicillium sp.* |
| | 10-15 age | |
| 1. | E.coli | *Mucor sp* |
| 2. | Bacillus sp | *Rhizopus sp* |

**Table-2. Isolation of microbes from nail sample (n=50)**

| S.No | Organisms isolated | Total (50) |
|---|---|---|
| 1. | *Aspergillus sp* | 12 |
| 2. | *Penicillium sp* | 6 |
| 3. | *Mucor sp* | 8 |
| 4. | *Rhizopus sp* | 7 |
| 5. | *E.coli* | 6 |
| 6. | *S.aureus* | 5 |
| 7. | *Alternaria sp* | 3 |
| 8. | *Fusarium sp* | 2 |
| 9. | *Bacillus sp* | 1 |

**Table-3. Number of positive cases according to the age group**

| Age group | Nail sample |
|---|---|
| 3-6 | 20 |
| 10-15 | 30 |

**Table-4. Antimicrobial activity of Henna (*Lawsonia inermis*), Aloe Vera (*Aloe barbadensis*) and Dettol solution.**

| S.No | Samples | Zone of inhibition (cm) |
|---|---|---|
| 1. | Henna (Lawsonia inermis) | 1.2 cm |
| 2. | Dettol solution | 1.0 cm |
| 3. | Aloe Vera (Aloe barbadensis) | 0.5 cm |

**Table-5. Total viable count of isolated microbes**

| S.No | Micro organisms | Cfu (ml) |
|---|---|---|
| 1. | *E.coli* | 3X10-4 |
| 2. | *Staphylococcus aureus* | 5X10-4 |
| 3. | *Bacillus sp,* | 4X10-4 |
| 4. | *Lactobacillus sp,* | 6X10-4 |

Cfu=colony forming unit

**DISCUSSION & SUMMARY**

The saprophytic microbes from the nail are made up of a total of 50 isolation representing species. Isolated fungi either have caused the disease or commensals. Out of the species like *Aspergillus sp, Alternaria sp, Fusarium sp, Penicillium sp, Rhizopus sp, Bacillus sp., E.coli and Staphylococcus aureus, and Mucor sp.*

In our study, the fungal flora isolated the nail samples were *Alternaria sp, Aspergillus sp, Fusarium sp, Rhizopus sp. The same finding the mold flora isolated like Alternaria sp, Aspergillus sp, Bipolaris sp, Cladosporium sp, Exophiala sp, Fusarium sp, Rhizopus sp, and Ulocladium sp*are usually common inhabitant of the household environment (Bokhary and Parvez, 1995). The pathogenicity of these mold flora is not restricted to nail infections. Alternaria sp could cause sub-cutaneous phaeohyphomycosis both in immune-deficient and immunocompetent patients. The present study carried out the evaluate the antimicrobial activity of Henna plant (Lawsonia inermis) against the Chennai antibiotic Dettol solution and Aloe vera soap (Aloe barbadensis). In our present investigation, the bacteria like E.coli, Staphylococcus sp, Lactobacillus sp, Bacillus sp, the fungal species were isolated from the nail samples of an infected person. The Henna plant (Lawsonia inermis) extract was prepared and testing its antimicrobial activity the bacterial species against the bacterial isolates as the antimicrobial activity tested Dettol solution, Aloe vera soap (*Aloe Barbados*). In our finding the Henna plant (*Lawsonia inermis*) shows (1.2 cm) zone of inhibition, Dettol solution shows (1.0 cm) zone of inhibition and Aloe vera soap (*Aloe barbadensis*). The Henna plant (*Lawsonia inermis*) (1.2 cm) zone of inhibition at Maximum observed. So, in our investigation to advised the person who have nail infections for preventing further secondary infection and also treatment by use of Henna Plant (*Lawsonia inermis*) because of its maximum zone of inhibition and also more antimicrobial activity.

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SP-22

# ISOLATION OF MICROBES FROM DENTAL CARIES AND ITS BACTERICIDAL ACTIVITY WITH CLOVE OIL AND *Ocimum sanctum(L.)*

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## ABSTRACT

Dental caries is the most problematic infection due to different types of microbes. These include Cracks, pits, or grooves in the back teeth, between teeth, around dental fillings or bridgework, Near the gum line the bacteria turn sugar and carbohydrates (starches) in the foods we eat into acids. They are too small to see at first. But they get larger over time. The oral *Streptococci., sp* play an important role in oral health. They are involved in Dental plaque development and formation of dental caries Nowadays; caries remains one of the most common diseases of the people worldwide. Dental caries is a significant public health problem affecting several children and adults throughout the globe. However, the prevalence of dental caries is increasing with increasing consumption of dietary sugar but several factors other than sugar are suggested for the etiology of dental caries including involvement of microorganisms and host factors. The microbes involved in dental caries convert dietary sugar into acid which leads to demineralization of tooth enamel and ultimately results in the development of dental caries. Comparative antimicrobial activity of chemical antibiotics with Herbal plant extract Clove Oil and *Ocimum sanctum* (L.)

**KEYWORDS**: Dental caries, Clove oil, *Ocimum sanctum* (L.), herbal plant

## INTRODUCTION

The oral *Streptococci sp.,* play an important role in oral health. They are involved in Dental plaque development and the formation of dental caries1. Dental caries can also cause bad breath and foul tastes. Complications such as cavernous sinus thrombosis and Ludwig 's angina can be life-threatening. There may be discomfort while eating sweet foods, hot or cool drinks. If left untreated the disease can lead to pain, tooth loss, if and in severe case death. Dental caries and periodontal diseases are among the most important global oral health problems, although other conditions like oral and pharyngeal cancers and oral tissue lesions are also of significant concern. Herbal medicines have less side-effect in comparison with traditional medicines, but side-effects do occur. Herbal products can vary in their potency[2]. The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immune-compromised individuals and financial considerations in developing countries. Today, caries remains one of the most common diseases of people worldwide. Individuals are susceptible to this disease throughout their lifetime. Worldwide, approximately 36% of the population has dental caries in their permanent teeth. In baby teeth, it affects about 9% of the population. Risk of caries includes physical, biological, environmental, behavioral and lifestyle-related factors[3]. Dental caries, one of the globally affecting diseases of the oral cavity is still prevalent in today's era despite knowledge of most advanced sciences and technologies in dental practice. The usage of these herbal extracts in clinical practice can benefit the oral hygiene of the patient[4].

## MATERIALS AND METHODS

### SAMPLE COLLECTION

The sample for the present investigation was collected from different areas in and around Kumbakonam,

Thanjavur. The Dental caries samples were collected from different patients attending a dental hospital in Bose Dental Hospital at Kumbakonam. Samples from a total of 60 dental caries patients were collected while 50 samples were collected from subjects with no clinical symptoms of dental caries or other oral diseases and used as control. The samples from occlusal pits and fissure caries and smooth surface caries were collected with the help of excavators by dentists. For the collection of root caries, the care was taken to avoid contamination by gingival flora.

**GROWTH MEDIA FOR BACTERIA ISOLATION**

No transport media was used and samples were immediately processed in a lab within 30 minutes. The samples were diluted tenfold in phosphate buffer saline and inoculated on nutrient agar.

**IDENTIFICATION OF ISOLATED BACTERIA**

**MORPHOLOGICAL CHARACTERIZATION OF BACTERIA**

The bacteria were gram stained and observed under a microscope. The isolated bacteria were first grouped based on Gram staining. The bacterial cells with cocci, bacilli, irregular, single, paired, chain or clusters shapes were found.

**BIOCHEMICAL CHARACTERIZATION**

The gram-positive cocci were first characterized by catalase test to differentiate between *Staphylococci sp,* and micrococci[7] (catalase-positive) from *Streptococci sp*, and *Enterococci sp.*,(catalase-negative). The *Streptococci sp,* were further characterized with the help of oral *Streptococci sp,* identification manuals[5] *Staphylococci sp,* were identified using coagulase test and growth on Manital salt Agar.

**COLLECTION OF LEAVES**

The infection-free leaves of *Ocimum sanctum* and Clove oil were collected from the village of T.Pallur, Tamilnadu.

**PREPARATION OF STERILE ANTIBIOTIC DISC**

Antibiotic disc preparation was done using standard procedures.

**MICROBIOLOGICAL ANALYSIS**

**BIOCHEMICAL TEST**[6]

Indole Production Test, Methyl Red, Voges-Proskauer Test, Citrate Utilization Test, Gelatin Hydrolysis, Catalase Test, Urease Test, Starch Hydrolysis.

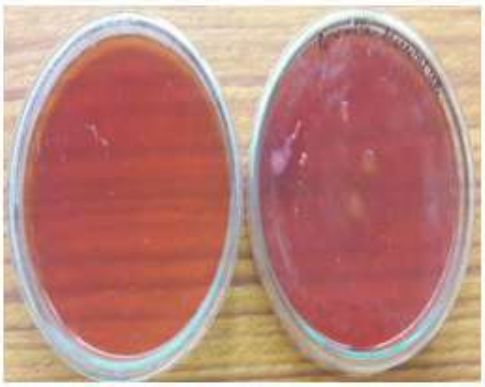
**ANTIBIOTIC SENSITIVE TEST- DISC DIFFUSION METHOD**

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at the desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.

**RESULT**

The sample for the present investigation was collected from Boss tooth hospital, Kumbakonam. Dental caries samples were collected from the dental clinic. The isolated organisms were confirmed by using the standard Bio-chemical test. The most predominant organisms were *Streptococcus sp*, *Staphylococcus aureus,* and *Escherichia coli.* The isolated organisms were tested by Clove oil, and *Ocimum sanctum* (Tulsi).

**FIG : 1 DENTAL CARIES SAMPLE**



**a) *Staphylococcus sp,* mannitol salt agar medium**

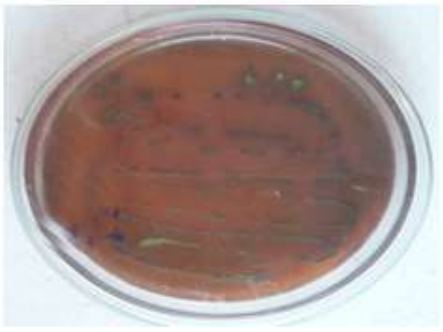
***b) Staphylococcus* son mannitol salt agar medium**

**FIG :2 ISOLATION OF MICROBES FROM DENTAL SAMPLES**

**c) Staphylococcus *sp*, medium on blood agar**

**d) Staphylococcus *sp*, blood agar medium on blood agar**

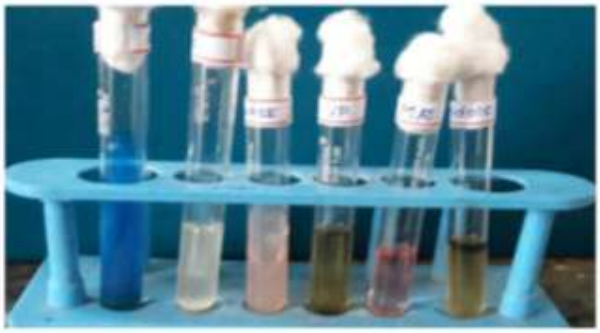
**e) *E.coil* On EMB agar medium**



**f) *E.coil* on EMB agar medium**

**Fig :3 BIO- CHEMICAL CHARACTERS *Streptococci Sp., E.coil* and *S. aureus***

*Streptococci Sp.,*



*Staphylococcus aureus.,* *E.Coli*

**HERBAL EXTRACTS**

*OCIMUM SANCTUM(L.)*

**CLOVE OIL**

**FIG 4 : ANTI MICROBIAL ACTIVITY OF *OCIMUM SANCTUM(L.)*& CLOVE OIL**


**TABLE : 1. BACTERICIDAL ACTIVITY OF HERBAL POWDER SAMPLES**

| ORGANISMS | AGAR WELL DIFFUSION METHOD | |
|---|---|---|
| | Clove oil | *Ocimum sanctum* |
| *Streptococcus sp,* | 12mm | Nil |
| *Staphylococcus sp,* | 5mm | 17mm |
| *Escherichia coli* | 5mm | 8mm |

**TABLE -2. ANTIBIOTIC SENSITIVITY TEST**

| Organisms | Disc diffussion method | | | |
|---|---|---|---|---|
| | Ampicillin | Tetracyclines | Erythromycin | Ciprofloxacin |
| *Streptococcus sp,* | 2mm | 14mm | 25mm | 18mm |
| *Staphylococcus sp,* | Nil | 10mm | 13mm | 22mm |
| *Escherichia coli* | 2 mm | 1 mm | 1.5 mm | 1 mm |

## SUMMARY AND CONCLUSION

In our findings, comparative antimicrobial activity of chemical antibiotics with Herbal plant extracts *Ocimum sanctum* (Tulsi) and clove oil. In testing Dental caries pathogens with commercial antibiotics by using the Disc diffusion method. The maximum zone of inhibition occurs in Erythromycin (25mm) against *Streptococci Sp.,* and *Staphylococcus sp.* Maximum zone of inhibition (22mm) Ciprofloxacin and in *Escherichia coli* maximum zone of inhibition (2mm) in Ampicillin was observed. In the present study, we conclude the *Ocimum sanctum* and clove oil shows the maximum zone of inhibition and compared with commercial antibiotics.

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SP-23

# FLUCTUATION OF TOTAL MICROBIAL LOAD OF *Staphylococcus* AND *Streptococcus* SPECIES IN DENTAL CARIES OF MAN (HOMOSAPIENS)

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## ABSTRACT

Dental caries, also described as tooth decay or dental cavities, is an infectious disease which damages the structures of teeth. The *Staphylococcus aureus* accumulates more in saliva who has tonsillitis and pharyngitis. So, they have a change of microbial load in Dental caries. *Streptococci* mutants are more important in the causation of dental caries. It breaks down dietary sucrose producing acid and tough adhesive dextran. The acid damages dentine and the dextran bind together food debris, epithelial cells, mucus, and bacteria to form dental plagues. The fluctuation of microbial load of *Staphylococcus aureus* and *Streptococcus* mutants studied. The reducing in microbial load in normal flora due to brushing. So due to this investigation, the people advised not to give the temptation of frequent snacking because the sugars load by bacteria and it converts sugars into acid it causes cavities. A toothbrush can remove most plaque, so total microbial load decrease by proper brushing and flossing. So, they are necessary to maintain Oral Hygiene.

**KEYWORDS:** Tooth decay, Human being, Dental caries, pathogens, Gram-negative species.

## INTRODUCTION

Tooth decay is caused by certain types of acid-producing bacteria which cause the most damage in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The resulting acidic levels in the mouth affect cause a tooth's special mineral content causes it to be sensitive to low $p^H$. Specifically, a tooth is in a constant state of back – end – forth demineralization and remineralization between the tooth and surrounding saliva when the $p^H$ at the surface to the tooth drops below 5.5, demineralization proceeds faster than remineralization. This results in the ensuing decay. Depending on the extent of tooth proper form, function, and aesthetics, but there is no known method to regenerate a large amount of tooth structure. Instead, dental health organizations advocate preventive and prophylactic measures, such as regular oral hygiene and dietary modifications, to avoid dental caries[1].

## MATERIALS AND METHODS

## GENERAL

1. SAMPLING AREA

The samples for the present investigation was collected from different areas in and around Kumbakonam, Puliampettai, Iyyavadi, Thirunageswaram, Karuppur, Marudanallur, Kottaiyur, etc,

2. GLASSWARE
3. CHEMICALS
4. SAMPLING PROCEDURE
5. MICROBIOLOGICAL ANALYSIS
6. DIFFERENTIATION OF *Streptococci* BY BACITRACIN TEST
7. DIFFERENTIATION OF *Streptococci* BY CAMP TEST
8. DIFFERENTIATION OF *Streptococci* BY BILE SOLUTION TEST

## MORPHOLOGY AND IDENTIFICATION

## MICROSCOPIC EXAMINATION

1. Hanging Drop Method
2. Grams' Staining

## BIO-CHEMICAL TESTS

1. Indole Production Test
2. Methyl Red Test
3. VP Test
4. Citrate Utilization test
5. TSI Test
6. Gelatin Hydrolysis
7. Starch Hydrolysis
8. Catalase Test
9. Urease Test
10. Lipid Hydrolysis
11. Antibiotic Sensitive Test

## TERMINATION OF *Lactobacillus acidophilus* BY SNYDER TEST

## COLUMBIA COLISTIN NALIDIXIC ACID AGAR TEST

Columbia Colistin Nalidixic Agar Test is used for the differentiation of *Staphylococcus sp* and *Streptococcus sp*. In this agar media, Gram-positive microbes grow luxuriantly with a clear defined hemolytic reaction. Columbia DNA plates were prepared and the *Staphylococcus* and *Streptococcus* colonies streaked on the agar surface of different plates. The plates were incubated at $35^0$C for 45 hours. The *Streptococci* form of small, white to greyish and beta or and beta or alpha hemolysin, whereas *Staphylococcus* shows large, white to greyish or cream to yellow colonies with or without hemolysin.

## MEDIUM EMPLOYED

Indole Medium

| | |
|---|---|
| Peptone | - 20g |
| Sodium chloride | - 5g |
| Distilled water | - 1000ml |
| $P^H$ | - 7.4 |

**Eosin Methylene Blue Agar**

| | |
|---|---|
| Peptic digest of animal tissue | - 10g |
| Dipotassium hydrogen phosphate | - 2.0g |
| Lactose | - 5.0g |
| Sucrose | - 5.0g |
| Eosin-y | - 0.4g |
| Methylene blue | - 0.065g |
| Agar | - 20g |
| $p^H$ | - 7.2 |
| Distilled water | - 1000ml |

**Showing Antibiotic Sensitive Pattern of *S.aureus* of Dental Carries In Man**

| S. No | Spectrum Of Antibiotics | *S.aureus* |
|---|---|---|
| 1. | Ciprofloxacin | S |
| 2. | Amikacin | R |
| 3. | Gentamycin | R |
| 4. | Chloramphenicol | S |
| 5. | Norfloxacin | S |
| 6. | Tobramycin | R |
| 7. | Cotrimoxazole | S |
| 8. | Lincomycin | R |

| | | |
|---|---|---|
| 9. | Vancomycin | R |
| 10. | Cefotaxime | R |

**Keys: S – Sensitive; R – Resistance**

**Biochemical Test For Differentiation Of Species *Streptococci sp.,***

| TESTS | Group A | | |
|---|---|---|---|
| | S. salivarius | S. mutants | *Staphylococcus aureus* |
| Haemolysis | Beta | Beta | – |
| Bacitracin | + | + | – |
| Camp Test | + | + | – |
| Bile esculin Hydrolysis | – | – | – |
| 6.5% NaCl Medium | NG | NG | – |
| At $10^0$C | NG | NG | – |
| At $45^0$C | NG | NG | – |

**Fungal Colony**

| S. No | Organisms |
|---|---|
| 1. | Actinomyces |
| 2. | Odontolyticus |

**Microbial Load of *Staphylococcus aureus, Streptococcus mutants* After Brushing**

| S. No | Organisms | Total Microbial Loan Before Brushing | Total Microbial Load After Brushing |
|---|---|---|---|
| 1. | *Staphylococcus aureus* | 250 x $10^{-4}$ | 200 x $10^{-4}$ |
| 2. | *Streptococcus mutants* | 210 x $10^{-4}$ | 190 x $10^{-4}$ |

## RESULT

The samples for the present investigation were collected from different areas in and around Kumbakonam like Puliampettai, Iyyavadi, Thirunageswaram, Karuppur, Marudanallur, Kottaiyur, etc., In our present investigation microbes like *Streptococcus salivarius, Streptococcus mutants, Streptococcus aureus* and the fungus *Actinomyces odontolyticus* was identified.

## DISCUSSION AND SUMMARY

Dental caries is initiated due to the decalcification and softening of dental enamel. The *Staphylococcus* found in all types of contaminated food kinds of stuff. It produces enterotoxin. Careless handling of food leads to illness. This species is differentiated by *Streptococci* by the production of coagulate[3]. Enterotoxin are heat stable. So heating after the toxin production may not prevent diseases[4]. The samples to the microbial analysis of Dental Caries was collected from Kumbakonam, Puliampettai, Iyyavadi, Thiruvarur, and Marudanallur from School Children belonging to the age group of 10-15. The organisms *Streptococcus mutants, Streptococcus salivarius, Staphylococcus aureus* and fungus *Actinomyces odontolyticus* were identified. The antibiotic sensitivity pattern of *Staphylococcus aureus* is detailed studied. It shows sensitivity to Ciproflaxacin, Chloramphenicol, Norfloxacin, Amikacin, Gentamycin, Tobramycin, Lincomycin, Vancomycin and Cefotaxime. The viridian *Streptococci sp, Streptococcus mutants, Streptococcus salivarius* differentiated from *Staphylococcus aureus.* Comp test, Bacitracin, and Bile esculin hydrolysis. The fungus species *Actinomyces odontolyticus* differentiated and the colony morphology was studied. The fluctuation of microbial load of *Staphylococcus aureus* and *Streptococcus mutants* studied[5,6 &7]. The reduction in microbial load in normal flora due to brushing. So due to this investigation, the people advised not to give the temptation of frequent snacking because the sugars load by

bacteria and it converts sugars into acid it causes cavities. A toothbrush can remove most plaque, so total microbial load decrease by proper brushing and flossing. So, they are necessary to maintain Oral Hygiene.

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SP-24

# ANTIBACTERIAL, ANTIFUNGAL AND PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *Tabernaemontana divaricata* SINGLE AND DOUBLE FLOWER VARIETIES

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## ABSTRACT

*Tabernaemontana divaricata* commonly called pinwheel flower, crape jasmine, East India rosebay, and Nero's crown is an evergreen shrub native to India and now cultivated throughout South East Asia and the warmer regions of continental Asia. In zones where it is not hardy, it is grown as a house/glasshouse plant for its attractive flowers and foliage. The stem exudes a milky latex when broken, whence the name milk flower. The plant contains several alkaloids and, like many other plants in the family Apocynaceae, is toxic and medicinal. Since it contains a lot of iboga alkaloids it may prove to be psychoactive and treat addiction. The present study confirms the presence of active phytochemicals in both the single and double flower varieties of *T.divaricata* with standard procedures. Based on the result this plant may be used for the production of new drugs, which are involved in an antimicrobial activity for curing several ailments. These might be advantageous to make use of this plant for biomedical relevance in pharmaceutical companies because of assured class of phytocompounds.

**KEYWORDS:** *Tabernaemontana divaricata*, antibacterial, antifungal, phytochemical screening

## INTRODUCTION

The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals. The (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available.The annual global export value of pharmaceutical plants in 2011 accounted for over US$2.2 billion[1]. *Tabernaemontana divaricata* is commonly used in the Chinese, Ayurvedic, and that t*raditional* medicine for the treatment of fever, pains, and dysentery[2]. It is a common garden plant found in tropical countries including Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia, and Thailand[3]. *Tabernaemontana divaricata (L),* a glabrous, evergreen dichotomously or small branched shrub tree. It bears attractive, white, a colored fragrant flower, single or double layered and may sporadically thought the year. The leaves are large, shiny and deep green and the size is about 6 inches in length and 2 inches in width. *Tabernaemontana divarticata* (L) is widely distributed throughout India as an ornamental plant. It is also found in Bangladesh and other parts of south East Asia. It possesses a wide range of valuable activities like anti-infection, antioxidant, anti-inflammation, anti-cancer, and anticonvulsant and -anti-diabetic properties. Important bacteria cause severe infection in human beings and animals. *Staphylococcus aureus* causes food poisoning, local abscesses' and superficial skin lesions. 95% of *s.aureus* strains are resistant to antibiotic penicillin around the world. *Escherichia coli* mainly affects the gastrointestinal tract and causes diarrhea especially in children. *K. pneumonia is* commonly found in the gastrointestinal tract and hands of hospital personnel. *P.aeroginosa* is an opportunistic human pathogen. It is "opportunistic" because it seldom infects healthy individuals. *Streptococcus* species are responsible for many cases of pink eye, meningitis, bacterial pneumonia, endocarditic, erysipelas, and necrotizing fasciitis the' flesh-eating 'bacterial infections[4]. Therefore, this

study aims to evaluate antibacterial properties for the above-mentioned bacteria and the fungi, *Candida albicans* from extracts of *T. divaricata* flowers, and to be employed for further development of health promotion pharmaceutical products.

## MATERIALS AND METHOD

### Collection of flowers & Extract preparation

The solvent methanol was used for the extraction of the flower extract by the cold extraction method. The stalk of the flowers were removed to get petals alone from both the varieties. From each flower variety, 250 gm of the petals were soaked in 500 ml of methanol in separate airtight containers. They are allowed to stand at room temperature for 5 days, with occasional manual agitation of the container using a sterile glass rod at every 2 – 3 hours. The extracts were separately filtered using sterile what man No.1 filter paper. The resulted in filtrates were then concentrated in a rotary evaporator (Laboratory 4000 –efficient, Heidolph, Germany) at 400 rpm/$50^0$ C. 10 ml of gummy extract were obtained upon evaporation of each extract. The gummy was stored at $4^oC$ for further studies.

### Preparation of inoculum

The pure microbial cultures were inoculated into the tubes of nutrient broth and potato dextrose broth, using inoculation or loops. Then the tubes were incubated at different temperatures and time duration (at $37^0C$ for 24-48 hours for bacteria; and at $28^0C$ for 48-72 hours for fungi). The young cultures were used for antimicrobial susceptibility tests.

### Preparation of discs

What man No;1 filter paper was taken and 6 mm discs prepared and are sterilized in a hot air oven. These discs were loaded with 10 µl of the flower extract and air-dried. This was carried out under sterile conditions inside a laminar airflow chamber**.**

### Media composition

**Muller- Hinton agar medium**

| | |
|---|---|
| Beef infusion | - 300 g |
| Casein acid hydrolyzed | - 17 g |
| Starch | - 15 g |
| Agar | - 17 g |
| Distilled water | - 1000 ml |
| $P^H$ | - 7.3±0.2 |

**Potato dextrose agar medium**

| | |
|---|---|
| Potatoes (infusion) | - 200 g |
| Dextrose | - 20.0 g |
| Agar | - 150 g |
| Distilled water | - 1000 ml |
| $P^H$ | - 5.6 |

**Nutrient broth**

| | |
|---|---|
| Peptone | - 5.0 g |
| Beef extract | - 3.0 g |
| Sodium chloride | - 5.0 g |
| Agar | - 3.0 g |
| Distilled water | - 5.0 g |
| $P^H$ | - 7.0 |

### Media preparation

19 gm of Muller-Hinton agar was suspended in 1000 ml of distilled water. The $p^H$ was adjusted to 7.3 and the agar was boiled until it dissolved into the medium completely. The medium was sterilized by autoclaving at $121^0C$ for 15 minutes and mixed well before pouring.

## ANTIBACTERIAL ACTIVITY ASSAY

Antibacterial activity of the methanol extracts of flowers of *Tabernaemontana divaricata* both single and

double varieties (both individually and in combination) were assayed using disc diffusion method[5]). Approximately 20 ml of sterile Muller -Hinton agar (MHA) medium was poured into sterile Petri plates and allowed to solidify. About 0.1 ml of 24 hours old culture of the above 5 bacterial cultures were maintain in the nutrient broth was inoculated into the plate and spread over the entire surface of the agar plate using an L-rod. Previously prepared discs using the two different extracts were placed and impregnated on to the seeded medium using sterile forceps. The culture plates were incubated for 24 hours at $37^0$C. The antibacterial activity of the flower extracts was noted by the formation of clear zones among the disc.

## ANTIFUNGAL ACTIVITY

The test organism *Candida albicans* was inoculated into a test tube containing potato dextrose broth and incubated at room temperature for 72 hours. Approximately 20 ml of sterile potato dextrose agar medium was poured into sterile Petri plate and allowed to solidify. Above 3.1 ml of the 72 hours old culture of the fungus was inoculated and spread over the entire surface of the agar plate using L-rod previously prepared discs using the two different extracts and its combination were placed and impregnated onto the seeded medium using sterile forceps. The culture plates were incubated for 24 hours at $28^0$C. The antifungal activity of the flower extracts was noted by the formation of clear zones around the analysis.

## PHYTOCHEMICAL ANALYSIS [6-9]

Qualitative phytochemical screening was carried out with the following methods.

**1. Test for steroids**

1 ml of the extract was dissolved in 10 ml chloroform and an equal volume of concentrated sulfuric acid was added by the side of the test tube. The upper layer turns red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids. 2ml of the extract and 0.2ml of HCl was used for this test. Yellow colour precipitate indicates the presence of alkaloids.

**2. Test for reducing sugar**

Benedict's test: 0.5 ml of the extract and 5 ml of Benedict's solution was taken in test tubes, boiled for 5 mins and allowed to cool spontaneously. A red colour precipitate of cuprous oxide indicating the presence of reducing sugar.

**3. Test for terpenoids**

2 ml of the extracts were added to 2ml of acetic anhydride and concentrated $H_2so_4$. The formation of the blue –green ring indicates the presence of terpenoids (Ayolla, 2008).

**4. Test for Fatty acids**

0.5 ml of the extracts were mixed with 5 ml of ether. The extracts were allowed for evaporation on filter paper and dried. The appearance of transparency on filter paper indicates the presence of fatty acid

**5. Test for flavonoids**

A few drops of concentrated hydrochloric acid was added to a small amount of the extracts. Immediate development of red color indicates the presence of flavonoids

**6. Test for Gums**

0.5 ml of the extract were taken and then Molisch's reagent and sulphuric acid were added. The red violet ring produced at the junction of two liquids indicates the presence of gums.

**7. Test for tannins**

To 2 ml of the extracts, a few drops of 1 % lead acetate was added. A yellowish precipitate indicated the presence of tannins.

**8. Test for saponins**

5 ml of the extracts were mixed with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. The formation of foam indicates the presence of saponins.

**9. Test for anthocyanins**

2 ml of the extracts 2 ml of 2N HCl and ammonia were added. The appearance of pink-violet indicates the presence of anthocyanins.

## RESULT & DISCUSSION

Fresh flowers of sugarcane and leaf of *Tabernaemontana divaricate* were used in the present study to evaluate the antibacterial, antifungal and photochemical properties. *Tabernaemontana divaricata* flowers

have been traditionally used in India and China in the prevention of eye and skin diseases. The methanol extract of *Tabernaemontana divaricata* single flower variety (pinwheel) has shown significant antibacterial activity against *Klebsiella pneumonia* (14.25±0.25) and *Candida albicans (*14.50±0.25) among all the tested bacterial pathogen. The flower extract has shown moderate antimicrobial activity against *E.coli (*10.50±1.25) *S.aureus,* (14±0.75), *S.pneumoniae* (11.25±0.75) and *P.aeruginosa* (11.50±1.75). The methanol extract of *Tabernaemontana divarticata* double flower variety (Flore H ) has shown significant inhibitory action against *Streptococcus pneumonia* (14.75±0.0) the flower extract as shown to have moderate antimicrobial activity against *E. coli* (11.25±1.25), *s.aureus* (11.75±0.75), *K. pneumoniae* (14±1.05) and *P.aeroginosa* (11.25±0.75) and *Candida albicans (13.25±0.75).* The combined extract of *T.divaricata* of both single and double flower varieties have shown significants antimicrobial activity against *Klebsiella pneumonia (14±0.75 )* and *Candida albicans (14±0.75).*The combined extract has also shown to possess higher inhibitory activity than the single and double flower verities, individually tested against the pathogenic bacteria and fungus.

## SUMMARY

Infection with environmental microbes is increasing alarmingly, the opportunistic microorganism can cause different infections and multidrug-resistant pathogens are commonly involved in infectious diseases are difficult to treat. To overcome and to manage, traditional medicines are known to be the best. Following that, antimicrobial activity and analyzed against five bacterial pathogens and viz., *E.coli*, *s.aureus,* and *S.aeruginosa* and a fungal pathogen, *Candida albicans*. The in vitro antimicrobial activity of methanol extract of *Tabernaemontana divaricata* single and double flower varieties and their combined activities were determined by the disc diffusion method. The single variety of flower methanol extracts showed a maximum zone of inhibition against *Streptococcus pneumonia* and *Candida albicans.* The double flower methanol extracts showed the maximum zone of inhibition against *Klebsiella pneumonia.* The combined flower methanol extracts showed the maximum zone of inhibition against *Klebsiella pneumonia* and *Candida albicans.* To that effect, the flowers of a single variety are considered to be more effective than the other. But in the combination of the both, the extracts reveal better antimicrobial property than their actions. Phytochemical analysis of methanol extract of single and double flower showed the presence of steroids, reducing sugar, terpenoids, gums, tannins and saponins. Considering the immediate reaction expressed by the single flower extract, it can be concluded that, that variety possess a high concentration of active phytochemicals. The present study also supports the medicinal usage of the flower extract which possesses compounds, that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by a pathogen. The most active extract can be subjected to isolation of the therapeutic microbial undergo for the pharmacological evaluation.

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# PREVALENCE OF MICROBES ON DOGS (Cannis lupus) AND TESTING THE MULTIDRUG RESISTANCE OF ISOLATES

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## ABSTRACT

Although many previous workers have also investigated the cultivable oral microbiota of dogs. Most have relied upon conventional identification methods, and the studies have been aimed at gaining a better understanding of the human microbiota. The bacterial communities of the skin have been well studied and computational and laboratory advances in the technology of microbial community profiling have enabled more accurate investigation of the communities commonly referred to as the microbiota or microbiome various studies using next-generation sequencing techniques have Beal in shown that the skin bacterial community of healthy humans is quite diverse and its composition. In the present study various pathogenic microbes like *Pseudomonas sp, Klebsiella sp, Staphylococcus aureus*, isolated from the dog skin and saliva. Two specimens were cultured from each subject. (Six times in 4 weeks). Data reported in the present study confirm the *Staphylococci* findings and coliforms. *Enterococci* and *Lactobacilli* proved to be among the six most frequently cultured organisms.

**KEYWORDS:** *Cannis lupus*, Multidrug resistance, isolates, microbiota

## INTRODUCTION

Microbial flora has spatial and temporal complexity that usually differs by individual body niche, age, geographic location, health status, diet, and type of host [1&2]. There is variation in the normal flora found in the oral cavity which depends on the area sampled (tooth enamel, tongue, gingival surface, saliva) and the state of periodontal health[3&4], colony counts of aerobic bacteria from moist areas such as the axilla or toes web spaces can reach $10^7$ bacteria per $cm^2$ (David, 2001). Nutrients like lipids and protein (Keratin) are provided by the skin for selected colonizing bacteria. This dry and slightly acidic environment may limit the types of microbes that can survive on normal skin. The organisms compete with each other for nutrients and space[5]Many external factors can alter the ecosystem of the skin, with resulting changes in microbial population[5-6]. Numerous bacteria that have been cultivated from normal bacteria have been cultivated from normal skin[7]. These include *Staphylococci sp, Micrococci sp, Corynebacterium sp, Brevibacteria sp, propionibacteria sp,* and *Acinetobacter sp*[8]. The research papers on antimicrobial resistance limited in isolates cats and dogs are very limited. There are very high numbers of *S.aureus* and *S.intermedius* isolated from dogs that were able to produce beta-lactamase enzyme, but they were sensitive to Cloxacillin or Oxacillin. Similar results were observed in our study that *Staphylococcus* isolated from dog saliva was also resistant to beta-lactams antibiotics.

## MATERIALS AND METHODS

### SAMPLE COLLECTION

Dog skin cannis lupus and saliva samples were collected from a pet dog in the house in area Sannapuram Kudiyana street Thanjavur (d.t) sample collection is done by swabbing with sterile cotton air buds from skin and mouth of dog then collected sample was brought to the laboratory and streaked on nutrient agar plats within 30 minutes. The dogs were of same age but different breeds.

## MICROSCOPIC EXAMINATION:[9]

The microbes mobility was determined by the hanging drop method and by gram staining method determine the staining nature of isolated microbes.

## BIOCHEMICAL TEST[9]

1. Indole Production Test
2. Methyl Red Test
3. VP-Test
4. Citrate Utilization Test
5. TSI Agar Test
6. Gelatin Hydrolysis Test
7. Catalase Test
8. Urease Test
9. Starch Hydrolysis Test
10. Antimicrobial Sensitivity Test

## RESULT

The Microorganisms *Staphylococcus aureus, E.coli, Klebsiella pneumonia, Pseudomonas aeroginosa, streptococcus sp, Bacillus sp, Enterobacter sp and fungi are Aspergillus sp, Penicillium sp, Rhizopus sp, Candida sp, Fusarium sp* were isolated from the dog saliva Microorganisms were isolated from the dog skin. The bacteria were *E.coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeroginosa,Fungi are Aspergillus sp, Penicillium sp,Candida sp.* The antimicrobial susceptibility testing against antibiotics on the Metrinadzole, Penicillin, Norfloxin, Gentamycdin, Amikacin, Carbenicillin, Tetracycline, Ciprofloxin, Ampicillin, sulphane, oxazole, Cerfuroxime, Chloramphenicol. The isolated organisms the antimicrobial activity was tested against Pseudomonas aeroginosa show the highest inhibition of (31mm), in amikacin and lowest inhibition (14mm), norfloxacin (8mm), metronidazole (9mm), and are resultant to amikacin, sulphamethoxazole, chloramphenicol, cefuroxime. The fungi colony like *Candida Albicans, Fusarium sp, Rhizopus sp, Penicillium sp, Aspergillus sp.*

**Table 1. Isolation microbes from dog (Canis lupus) skin**

| S.NO | Isolated Microbes Bacterial sp | Isolated Microbes Fungal sp |
|---|---|---|
| 1. | *Staphylocaccus aureun* | *Aspergillus sp* |
| 2. | *E.coli* | *Penicillium sp* |
| 3. | *Klebsilla pneumoniae* | *Rhizopus sp* |
| 4. | *Pseudomonas Aeroginosa* | *Candida sp* |
| 5. | *Streotococcus pyogens* | *Fusarium sp* |
| 6. | *Bacillus sp* | |
| 7. | *Enterobacter sp* | |

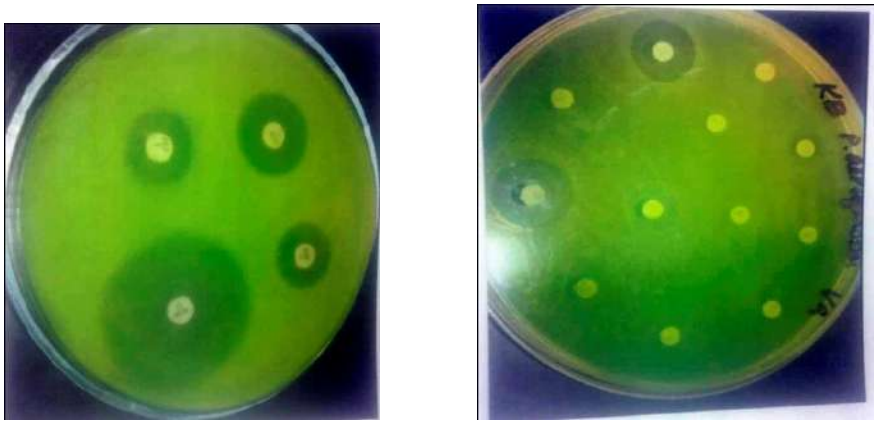
**Table 2. Isolated Microorganisms the antimicrobial activity was tested against *Pseudomonas aeroginosa***

| S.No | Antibiotic activity | Zone of inhibition |
|---|---|---|
| 1. | Metrinadazole | 9mm |
| 2. | Penicillin | 8mm |
| 3. | Norflaxacin | 14mm |
| 4. | Gentamycin | 22mm |
| 5. | Amikacin | 31mm |
| 6. | Carbencillin | 20mm |
| 7. | Ampicillin | Resistant |

| | | |
|---|---|---|
| 8. | Sulphame throxazole | Resistant |
| 9. | Chloramphenicol | Resistant |
| 10. | Cifrofloxin | Resistant |
| 11. | Cefuroxime | Resistant |
| 12. | Tetracycline | Resistant |
| 13. | Vanomycin | Resistant |


**Fig.1 (A). Isolation of microbes dog (Canis lupus) saliva**


**Fig.1 (B). Isolation of microbes on dog (Canis lupus) skin**



**(A)** **(B)**

**Fig 2 Antibiotic Sensitivity of *Pseudomonas aeruginosa* on muller hinton agar**

## DISCUSSION & SUMMARY

In the investigation like *Staphylococcus aureus, E Coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Streptococcus pyogens, Bacillus sp, Enterobacter sp* and the fugal species like *Aspergillus sp, Penicillium sp, Rhizopus sp, Candida sp,* and *Fusarium sp* is isolated from Dog Canis lupus Saliva. In the present

study, Pseudomonas aeruginosa was selected for testing its antimicrobial activity. It shows resistance to ampicillin, sulfamethoxazole, Chloramphinical, ciprofloxacin. In the present study, various pathogenic microbes like *Pseudomonas sp, Klebsillasp, Staphylococcus aureus,* isolated from the dog skin and saliva, this work was agreed by Hanel and They examined[10] the fecal flora of several animals including dog and man. Two specimens were cultured from each subject. (six times in 4 weeks). Data reported in the present study confirm the *Staphylococci* findings and coliforms. *Enterococci* and *Lactobacilli* proved to be among the six most frequently cultured organisms. The fecal flora of dogs to consist chiefly of acidogenic *Streptococci coliforms*, and lactic acid bacilli, and another group that *E.coli* was a common organism in the survey reported in the paper *E.coli, Streptococcus mitis*, *Streptococcus lactis* and *Enterococci* were the bacteria isolated most common often. Bacteroides of coliforms being so considered by others it might be useful. Cefruxine, tetracycline, vancomycin and higher zone of inhibition in 31(mm) in AmikacinThe present study carried out on Isolation and Identification of domestic dog (Cannis lupus) bacterial and fungal species on, skin and saliva. Microorganisms like *Staphylococcus aureus, E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus pyrogens, Bacillus sp and the fungi Aspergillus sp, Rhizopus sp, Candida sp, Fusarium sp.* Isolated from domestic dogs (Canis lupus) skin. Usually the dog (Canis lupus) spends most of the timing licking their genital and anal. A different type of microbes presents in healthy dogs also. If the dog has an open cut or wound it lick and spread many diseases. In the present investigation the multidrug resistance of *Pseudomonas aeruginosa* was tested, even many microbes isolated *Pseudomonas aeruginosa* selected for antibiotic susceptibility test because of its virulence. In our findings, *Pseudomonas aeroginosa* resistant to many antibiotic, like Ampicillin, Sulphanme Throxazole, Chloramphenicol, Cifroflxin, Cefuroxime, Tetracycline, Vancomycin, and sensitive to Amikacin (31mm) higher zone of inhibition followed bvy Gentamycin (22mm), Caarbencillin (20mm), Norflcacin (14mm), Metronidazole (9mm), Penicillin (8mm). In the present investigation of our report the dog (Cannis lupus) for many pathogenic microbes in its skin and saliva but bacterial isolated Pseudomonas aeruginosa also multidrug resistance so, care should be taken for handling dog, (Cannis lupus) and kisses of pet animals must be avoided for the prevention of zoonotic diseases.

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SP-26

# ISOLATION OF MICROBES FROM DENTAL CARIES AND ITS BACTERICIDAL ACTIVITY WITH *PSIDIUM GUAJAVA* AND *PUNICAGRANATUM*

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## ABSTRACT

Dental caries is a significant public health problem affecting several children and adults throughout the globe. However, the prevalence of dental caries is increasing with increasing consumption of dietary sugar but several factors other than sugar are suggested for the etiology of dental caries including involvement of microorganisms and host factors. The microbes involved in dental caries convert dietary sugar into acid which leads to demineralization of tooth enamel and ultimately results in the development of dental caries. comparative antimicrobial activity of chemical antibiotics with Herbal plant extract *Punica granatum*(Pomegranate), *Pisidium guajava*(Guava).The maximum zone of inhibition (16mm) in *Pisidium guajava* were observed in *Streptococci sp,* and (14mm) in *Punica granatum* observed in *Staphylococcus sp,* and *Escherichia coli* (9mm) in *Punica granatum* observed. In testing Dental caries pathogens with commercial antibiotics by using the Disc diffusion method. The maximum zone of inhibition occurs in Erythromycin (25mm) against *Streptococci sp,* and in *Staphylococcus sp,* the maximum zone of inhibition (22mm) Ciprofloxacin and in *Escherichia coli* maximum zone of inhibition (2mm) in Ampicilin were observed. In the present study we conclude the *Pisidium guajava*and *Ocimum sanctum* shows a maximum zone of inhibition and compared with commercial antibiotics.

**KEYWORDS:** Herbal plant, *Punica granatum*, *Pisidium guajava.* dental caries

## INTRODUCTION

Dental caries is a very common problem. The mouth contains a wide variety of oral bacteria, but only a few specific species of bacteria be lived to cause caries *Streptococcus mutants, Streptococcus sale various, Streptococcus sanguis, Streptococcus mitis, Actinomyces viscosus, Actinomyces isrealii, Nocardia sp*[1]. In baby teeth, it affects about 9% of the population[2]. Risk of caries includes physical, biological, environmental, behavioral and lifestyle-related factors[3].Dental caries is one of the widespread devastating diseases affecting manhood. Oral microorganisms play a vital role in their initiation and progression. It can be prevented by mechanical plaque removal or by the use of chemical agents (dentifrices or mouth rinses)[4]. Although chemical plaque control methods are effective, they cause side effects like staining of teeth, altered taste sensation, toxic effect on connective tissues, dryness and soreness of oral cavity, and oral desquamation, especially observed in children[5]. The use of plant resources for medicinal purposes is used in all civilizations and cultures and hence plants have played a key role in health care systems worldwide. In another study pomegranate, *Punica granatum* (Punicaceae), in the prevention of dental plaque was investigated and it was concluded that the plants extract was useful in the prevention of diseases caused by plaque bacteria[6]. These results also supported an *in vitro* study of a phytotherapeutic gel containing *Punica granatum*plant powder, which was able to inhibit the adherence of *Streptococcus sp.,* to glass in the presence of sucrose[7]. Listed below is the figure to show the potential application of plant extracts in the prevention and treatment of oral diseases caused by cariogenic and periodontal microbial pathogens.

## MATERIALS AND METHODS

### SAMPLE COLLECTION

The sample for the present investigation was collected from different areas in an around Kumbakonam, Thanjavur. The Dental caries samples were collected from different patients attending a dental hospital in Bose Dental Hospital at Kumbakonam. Samples from a total of 60 dental caries patients were collected while 50 samples were collected from subjects with no clinical symptoms of dental caries or other oral diseases and used as control. The samples from occlusal pits and fissure caries and smooth surface caries were collected with the help of excavators by dentists. For the collection of root caries, the care was taken to avoid contamination by gingival flora.

### GROWTH MEDIA FOR BACTERIA ISOLATION

No transport media was used and samples were immediately processed in the lab within 30 minutes. The samples were diluted tenfold in phosphate buffer saline and inoculated on nutrient agar.

### IDENTIFICATION OF ISOLATED BACTERIA

### MORPHOLOGICAL CHARACTERIZATION OF BACTERIA

The bacteria were gram stained and observed under the microscope. The isolated bacteria were first grouped based on Gram staining. The bacterial cells with cocci, bacilli, irregular, single, paired, chain or clusters shapes were found.

### BIOCHEMICAL CHARACTERIZATION

The gram-positive cocci were first characterized by a catalase test to differentiate between *Staphylococci sp,* and micrococci (catalase-positive) from *Streptococci sp*, and *Enterococci sp.*,(catalase-negative). The *Streptococci sp,* were further characterized with the help of oral *Streptococci sp,* identification manuals[8]. *Staphylococci sp* were identified using a coagulase test and growth on Manital salt Agar.

### COLLECTION OF LEAVES

The infection-free leaves of Pomegranate were collected from the village of T.Pallur, Tamilnadu.

### PREPARATION OF STERILE ANTIBIOTIC DISC

The extracts of leaf samples were incorporated into a sterile disc, which was prepared 5mm in diameter of Whatman No.1 filter paper by using a punching machine. Each sterile disc was incorporated individually with 50,100 and 150µl of the extract using a micropipette. This can be achieved by adding a small quantity of extracts and the disc was allowed to dry in the laminar airflow chamber. Another dose of the extract was applied to already prepared disc Control was maintained by adding distilled water on the discs.

### MICROBIOLOGICAL ANALYSIS

### BIOCHEMICAL TEST

Indole Production Test, Methyl Red Test, Voges-Proskaure Test, Citrate Utilization Test, Gelatin Hydrolysis, Catalase Test, Urease Test, Starch Hydrolysis, Antibiotic Sensitive Test, Disc Diffusion Method was performed and compared with a data value. However in this study, we have found many mutans *Streptococci sp,* from healthy patients, while many dental caries samples were found without the growth of mutans *Streptococci sp,* In has been already investigated in several studies that mutans *Streptococci sp,* are involved in dental caries etiology without any unique association.

## RESULTS

Results of the qualitative biochemical test and antibacterial activity were showed in the table and compared.

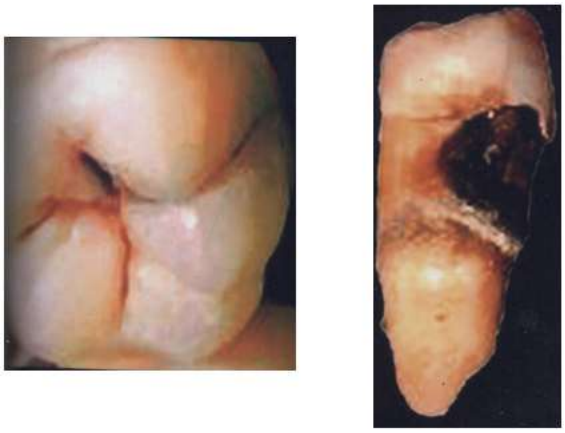
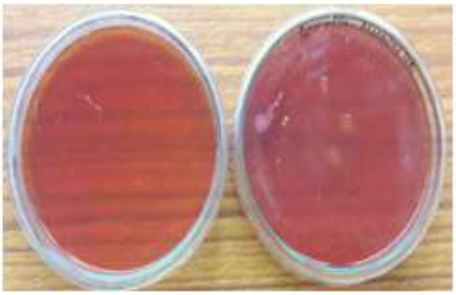


**FIG: 1 DENTAL CARIES SAMPL**



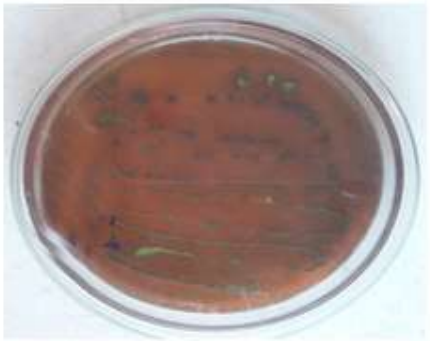
**a) *Staphylococcus sp,* on mannitol salt agar medium**

***b. Staphylococcus sp, on* mannitol salt agar medium**

**c) Staphylococcus *sp,* blood agar medium**

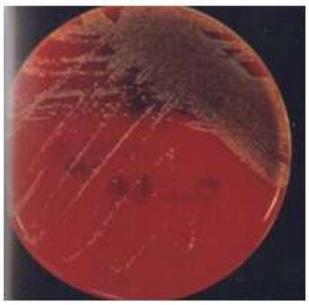
**d) Staphylococcus *sp,* on blood agar medium**

e)) ***E.coil* On EMB agar Medium**

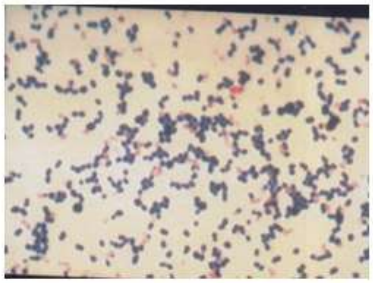


**f) *E.coil* On EMB agar medium**

**Fig :2 ISOLATION OF MICROBES FROM DENTAL SAMPLES**

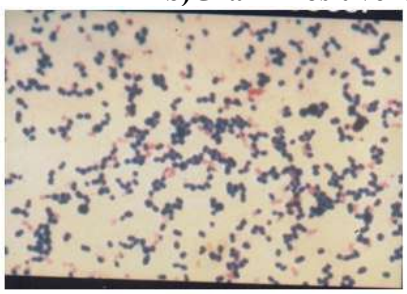


a)*Streptococcus sp*,

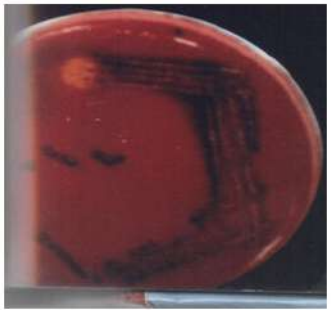


b)Gram- Positive cocci

c) *Staphylococcus aureus*



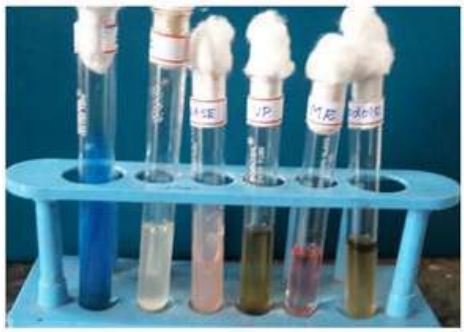
*d)* Gram- Positive Cocci



e) *Escherichia coli*

*f)* Gram–Negative rod

**Fig : 3 ISOLATION OF MICROBES ON DENTAL CARIES**



a) *Streptococcus sp,*

b) *E-Coil*

(c) *Staphylococcus aureus*

**Fig :4 BIO- CHEMICAL CHARACTERS *Streptococci sp, E.coil* and *Staphylococcus aureus***

*a) Psidium guajava* *b) Punica granatum*

**Fig 5 : HERBAL POWDERS**

*a) Staphylococcus aureus* *b) Streptococcus sp,*

**c) *E.coil***

**FIG 6 : ANTI MICROBIAL ACTIVITY OF *Psidium guajava* AND *Punica granatum***

**TABLE: 1 BACTERICIDAL ACTIVITY OF HERBAL POWDER SAMPLES**

| Organisms | Agar Well diffusion method | |
|---|---|---|
| | *Punicagranatum* | *Pisidiumguajava* |
| *Streptococcus sp,* | 12mm | 16mm |
| *Staphylococcus sp,* | 14mm | 10mm |
| *Escherichia coli* | 9mm | 7mm |

**TABLE -2 ANTIBIOTIC SENSITIVITY TEST**

| Organisms | Disc diffusion method | | | |
|---|---|---|---|---|
| | Ampicillin | Tetracyclines | Erythromycin | Ciprofloxacin |
| *Streptococcus sp,* | 2mm | 14mm | 25mm | 18mm |
| *Staphylococcus sp,* | Nil | 10mm | 13mm | 22mm |
| *Escherichia coli* | 2 mm | 1 mm | 1.5 mm | 1 mm |

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SP-27

# PRODUCTION AND QUALITY ATTRIBUTES OF MIXED WINE FROM GRAPES *(VITIS VINIFERA)* AND DATES *(PHOENIX DACTYLIFERA)*

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## ABSTRACT

Fruit wines are undistilled alcoholic beverages usually from grapes with dates. Which are nutritive, tastier, and milder stimulates. Being fruit based fermented and undistilled product, wine contains most of the nutritive value of the wine is increased due to the release of amino acids and other nutrients from yeast fermentation. The final analysis of wine of various parameters alcohol content, PH, acidity, TSS, clarity was conducted and showed a PH range of 6, and the alcohol content was 10%. The results of process monitoring and final analysis will help a small-scale wine industry or can refer the results to develop a small-scale wine industry.

**KEYWORDS**: fermentation, wine,

## 1.INTRODUCTION

The wine was derived from the Greek word 'oines' meaning wine while the science of wine is called 'enology'. The proper dosage, or moderate intake of wine, in addition to affecting cholesterol levels favorably, decreases the tendency of blood to clot and assists in dissolving clots, and all important factors in protecting against heart disease. Decreasing ovarian cancer risk in women and making the bones stronger**.** Findings show that wine is active against oral *Streptococci* and *S.pygogenes* and suggest that it enhances oral health[1]. Research also indicates that moderate wine drinking may reduce the tendency of arteries to constrict during stress, lower blood pressure, and increase coronary artery diameter and blood flow. It serves as an important adjunct to the human diet as it increases the satisfaction by providing relaxation necessary for proper digestion and absorption of food[2].

### Antibacterial activity

The consumption of wine has also been reported to have an antimicrobial effect against various pathogens. Wine has bactericidal activity[3] against*, Acinetobacter, Escherichia coli, and Klebsiella.*

## 2.MATERIALS AND METHODS

### 2.1 SITE OF COLLECTION

Dates and Grapes are collected from the Kumbakonam market, Thanjavur (Dt), Tamilnadu. At evening time in the sterile plastic cans, the samples were kept in clean dry bottles previously washed and rinsed by distilled water. The sample was then stored at 4° C and it was used for further studies.

### 2.2 Preparation of Sample

**1.Sterilization of sample**

The sample of dates and grapes are sterilized by using hot water.

**2. Inoculum Preparation**

Twenty-five ml of sterilized GYE(Glucose Yeast Extract)broth (Yeast extract, 0.3%, malt extract, 0.3%, peptone, 0.5%, and glucose, 1%, pH 4.5), dispensed in 100 ml flask was inoculated with the loopful culture of *Saccharomyces cerevisiae*. The flask was incubated at 30°Conc.

### 2.3 Wine Preparation

**FERMENTATION**

Freshly harvested grapes are crushed and the resulting juice (must) is collected. The must contain fermentable sugars and natural yeasts which, either by themselves or with the help of additional yeast cultures, start the fermentation process in which mainly ethyl alcohol and carbon dioxide are formed. The latter is a gas and escapes from the must. The fermentation process comes to a halt when all of the sugars are fermented or the alcohol concentration becomes too high and kills scoff the yeasts. At this point the must have turned into wine. Fermentation was terminated after four weeks; the wine was then stored to allow the yeast to flocculate. The resulting wine was then aged for two months. The aged wine was then filtered using a pressurized filtering kit, decanted into sterile bottles and corked.

**Maturation of wine**

The prepared wine is tested for antibacterial activity, Physico-Chemical parameter, [4]Total soluble solids (TSS), pH, Acidity, Alcohol by oxidation method[5], Estimation of Protein, Estimation Of carbohydrate, Microbial analysis[7].

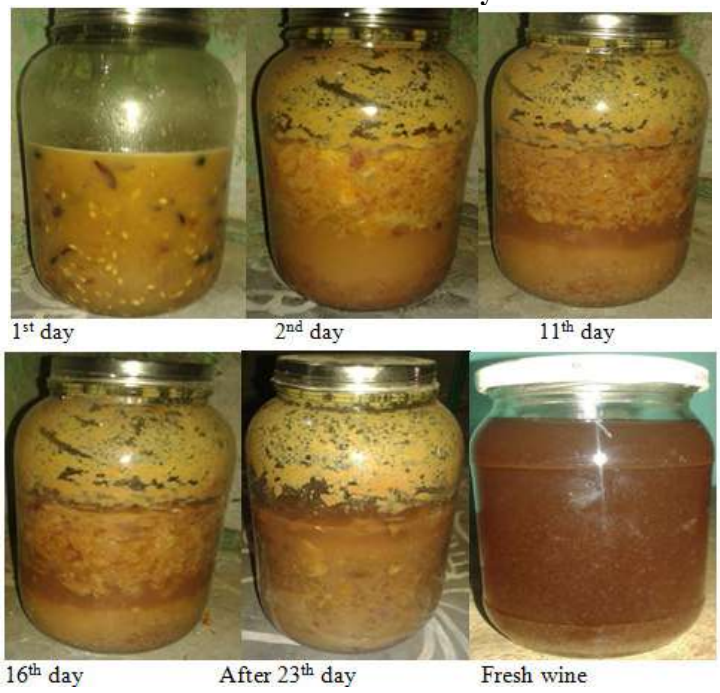
## 3. RESULT

Dates and grapes provided a good growth medium for *saccharomyces cerevisiae* and were converted into wine. The yeast strain grew well and utilized sugar at the rate of 750g/days/1000ml with over 90% fermentation efficiency thus yielding a brownish color alcoholic beverage. During the fermentation, the pH decreased with days of fermentation.

**Estimation of nutrients from wine**

The range of protein content in wine is 0.1%. Carbohydrate content is 2.5%. In Microbial analysis, the result shows that the beverage was produced under hygienic conditions and is safe for human consumption.

**Production of wine on a daily interval**




**Physico-chemical analysis of wine**

| S.No | Parameters | Value |
|---|---|---|
| 1. | Ph | 6.0 |
| 2. | Temperature | 37ºC |
| 3. | Acidity | 0.63 |
| 4. | Clarity(%absorbance) | 0.278 |
| 5. | TSS | 11.4 |

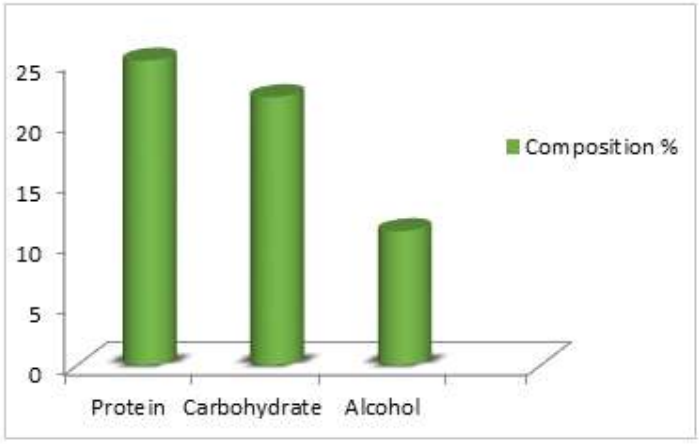
**Nutritional analysis of wine**

| S.No | Nutrients | Composition % |
|---|---|---|
| 1. | Protein | 25% |
| 2. | Carbohydrate | 22% |
| 3. | Alcohol | 11% |

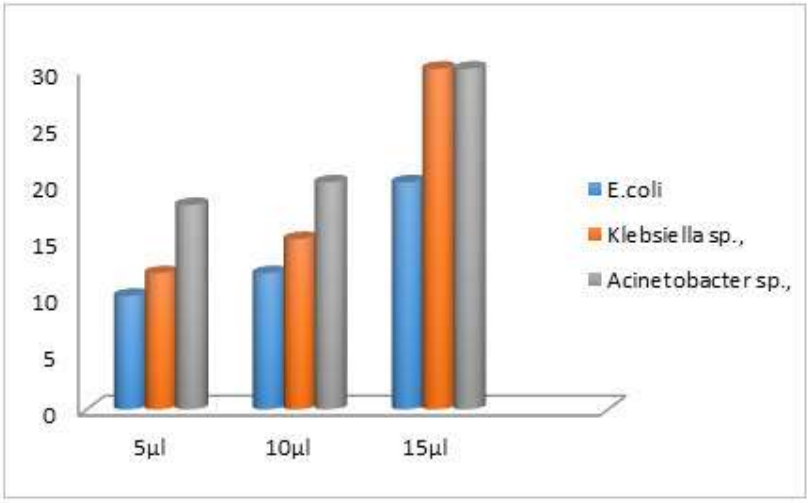
**Antibacterial activity of wine**

| S.No | Different concentration of wine | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | *Escherichia coli* | *Klebsiella sp.,* | *Acinetobacter sp.,* |
| 1. | 5µl | 10 | 12 | 18 |
| 2. | 10µl | 12 | 15 | 20 |
| 3. | 15µl | 20 | 30 | 30 |

**Nutritional analysis of wine**




**Antibacterial activity of wine**




## 4. DISCUSSION AND SUMMARY

The fermentation temperature commonly employed by the winemaker's ranges between 50°F to 70°F. The wine was prepared after 21 days of fermentation[8]. In the previous investigation fermentation process occurred in two stages; the primary stage, characterized by a high rate of fermentation, and a secondary stage which showed a slowdown of the fermentation rate as a result of the effect of alcohol concentration

on yeast cells. The high rate of fermentation in the primary stage is due to the presence of yeast nutrients at the beginning of fermentation which was gradually used up as fermentation progressed. This is in agreement with previous[9]. The low TTA of the wine is a reflection of the initial low pH of the Roselle calyx extract[10]. The different free sugars reported in grapes and dates have been sucrose, xylose, and fructose[11]. The final analysis of wine was conducted. The titrable homemade wines have relatively low alcohol content than the commercially available wine and there is no usage of either any preservative or any additives, so homemade wines are not harmful for health and are acceptable for daily usage. Dates and grapes both in fresh as well as in processed form not only improve the quality of our diet but also provide essential ingredients like vitamins, minerals, carbohydrates, proteins.

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SP-28

# UTILIZATION OF DORMANT STAGE OF *Bacillus megatherium* AS AN INOCULANT

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## ABSTRACT

Phosphorus is one of the essential nutrients next to nitrogen required for plant growth. Phosphorus is present soil in two forms such as inorganic phosphorus compounds. Around 40 to 95% of phosphorus is present in an inorganic form in the soil either as calcium phosphate as iron and Aluminium phosphate or apatite fluorapatite. The important calcium phosphate compounds are monocalcium phosphate, Dicalcium phosphate. The most important are phytin and phytin derivatives, Nucelic and phospholipid. In this study, experiments were conducted to assess the conditions favoring the *Bacillus megatherium* to form spores and their tolerance towards high temperature. The regeneration ability of *Bacillus megatherium* was also studying. The sporulated *Bacillus megatherium* culture may be used as a bio inoculant for phosphorus solubilization since it has a longer shelf life, regeneration ability, and capable of withstanding the unfavourable conditions.

**KEYWORDS:** *Bacillus megatherium*, Phosphorus, inoculant, gram-positive

## INTRODUCTION

The soil releases the phosphorus, but in very small quantities. Many of our soils contain a good amount of total phosphate. Whatever the quantity of phosphorus applied to soil through phosphatic fertilizers, only 20 to 25% phosphorus is available to plant. The most available forms of phosphorus are moderately available as HPO42 and least available as Po3. In neutral or alkaline soils having a high content of calcium and magnesium, the precipitation of calcium phosphate and magnesium takes place. In acidic soils, the phosphate ion will soluble iron and aluminum ions to form insoluble phosphate. Some microorganisms can solubilize the fixed phosphate and they are called phosphate solubilizing microorganisms. Mature spores have no detectable metabolism a state that is described as cryptobiotic They are highly resistant to environmental stresses such as high temperature. Sporulation temperature and heat resistance also *Bacillus* species. Sporulation temperature also influences the effect of pH of heating menstruum and the effect of different acids on spore heat resistance lactic acid decreases spore heat resistance more than other acidulants, but only when spores had been sporulated at spores if *Bacillus* and *Clostridium* species as these spores require incubation at temperature 30ºC to 40ºC higher to achieve inactivation equivalent to that of growing cells of some organisms. *Bacillus subtilis* spores can survive moist heat with a D value of 20 to 30 minutes. More ever, spores survive approximately 1000-fold longer in day heat than in moist heat. The proteins are uniquely present in spores which are dedicated to the repair during germination and outgrowth of the major lesion caused by UV radiation of spores. The second theory[1] of spore formation and their view of the arrangement of chromatin, when first seen during germination closely resembles the open ring structure found in sliced or smashed resting spores[1].

## MATERIALS AND METHODS

## SITE OF COLLECTION

For microbial analysis, the sample was collected from Tamil Nadu Agricultural College Trichy. Rhizosphere soil samples were collected and to the laboratory for further investigation.

## TRANSPORT OF SAMPLE

The samples were collected in and around the rhizosphere soil of Tamil Nadu Agriculture College, Trichy. They were kept in a polythene bag and transported to the laboratory.

## ISOLATION OF *Bacillus megatherium* FROM SOIL MATERIAL

For isolation of *Bacillus megatherium* from soil Katznelson and Bose medium was prepared. 100 gm of and rhizosphere soil suspension was prepared. Serial dilution was made. 10º5 & 10º6 dilutions in the case of rhizosphere samples were pipetted out and kept 4 plates for each treatment. The plates incubated at 48 hours. The clear halo zone was observed around bacterial colonies surrounded by a turbid white background after 5-7 days of incubation. The numbers of halo were counted in each plate and arrive at the average number of phosphate dissolving bacteria in each case. The population of *Bacillus megatherium* per gram of soil or rhizosphere sample is calculated and empress on a dry weight basis.

## PREPARATION OF SPORULATION MEDIUM

## SPORULATION OF *BACILLUS MEGATHERIUM* USING AMENDMENTS AND TEMPERATURE.

For testing the sporulation in the medium, the broth was amended with K2HPo4, HCL. About 50ml of broth, which was amended by using the above chemicals was poured into an appropriate conical flask (9.0,5.5) about 50ml of broth poured into a conical flask. These flasks were maintained.

## GRAM STAINING

## TEMPERATURE TOLERANCE OF BACILLUS CULTURES

## BIOCHEMICAL TESTS[2]

Carbohydrate test, Indole production test, Methyl red test, VP test, Citrate utilization test, Gelatin hydrolysis, starch hydrolysis, Catalase test, Urease test, Lipid hydrolysis.

## RESULT

In this present study, the *Bacillus megatherium* was observed from rhizosphere soil in Tamil Nadu Agricultural College, Trichy. In the present investigation, sporulation observed on the 3-rd day was 912 which gradually increased up to 7$^{th}$ day reaching a maximum of 1112 and then declined on the 9$^{th}$ and 11$^{th}$ day of inoculation. The buffer K2HPo4 has good potential in increasing the spore load rapidly.

## MODIFICATION OF PEPTONE BEEF EXTRACT MEDIUM WITH CALCIUM CARBONATE

The peptone beef extract medium was modified by adding the calcium carbonate at the rate of 0.01 gm per 500 ml of the medium prepared. The calcium availability in the medium promotes sporulation. The observation was recorded. The spore numbers gradually increased from 3$^{rd}$ day up to 9$^{th}$ day reaching a maximum of 198 spores. Then the spore load decreased on the 11$^{th}$ day of incubation.

## EXPOSE OF PEPTONE BEEF EXTRACT MEDIUM TO A TEMPERATURE

The prepared peptone beef extract medium was subjected to temperature. This temperature was identified to be the best sporulation temperature. The finding is listed. The spores load was found to be in the increasing trend up to 7$^{th}$ day followed by a decline any higher temperature above 50C has not offered good sporulation.

## MODIFICATION OF PEPTONE BEEF EXTRACT MEDIUM WITH DILUTE HYDROCHLORIC ACID AND ALTERING THE PH OF THE MEDIUM TO ACETATE CONDITION

The peptone beef extract medium was modified by the addition of dilute hydrochloric acid. The pH of the medium was altered to 5.5. The observations recorded were as follows. The spore load increase was noticed up to 7days followed by the decline in spore numbers. Maximum sporulation was witnessed on the 7$^{th}$ day. Regeneration of *Bacillus megatherium* spores was studied using bore well water and distilled water. Hundred percent regeneration was observed in the spores with the addition of bore well water

followed by sterile water added treatment. It is interesting to note that all the spores could regenerate by the addition of water.

**STUDIES ON THE SPORULATION ABILITY USING DIPOTASSIUM HYDROGEN PHOSPHATE**

| SI.NO | pH level | Total number of spores from days of inoculants | | | | |
|---|---|---|---|---|---|---|
| | | $3^{rd}$ | $5^{th}$ | $7^{th}$ | $9^{th}$ | $11^{th}$ |
| 1. | 9.0 | 912 | 1098 | 1112 | 1100 | 908 |

**STUDIES ON THE SPORULATION ABILITY USING CALCIUM CARBONATE**

| SI.NO | NAME OF THE CHEMICAL | Spores observed (in numbers) | | | | |
|---|---|---|---|---|---|---|
| | | $3^{rd}$ Day | $5^{th}$ Day | $7^{th}$ day | $9^{th}$ day | $11^{th}$ Day |
| 1. | Calcium carbonate | 53 | 61 | 176 | 198 | 112 |

**STUDIES ON THE SPORULATING ABILITY USING TEMPERATURE (50$^{o}$C)**

| SI.NO | Temperature | Spores count (in numbers) | | | | |
|---|---|---|---|---|---|---|
| | | $3^{rd}$ day | $5^{th}$ day | $7^{th}$ day | $9^{th}$ day | $11^{th}$ Day |
| 1. | 50C | 181 | 210 | 402 | 398 | 312 |

**STUDIES ON THE SPORULATING ABILITY USING HCL**

| S.NO | $P^{H}$ | Spores Count (in numbers) | | | | |
|---|---|---|---|---|---|---|
| | | $3^{rd}$ Day | $5^{th}$ Day | $7^{th}$ Day | $9^{th}$ Day | $11^{TH}$ Day |
| 1 | 5.5 | 28 | 42 | 54 | 41 | 38 |

**COMPARISON OF THE TREATMENTS**

| S.NO | Name of the treatment | Spores Count (in numbers) | | | | |
|---|---|---|---|---|---|---|
| | | $3^{rd}$ Day | $5^{th}$ Day | $7^{th}$ Day | $9^{th}$ Day | $11^{th}$ Day |
| 1 | PBE modified with $K_2Po_4$ (pH – 9.0). | 912 | 1098 | 1112 | 1100 | 908 |
| 2 | PBE modified with $CaCo_3$ | 53 | 61 | 176 | 198 | 112 |
| 3 | PBE exposed to 50$^{o}$C | 181 | 210 | 402 | 398 | 312 |
| 4 | PBE modified with dilute HCL | 28 | 42 | 54 | 41 | 38 |

**STUDY ON SPORE GERMINATION (1%)**

| S.NO | Treatments | Spore Germination (%) | |
|---|---|---|---|
| | | Bore well | Sterile water |
| 1 | T1 | 100 | 96.8 |
| 2 | T2 | 92.4 | 88.4 |
| 3 | T3 | 97.6 | 94.82 |
| 4 | T4 | 96.7 | 88.2 |

**DISCUSSION AND SUMMARY**

According to our current understanding, the developmental pathway leading from a vegetatively growing bacterial cell to a spore is triggered by depletion from the bacterium's local environment of a readily metabolized form of carbon, nitrogen, or phosphate. Biological control offers an environmentally friendly alternative to the use of pesticides for controlling plant diseases. Knowledge of the biological environment in which the agent will be used and of how to produce a stable formulation is critical to successful biocontrol[3-7]. In the dormant state, spores undergo no detectable metabolism and exhibit a higher degree of resistance to inactivation by various physical insults, including wet and dry heat, UV and gamma radiation, extreme desiccation, and oxidizing agents.

## CONCLUSION

In this present study, the use of dipotassium hydrogen phosphate, calcium carbonate, dilute hydrochloric acid exposure to a temperature of 50ºC have given excellent spore formation in *Bacillus Megatherium.* These chemical treatments and modifications made to the peptone beef extract medium have yielded a maximum number of spores. In this study, experiments were conducted to assess the conditions favoring the *Bacillus Megatherium* to form spores and their tolerance towards high temperature. The regeneration ability of *Bacillus Megatherium* was also studied. The sporulated *Bacillus Megatherium* culture may be used as a bio inoculant solubilization since it has a longer shelf life, regeneration ability and capable of withstanding the unfavorable conditions.

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SP-29

# STUDY OF SECONDARY METABOLITES AND ANTIBACTERIAL ACTIVITY OF *Coleus forskohlii* AGAINST HUMAN PATHOGENS

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## ABSTRACT

Medicinal plants are important for pharmacological research and drug development. Medicinal plants are *Coleus forskohlii* are collected in the villages of Salem district, Tamil Nadu [India]. Plants cultivated in this region are not systematically tested for the antibacterial activity. The crude root extract was collected for this research. Three different solvents include methanol, ethanol, and aqueous extracts of roots that were examined for qualitative analysis of secondary metabolites. The extract was subjected for antibacterial effect against the human pathogens by the Disc diffusion method.

**KEYWORDS:** *Coleus forskohlii,* root extract, secondary metabolites, Human pathogens & antibacterial activity.

## INTRODUCTION

*C.forskohlii* brig is a member of the mint family Lamiaceae. It is indigenous to India and is recorded in Ayurvedic material under the Sanskrit name "Makandi" and "Mayani"[8]. The experimental plant of the present study *coleus forskohlii* root is mint family has long been cultivated in India. The plant contains its active alkaloid components in the root system. The antibacterial effect of *Coleus* root against pathogens *Escherichia coli, Klebsiella, staphylococcus aureus.* The disc diffusion method has been adopted in this study and Petri dishes containing nutrient agar medium with the test pathogens were used for the antibacterial assay. Then the plates were incubated at 37°c for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition. The antibacterial screening showed that the root extract of *Coleus forskohlii* showed antibacterial activity against test pathogens used in this study depends on the active alkaloid compound in the extract.WHO indicates that about70 - 80 % of the world population in the developing countries depends on herbal sources. Phytoconstituents such as flavonoids alkaloids. Fannies & triterpenoids are a rich source of many medicinal plants that challenges modern medicine. The plant has ethno medicinal importance for the treatment of eczema, asthma, psoriasis, cardiovascular disorder and hypertension.

## MATERIALS &METHODS

### PLANT SAMPLE COLLECTION

*Coleus forskohlii* root sample was collected from Salem city using sterilized bag and was transferred to the lab within 7 hours after collection the sample was immediate analyze were not possible. The sample was preserved at 4°c[6].

### PLANT IDENTIFICATION

The *Coleus forskohlii* plant was identified with the help of published regional flora (Gamble et al., 1925; Matthew et al., 1983) and the identified were verified with Salem.

### PREPARATION OF PLANT EXTRACT

The roots of *Coleus forskohlii* were collected and were shade dried. Powered and extracted in the soxhlet apparatus. Successively with methanol, ethanol & water respectively due to their nature of polarity. After extraction, the extracts were filtered through Whatman no: 1 filter paper & stored for further use. From the stock solution do&conc. 25% (0.5ml extract + 1.5 ml distilled water) 50% (1.0 ml extract + 1 ml

distilled water) and 100 %( 2ml extract only) of the extracts were prepared.

## PHYTOCHEMICAL SCREENING

The root extract of *Coleus forskohlii* was analyzed for the presence of Terpenoids, Flavonoids, Steroids, Anthroquine. Cyvcosides, sugar, alkaloid, quinone, phenols, tannins, saponins, protein, and lipids according to standard methods[7].

## STERILISATION

All the glasswares used were washed dried and sterilized in a hot air oven at a temperature of 160°c for 1 hour according to the method described by Adobe and eze (2004)

## SAMPLE COLLECTION

*Coleus forskohlii* root sample was collected from Salem city using the sterilized bag and was transferred to the lab within 7 hours after collection the sample was immediate analyze were not possible. The sample was preserved at 4°C (Pavendan et al., 2011)

## MICROBIAL SCREENING

The samples were serially diluted and spread on to the sterile nutrient agar, McConkey agar, EMB agar. All the plates were incubated at 37°c for 24-48 hours. The isolated bacteria colonies were identified by using Biochemical standard procedure (Holt et al.)

## DISC DIFFUSION METHOD USING ROOT EXTRACT (LIQUID)

The root of *Coleus forskohlii* was collected and were air-dried, powdered using a mortar and pestle and were further the powder was transferred into the closed container. Each of the powdered air-dried plant root material was extracted with water, methanol, and ethanol 25 gm of each powdered sample was mixed in a conical flask with 100 ml distilled water. Plugged, then shaken at 120 rpm for 30 minutes and kept for 24 hours. After 24 hours each of the extracts was filtered through whatman no: 1filterpaper.

## DETERMINATION OF MIC AND MBC

The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates. To 0.5 ml of varying concentration of the extracts (25% , 50%, 75% , & 100%),2 ml of nutrient broth was added and then added test organism. Tubes containing bacterial cultures were than incubated at 37°c for 24 hours. After incubation, the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination. Nutrient agar was streaked with the test organism respectively to serve as control. Plates incubated with bacteria then incubated at 37°c for 24 hours. After incubation the concentration in which no visible growth was seen was noted as the minimum bactericidal concentration.

## RESULTS AND DISCUSSION

Plants are known to have beneficial therapeutic effects in the traditional Indian system of medicin[2] much work has been done on ethnomedicinal plants in India[9]. The plant photochemically extracts from plants to be used in allopathic medicine as they are potential sources of antimicrobial agent[5] medicinal plants possess high antioxidant property containing a high amount of flavonoid and phenolics[4]. The results of the antibacterial activity of *Coleus forskohlii* crude extract assayed in vitro by the disc diffusion method. The growth inhibitory effect of *Klebsiella pneumonia , Escherichia coli,* are presented in Table-1. In our study remarkable antibacterial activity against gram-negative bacterial strains than gram-positive

**Table1: Biochemical analysis**

| S.NO | Biochemical Reaction | *E.Coli* | *Klebsiella Sp.,* | *Pseudomonas Sp.,* |
|---|---|---|---|---|
| 1 | Indole | + | - | - |
| 2 | Methyl Red | + | - | - |
| 3 | Citrate | - | + | + |
| 4 | Urease | - | + | - |
| 5 | Oxidase | - | - | + |
| 6 | Catalase | + | - | + |

**Table2: Biochemical analysis**

| S.NO | Character | *E.Coli* | *Klebsiella Sp.,* | *Pseudomonas Sp.,* |
|---|---|---|---|---|
| 1 | Gram reaction | - | - | - |
| 2 | Morphology | Rod | Rod | Rod |
| 3 | Motility | Motile | Motile | Motile |
| 4 | Colony Character | Metalic seen in Macconkey | Metalic seen in Macconkey | Produce yellow pigment |

**Table3: Qualitative analysis of secondary metabolites on**

| S. No | Extracts | Analysis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Alkaloid | | Flavonoids | | Terpnoids | Steroids | Anthroquinone |
| | | T1 | T2 | T1 | T2 | | | - |
| 1 | Aqueous | - | + | - | - | - | - | - |
| 2 | Ethanol | - | - | + | + | + | + | - |
| 3 | Methanol | - | - | - | - | - | - | - |

| Glycosides | Sugar | Phenols | Tannins | Saponing | Protein | Lipids |
|---|---|---|---|---|---|---|
| + | + | - | + | - | - | + |
| - | + | + | - | + | + | + |
| + | + | + | + | - | - | - |

**Table4: Antibacterial activity of coleus frskohlii extracts**

| S.No | Plant extract | Zone of inhibition (mm) *E.coli* | | | | |
|---|---|---|---|---|---|---|
| | | Positive control | 25µl | 30µl | 35µl | 40µl |
| 1 | Aqueous | 17 | 0 | 0 | 05 | 07 |
| 2 | Ethanol | 17 | 0 | 0 | 6 | 11 |
| 3 | Methanol | 17 | 0 | 0 | 06 | 07 |
| **Zone of inhibition (mm)*Klebsiella Sp.,*** | | | | | | |
| | | Positive control | 25µl | 30µl | 35µl | 40µl |
| 1 | Aqueous | 22 | 0 | 01 | 03 | 04 |
| 2 | Ethanol | 22 | 04 | 06 | 11 | 15 |
| 3 | Methanol | 22 | 01 | 02 | 05 | 07 |
| **Zone of inhibition (mm)*Pseudomonas Sp.,*** | | | | | | |
| | | Positive control | 25µl | 30µl | 35µl | 40µl |
| 1 | Aqueous | 22 | 0 | 01 | 03 | 04 |
| 2 | Ethanol | 22 | 04 | 06 | 11 | 15 |
| 3 | Methanol | 22 | 01 | 02 | 05 | 07 |

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SP-30

# PHYTOCHEMICAL SCREENING AN ANTIBACTERIAL ACTIVITY OF *Acalypha indica Linn.*

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## ABSTRACT

The medicinal plants have similar properties as conventional pharmaceuticals and are applied for humans to cure or reduce symptoms from sickness. One of the traditional medicinal plants that is *Acalypha indica* which belongs to Euphorbiaceae can be used to treat multiple infections. The phytochemical analysis of leaves of *Acalypha indica* was carried out by preparing aqueous and ethanolic extract. The components analyzed were saponins, flavonoids , terpenoids as cardiac glycosides. The medicinal properties of these components were characterized.

**KEYWORD:** *Acalypha indica,* phytochemicals, saponins, tannins, phlorotannins, terpenoids, flavonoids and steroids.

## INTRODUCTION

The number of traditions came to dominate the practice of herbal medicine at the end of the twentieth century[2]. It was the advent of antibiotics in the 1950s that led to the decline of the use of plant derivatives as antimicrobials[1]**.** Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century [16.] Medicinal components from plants play an important role in conventional as well as western medicine. Plant-derived drugs have been a part of the evolution of human, healthcare for thousands of years. Plant-based drugs were commonly used in India and China[8.] It has been reported to be useful in treating pneumonia, asthma, rheumatism, and several other ailments[9.]

### CHARACTERISTICSOFAcalypha Indica

Acalypha indica commonly known as "Kuppai meni" in Tamil, belongs to the family. Euphorbiaceae. It is a slender climbing shrub that grows to about 6 m high in marshy places [Jaures et al 2013], that has catkin-like inflorescences with cup-shaped involucres surrounding the minute flowers. It occurs throughout the tropics. The leaves are broad ovate, 1.2 cm–6.5 cm × 1 cm–4 cm (0.47 in–2.56 in × 0.39 in–1.57 in).The tiny male flowers are white-green, located on the upper part of the flower spikes, and are ebracteate. It is used as emetic, expectorant, laxative, for the treatment of Bronchitis, pneumonia, and pulmonary tuberculosis.

## CHEMICAL CONSTITUENTS

### LEAVES

The aerial part of the leaves contains acalyphin and flavonoids. They include clitoris, nicotiflorin, tannins, aurantiamide, biorobin, finders in and succinimide, etc.

## SCIENTIFIC CLASSIFICATION

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Order | : | Malpiigiales |
| Family | : | Euphorbiaceae |
| Genus | : | *Acalypha* |
| Species | : | *indica* |

## TRADITIONAL USES

The crushing plant is used for skin parasites. The sap of crushed leaves mixed with salt, or a decoction of plant, is used for scabies and other skin problems. The root decoction is also taken for intestinal worms and stomach ache. The leaf sap is taken as an emetic. *Acalypha indica* is listed in the Pharmacopoeia of India as an expectorant to treat asthma and pneumonia [Nameirakpam *et al*., 2002]. It was formerly listed in the British Pharmacopoeia.

## NUTRITIONAL VALUE OF *Acalypha indica*

A variety of compounds from plant sources like polysaccharides flavanoids, alkaloid terpenoids, lectins, proteins, peptides etc., are responsible for antimicrobial activity[11].

## SIDE EFFECTS & ALLERGIES OF *Acalypa indica*:

Ingestion of Acalypha indica may lead to hemolysis in people suffering from glucose-6-phosphate dehydrogenase deficiency. Acalyphin is used as a substitute for ipecacuanha, a vermifuge, expectorant and emetic. Some of the chemical compounds in Acalypha indica cause dark chocolate-brown discoloration of blood, and gastrointestinal irritation in rabbits[4].

## 2. MATERIALS AND METHODS

## COLLECTION OF CLINICAL SAMPLE

The clinical sample (urinary tract infection) collected aseptically was the clinical laboratory from Kumbakonam, Tanjore Dt, (Tamilnadu).

## ISOLATION OF PATHOGENIC ORGANISMS FROM CLINICAL SAMPLES

Isolation of clinical samples was performed by serial dilution technique using nutrient agar media. 1gram sample was serial dilution up to 10 power - 7 and 1ml of aliquots were streaked over the nutrient agar plates and incubated at 30°c fur 48hrs. After the incubation period, selected colonies of clinical samples were transferred from the mixed culture of the plate onto respective agar plates and agar slants and incubated at 30°c for 48hrs. Slants containing pure culture were stored at 4°c for further examination.

## PREPARATION OF MEDIA AND PLATING METHOD

The samples were serially diluted and then it was inoculated into a Petri plate containing nutrient agar media that was recorded after incubation period for 30°c for 48hrs. The morphological method consists of macroscopic and microscopic methods. The microscopic characterization was done by light microscopy. By using the microscopic method appearance of organisms was identified as colour, shape and morphology.

## COLLECTION OF PLANT

The *Acalypha indica* plant leaves were collected from the local area in Maharajapuram, Thiruvidaimarithur taluk, nearby Kumbakonam, Thanjavur District.

## PREPARATION OF PLANT EXTRACT

The full plants were cleaned and coarsely powdered after shade drying. The powder (100g) was extracted using Ethyl acetate, Petroleum ether and Toluene as a solvent. For extraction soxhlet apparatus was used. The extracts were concentrated under reduced pressure in a rotary evaporator. The solvent was removed in vacuo and the extract was used for the antibacterial assay. For these fractions, an antibacterial assay was carried out using four types of NICM bacterial cultures and four types of fungal strains. Using the extracts, stock solutions for each type of organic solvent was prepared by mixing well an appropriate amount of dried extracts with Dimethyl sulphoxide (DMSO) to obtain a final concentration of 100mg/ml. This was used for the evaluation of antibacterial and antifungal activities. Each solution was stored at 4°C after collecting in sterilized bottles until further use[14].

## STERILIZATION

All the glasswares were washed, dried and sterilized in a hot air oven at a temperature of 160°c for 1hr according to the method described by Adible and Eze (2014).

## ETHANOL AND AQUEOUS EXTRACTION

The plant materials were dried in the shade and powdered by a mechanical grinder[11.] The powder of *Acalypha indica* was initially defeated with Petroleum benzene(60-80°c) followed by 1000ml of ethanol, by using soxhlet extractor for 72hr at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper and then concentrated in a vacuum and dried at 45°c for ethanol elimination. The extracts were kept in a sterile bottle under refrigeration condition of about 2-8°c[13].

## TEST FOR PHYTOCHEMICAL SCREENING

The freshly prepared extracts (Aqueous and Ethanolic) were subjected to phytochemical screening to test for the presence of the phytoconstituents such as tannins, phlorotannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids following standard methods described by Harborne (1973) and Sofowara (1993).

## MICROSCOPIC EXAMINATION

The microbes motility was determined by the hanging drop method gram staining method determines the staining nature of isolated microbes

## RESULT

Medicinal plants are a very important and widely available resource for primary health care and complementary health care systems. The literature in this area of research showed that the plant Kingdom contains many species of plant harboring substance of medicinal value that have yet to be discovered[8]. Though a large number of plants show that they are a very rich source of compounds with possible antimicrobial activities, but more investigation is necessary.

## ANTIBACTERIAL ASSAY

In vitro antibacterial activities of all crude extracts of Ethyl acetate, Petroleum ether, and Toluene fractions were determined by standard agar disc diffusion assay. Petri dishes (100mm) containing 25 ml of MHA were seeded with 100 µl inoculums of the test strain (inoculums size was adjusted to deliver final inoculums of approximately (106 CFU/ml) and allowed to solidify. Discs of 6 mm diameter were placed on the solidified agar media with a help of a sterilized forceps[11].From the stock concentration of crude and fraction of extracts (100mg/ml), three different volumes of 50 µl, 75 µl, and 100 µl were used to load the sterile disc under aseptic conditions. The plate were incubated at the plates were incubated at 37̊C 24h. DMSO and sterilized distilled water were used as a positive control. Streptomycin (30 mg/disc/ was used as a standard antibiotic disc for crude extract testing.The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each extract was expressed in terms of the mean diameter of zone of inhibition (mm) produced by respective Acalypha indica extracts [15.] The tested organisms *E. coli* shows the zone of inhibition in *Acalyphaindica* leaf ethanol extract ranging from 10.2 ± 12.1, Aqueous 14.0± 16.3. Whereas the *Klebsiella sp.,* leaf ethanol extract ranging from 13.2 ± 18.73, Aqueous 8.1±10.42.The antibacterial activity of *Acalyphaindica* extract was presented in the table.

## ANTIBACTERIAL SENSITIVITY TEST OF *E. coli* and *Klebsiella sp.,* BY USING LEAF EXTRACT OF *Acalypha indica* IN ETHANOL AND AQUEOUS.

**TABLE:1- WELL DIFFUSION OF *Acalypha INDICA* METHOD FOR ZONE OF INHIBITION**

| BACTERIAL SPECIES | LEAF EXTRACT OF *Acalyphaindica* | |
|---|---|---|
| | ETHANOL ( mm) | AQUEOUS (mm) |
| *E. Coli* | 10.2 ± 12.1 | 14.0 ± 16.3 |
| *Klebsiella sp.,* | 13.2 ± 18.73 | 8.1 ± 10.42 |

**FIGURE1:- WELL DIFFUSION OF *Acalypha INDICA* METHOD FOR ZONE OF INHIBITION**

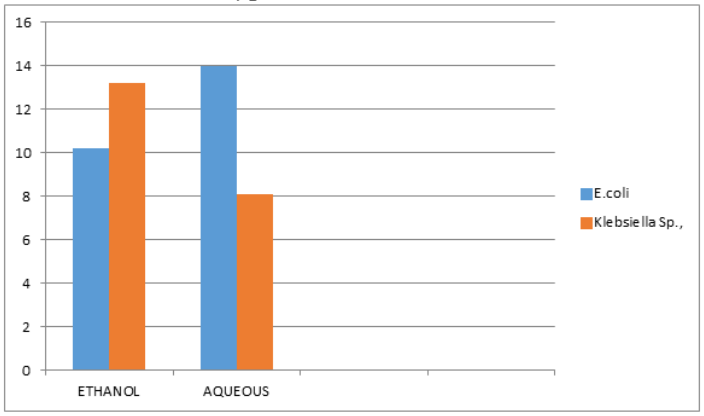
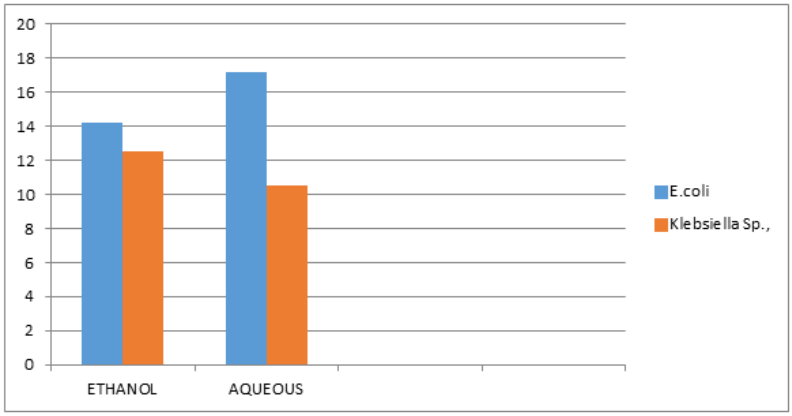



**TABLE: 2 ANTIBACTERIAL SENSITIVITY TEST OF *E. coli* and *Klebsiella sp.,* BY USING LEAF EXTRACT OF *Acalypha indica* IN ETHANOL AND AQUEOUS.( WHATTMAN NO. 1 FILTER PAPER) DISC METHOD FOR ZONE OF INHIBITION.**

| BACTERIAL SPECIES | LEAF EXTRACT OF *Acalyphaindica* | |
|---|---|---|
| | ETHANOL (mm) | AQUEOUS( mm ) |
| ***E. Coli*** | 14.2 ± 20.20 | 17.2 ± 24.20 |
| ***Klebsiella sp.,*** | 12.5 ± 18.22 | 10.5 ± 15.70 |

**Fig-2:- ANTIBACTERIAL SENSITIVITY TEST OF *E. coli* and *Klebsiella sp.,* BY USING LEAF EXTRACT OF *Acalypha indica* IN ETHANOL AND AQUEOUS. ( WHATTMAN NO. 1 FILTER PAPER) DISC METHOD FOR ZONE OF INHIBITION.**




## DISCUSSION

Hexane, chloroform, ethyl acetate and methanol extracts from the leaves of Acalypha indica were tested against Gram-positive (Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis) and Gram-negative (Klebsiella pneumoniae, Escherichia coli, Proteus Vulgaris, Pseudomonas aeruginosa) bacteria. All the extracts exhibited antibacterial activity against Gram-positive organisms with minimum inhibitory concentrations (MIC) between 0.156 to 2.5 mg/ml. Among the Gram-negative bacteria, only the Pseudomonas aeruginosa was susceptible to the extracts[10]. Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects. According to the World Health Organisation, medicinal plants would be the best source to obtain a variety of drugs. *Acalypha indica* is an indigenous medicine for the treatment of eye infection, wounds, joint pain, arthritis, and many other diseases[4.] The results of the phytochemical screening of A.indica leaves show that they are very rich in tannins, saponins, terpenoids, alkaloids and phlorotannins which are best known for their antimicrobial and

antiviral property[2.] Flavonoids are present in all vascular plants and have been reported to exert multiple biological effects including anti-inflammatory, antiulcerogenic, antiallergic, antiviral and cancer activities[5.] Medicinally, tannins are used in antidiarrhoeal, hemostatic and anti haemorrhoidal peparations. The plant contains more metabolites and there is a need for further investigation using fractionated extracts and purified chemicals compounds[7.] These results provide evidence that *Acalypha indica.* leaf extract possesses vital phytochemicals, antimicrobial and antioxidant properties.

## SUMMARY AND CONCLUSION

The *Acalypha indica* Linn leaves were collected and powdered. The powder was extracted by using solvents include ethyl acetate, petroleum ether, and toluene by soxhlet apparatus.In the antibacterial sensitivity test by the well diffusion method the ethanolic leaf extract show the minimal effect on *E.coli* instead aqueous leaf extract gives a good effect.In the case of *Klebsiella* species which is highly controlled by ethanolic leaf extract than aqueous extract.The disc diffusion method in which the aqueous leaf extract of *Acalypha indica* Linn effectively controls *E.coli* than ethanolic extract.In case of analyzing ethanolic leaf extract against *Klebsiella* species produce effective results than aqueous extract. Therefore for further analysis of medicinal properties, both ethanol and aqueous extract can be examined for any drug designing.

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# THE EFFECT OF PROBIOTIC BLEND ON GROWTH AND HISTOPATHOLOGICAL ANALYSIS OF FRESH WATER FISH *Labeo rohita* FINGERLINGS.

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## ABSTRACT

The probiotic effects of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* (probiotic blend) in fish have been documented recently. In this study, the effects of different levels of the probiotic blend on the growth performance, histopathological changes of *Labeo rohita* fingerlings were evaluated. Fish was fed with basal diet containing formulated feed (control), Exp-I-0.5gm, Exp-II-1gm, Exp-III-1.5gm probiotic blend for 60 days. Growth performance indices and histopathological changes were examined at 60 days of study. Growth performance is evaluated on days 30 and 60 of studying. Fish fingerling samples were taken at the same days of study and histological study including Gill, Liver, Muscle, and kidney effects are compared among the control. Results showed that oral administration of probiotic blend for 60 days had a significant impact on the growth rate of *Labeo rohita* fingerlings. Dietary administration of probiotic blend (Exp-III) increased considerably the growth rate compared to the control at 30 days of study. However, Exp-III showed significantly increased in growth rate compared to the control at day 60. Similarly, histopathological changes (Gill, Liver, Muscle and Kidney) were significantly no effects in Exp-I, Exp-II, Exp-III at day 60 of the study compared to the control. These results suggest that dietary supplementation of food with 1.5gm probiotic concentration in day 60 is suitable for enhancing the growth and histopathological changes of (*Labeo rohita*) rohu fish fingerlings.

**KEYWORDS:** *Lactobacillus acidophilus, Lactobacillus rhamnosus, Labeo rohita,* growth performance, histopathological.

## INTRODUCTION

Healthy food from aquatic resources is an important dietary component in many countries, the demand for which will continue in the forthcoming years[1,2,3,4,5]. Aquaculture is a fast developing industry and food sector in India. This development of aquaculture is primarily depended on availability of compatible and suitable diets. Fish meal has transformed into an important source of protein in fish diets because of its high protein quality and palatability. Nutrients derived from fish include vitamins, calcium, phosphorus and unsaturated fat. These nutrients when provided naturally or artificially in aquaculture enable the fish to rise adequately for the enhancement of health in humans. World aquaculture has grown[6] tremendously during the last few years growing up to be an economically significant industry. Nowadays it is the fastest growing food-producing sector in the world with the greatest potential to react to the growing demand for aquatic food[7]. Aquaculture has grown into one of the most promising and fastest growing industries. It provides high-quality and rich animal protein, raises nutritional levels, generates income and provides employment around the globe[8].

### Habit, Habitat & Distribution

Fresh water fish *Labeo rohita* being aqua farmed nowadays due to their taste and flesh[9].The fish *Labeo rohita* comes under the family Cyprinidae, commonly found in rivers and freshwater lakes in and around South-East Asia. It is an herbivore and eats mainly zooplankton, more phytoplankton, and as a juvenile or adult is a herbivorous column feeder, eating mainly phytoplankton and submerged vegetation.

**Probiotic in aquaculture**

The term "probiotic" was first used to denominate microorganisms that affect other microorganisms[10]. Etymologically, the term "probiotic" was originated from the Latin word "pro" which means "for" and the Greek word "bios" which means "life". The use of probiotics in aquaculture is now widely accepted with an increasing demand for neighborhood friendly aquaculture[11,12,13,14.]Recently, several preparations of probiotics are locally available and have been introduced to fish, shellfish, and molluscan farming as feed additives or incorporated in pond water[15,16.]. Probiotic is a relatively new term that is used to name microorganisms that are associated with the beneficial effects for the host. The probiotic supplement in aquaculture is considering the benefits of humans, animals, and poultry[17]. To avoid or reduce the use of certain antimicrobials, biological control was tested, described as the use of natural opponents to reduce the damage caused by harmful organisms. Strictly speaking, a probiotic should not be classified as a biological control agent because it is not necessarily a natural enemy of the pathogen[18].

***Lactobacillus acidophilus***

Preliminary studies of *L. acidophilus* were made on strains isolated from the fecal material of humans, pigs, and chickens[19].*Lactobacillus acidophilus* is part of the most effective forms of probiotic bacteria. Health benefits of *Lactobacillus acidophilus* include a reduction in the occurrence of diarrhea in humans, enhancement of the immune system, reduction in cholesterol and improved symptoms of lactose intolerance[20] and antitumor effects[21].

***Lactobacillus rhamnosus***

*Lactobacillus rhamnosus*, is a type of probiotic bacteria. Probiotic, set out by the Food and Agricultural Organization of the United Nations, are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host[22]." *L. rhamnosus* was first isolated in the intestine of a healthy human by scientists and when it was shown to have a remarkable tolerance for the harsh acid normally found in the stomach and digestive tract.

**Histological studies on fish**

The histopathological analysis seems to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as the gills, muscle, liver and kidney[23]. Pathogens produce pathological changes in fish such as necrosis in the liver, tubular damage of kidney and gill lamellar abnormalities[24]. Histology of fish liver could therefore serve as a model for examining the interactions between stress factors which include bio-toxins, parasites, infectious germs, physicochemical parameters and pollutants[25]. Pathogens produce pathological changes in fish such as necrosis in the liver, tubular damage of kidney and gill lamellar abnormalities. Therefore, histopathological studies are needed for the description and evaluation of potential lesions in aquatic animals exposed to various infections and toxicants in aquaculture.

## MATERIALS AND METHODS

**Procurement of fish**

Experiments were performed by the study of the probiotic acceptability. Growth rate and its histopathological aspects of *Labeo rohita* fingerlings with locally available ingredients were used. At the beginning of this study, fish specimens of *Labeo rohita* (average length 4–6cm and weight 4gm) were procured from Tamilnadu Fisheries Development Corporation Limited, KRP Dam Fish Farm, Krishnagiri, TamilNadu, India. 100 fingerlings were positioned in 5 polythene bags containing 10 liters of oxygenated water were transported with lesser disturbances. The collection and transportation of fingerlings needed to be done during evening hours. The cement tank was prepared prior by washing thoroughly with detergents and rinsed several times with water. The tank was then dried for two days and filled with bore well water. Water was aerated 24 hours prior to the introduction of the fingerlings. In the laboratory after initial placements of the polythene bag containing fingerlings for one hour in the aerated cement tanks, they were carefully discharged into the tank.

**Acclimatization of fish**

Fish was acclimatized for about 20 days before the commencement of the experiment. During acclimatization, fish were fed rice bran and groundnut oil cake. Water was substituted daily to minimize

contamination from metabolic wastes and also to secure a healthy environment. The feeding was withheld for 24hrs before the experiment and to keep the specimens more or less in the same metabolic state.

**Mortality rate**

To observe the mortality rate, the Fish was randomly selected from the stock and exposed to different concentrations of probiotic blend in unique 20-liter plastic troughs. Dead fish from the plastic trough were removed immediately. Death was indicated by the failure of the fish to react to gentle prodding with a glass rod and cessation of the opercula movement. The present study and were selected based on the survival/mortality ratio and the experiment was performed for 60 days.

**Feed**

The fingerlings were adopted in the five plastic troughs after measuring their initial length and weight. The fingerlings were fed with Grow-best fish pellet feed at the rate of 5gm per body weight through twice daily. For experiments, five batches of *Labeo rohita* containing fifty fingerlings were transferred to plastic troughs with 15 liter of aerated water. The following five types of feeding were offered to the *Labeo rohita* fingerlings.

1. Common feed.
2. Formulated feed.
3. Probiotic Blend (200mg) with (Figure-4.1) formulated feed-Exp.1
4. Probiotic Blend (300mg) with (Figure-4.2) formulated feed-Exp.2
5. Probiotic Blend (400mg) with (Figure-4.3) formulated feed-Exp.3

Formulated Feed Ingredients are Oilcacke (240 gm), Rice bran (680 gm), Tapioca powder (80 mg), Vitamins and minerals (15%), Protein-54% (min), Fat 14% (min), Moisture 12 % (max.) and Fibre 5% (max.).The ingredients were powdered and mixed clearly. A Prepared 10mg of feed (Control, Formulated, Exp.1, Exp.2, and Exp.3) was mixed with 20ml of RO water in beaker thoroughly, and then divided into five parts, control feed was common, formulated feed second trough and after few minutes probiotic blend is added and without formulated feed (Exp. I - 200mg probiotic, Exp.II - 300mg probiotic and Exp.III - 400mg probiotic (probiotic blend) was further added. The feed applications were started and given every morning from 9.00 to 10.00 am and from 4.00 to 5.00 pm daily up to the end of the experiment. Experiments were conducted with these feeds for 60 days. During the experimentation period also 50% of water was altered every day and two days once total water was completely renewed. Aeration and cleaning were done as mentioned for the acclimatization period. During the period every day fecal matter and other debris were abducted in the morning. Aeration was given continuously during the experimental period. During the cleaning and feeding time, the aerator was arrested and the fish were transferred in to further plastic trough, and then it was changed into their home through after cleaning. Monthly once weight and length of the fish were calculated with the help of electronic balance and values were noted, after 60 days 5 fish in each trough were taken for histological analysis. For the histological studies on gill, liver, muscle, and kidney were dissected from both control and experimental fingerlings of *Labeo rohita*. Gill, liver, muscle, and kidney from the body of control and experimental fish were removed and fixed in 10% formalin for 24 hours. The dissected fish organs were well analyzed.

**Histopathological study**

For histopathological studies of different tissues such as gill, liver, muscle and kidney were dissected from both control and treated fingerlings of *Labeo rohita*. The isolated tissue samples were as showed in Bouin's fixative for 24 hrs and washed with distilled water. The samples were dehydrated in different grades of alcohol series and treated further. Sections of 5-6µm thicknesses were taken using a microtome and stained using hematoxylin and eosin. Respectively mounted using DPX and observed under a compound microscope[26].

**Statistical Analysis:**

The data thus obtained was subjected to statistical analysis. The variation for various parameters and the significance of their interaction among the different experiments for the growth tested by using mean, standard deviation and one way ANOVA.

**RESULTS AND DISCUSSION**

**Growth Performance of Fish**

The growth performance of *Labeo rohita* fingerlings after one month similarly increased the growth rate in all experiments when compared with control. After two months of experiments, the growth rate significantly increased in all experiments when compared with control. The probiotic as better growth and survival conditions were observed in the zebrafish, it will help in improving the health and also reduce the rate of mortality[27]. Similarly the use of probiotic as biological control agents or as dietary live microbial supplements in commerce function and improve the growth of the fish. In the present study, the formulated, an experiment I, II and III we obtained growth rate increase in weight and length of the fish *Labeo rohita*, which was significantly higher than the control fed diets. This is consistent with the earlier findings by different authors[28]. The probiotic (Bacillus) supplemented diet significantly increased the weight and length of fish than the control diet without probiotic supplementation[29]. We had a higher growth rate in probiotic supplemented experiments (Exp-I, II and III)[30]. In the present study, the best growth and length values were noted in probiotic fed formulated feed, Exp-I, II and III (probiotic blend).

**Table. No 1. Mean and sd values for Initial weight, the final weight of the first month and final weight of the second month for the fish labeo*rohita* fingerlings.**

| Experiment (Mean Values) | Control | Formulated Feed | Experiment I | Experiment II | Experiment III |
|---|---|---|---|---|---|
| **Initial Weight** | 4.01 ± 0.3729 | 4.22 ± 0.5436 | 4.002 ± 0.3518 | 4.19 ± 0.3910 | 4.005 ± 0.4759 |
| **30 Days Weight** | 4.51 ± 0.3016 | 4.76 ± 0.5043 | 4.69 ± 0.3330 | 4.89 ± 0.3974 | 5.007 ± 0.4270 |
| **60 Days Weight** | 5.49 ± 0.4459 | 5.74 ± 0.4294 | 5.67 ± 0.3249 | 5.91 ± 0.2042 | 6.2 ± 0.3475 |

Each value is a mean ± SD of significantly different (P<0.05)

**Table. No 2.Mean values for Initial length, the final length of the first month and final length of second month for the fish labeo*rohita* fingerlings**

| Experiment (Meam Values) | Control | Formulated Feed | Experiment I | Experiment II | Experiment III |
|---|---|---|---|---|---|
| **Initial Length** | 4.94 ± 0.1907 | 5.03 ± 0.1586 | 4.97 ± 0.1791 | 4.9± 0.1732 | 4.89 ± 0.1573 |
| **30 Days Length** | 5.84 ± 0.2457 | 5.86 ± 0.1997 | 5.95 ± 0.1851 | 6.01 ± 0.1877 | 6.11 ± 0.1841 |
| **60 Days Length** | 7.005 ± 0.253 | 7.09 ± 0.2072 | 7.17 ± 0.2238 | 7.25 ± 0.2112 | 7.42 ±0.2227 |

Each value is a mean ± SD of significantly different (P<0.05)

**Table No.3: Significance of ANOVA weight and length of the fish *Labeo rohita* fingerlings**

| Experiment | Between Days | Significance |
|---|---|---|
| **Fish Weight** | **Initial** | 0.4952 |
| | **30 Days** | 0.0844 |
| | **60 Days** | 0.0050 |
| **Fish Length** | **Initial** | 0.6181 |
| | **30 Days** | 0.0387 |
| | **60 Days** | 0.0072 |

Each value is a mean ± SD (P<0.05)

The effects of probiotics have been investigated in many aquatic animals. Improvement of the growth has been recorded by feeding of *Bacillus sp.* In the Tilapia[31], *Catlacatla*[32], *Labeo rohita*, and *Penaeus monodon*. Growth enhancement as a result of probiotic administration has been reported in several previous studies on a variety of fish and shellfish species fed dietary probiotic[33,34,35,36,37,38]. The function of probiotic blend in the improvement of growth and feed utilization in fish was important to note as related to the improvement of nutrient digestibility. Probiotic to induce beneficial microflora into the larval intestine and cause high growth performance[39]. In the present study, better growth performance (weight and length) was observed in the *Labeo rohita* fingerlings with a fed probiotic blend.

**Histopathological studies**

In the present study, no histological alterations were found in different tissues namely liver, kidney, muscle and gills treated with probiotic blend on freshwater fish fingerlings *Labeo rohita*. Probably related to an increase in suitable attachment sites as a result of histological and functional development of fry and improved internal environmental conditions for bacterial growth.

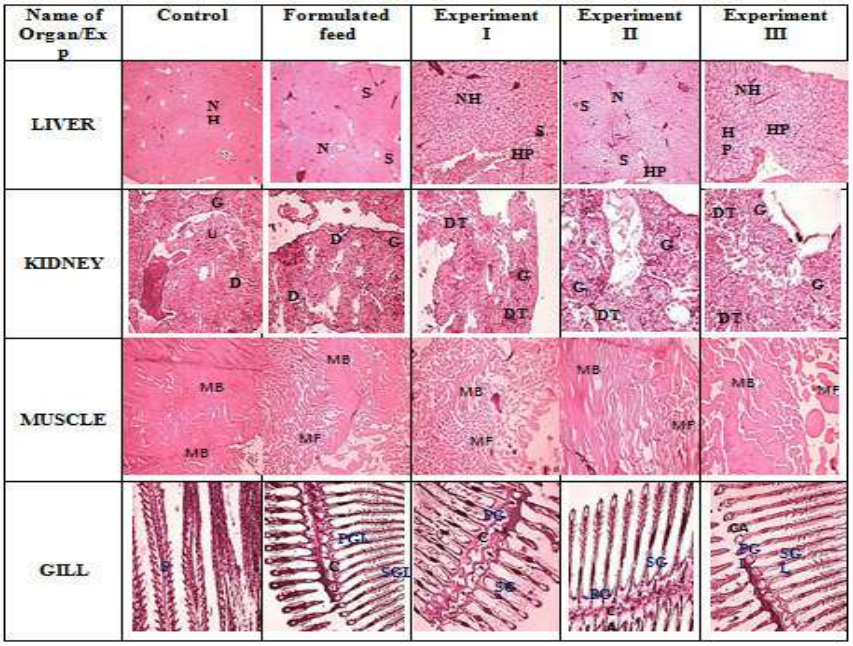
**Liver**

Liver of *Labeo rohita* fingerlings fed with control feed showed normal hepatic cells, polygonal in shape with almost centrally placed nuclei. Hepatic plate, hepatocytes with sinusoids, and nuclei were also pointed out. The liver derived from formulated, experimental (Exp-I, II and III) fingerlings showed no structural damage. Normal hepatic cells, almost centrally placed nuclei, hepatic plate and hepatocytes were also observed. However, the formulated, experimental fingerlings of probiotic fed challenged with probiotic blend showing the normal architecture, as saw in liver tissue of control. The histology of the liver showed normal hepatocytes, sinusoids and hepatic plate cells. Histologically there is no change such as any vacuolation. No necrosis and no nuclear condensation in hepatocytes were seen in the fingerlings. That fact shows that the probiotic promoted an increase in the epithelial layer of the middle intestine of those fish[40]. The results of hepatic enzymes analysis which decreased in fish kept on probiotic in comparison to control treatment indicating a normal, positive and beneficial effect of both probiotic on the maintenance of the integrity of hepatocytes and their roles in the improvement of liver histology. Analogous results observed by received the support of several authors[41,42,43].

**Kidney**

The Kidney of *Labeo rohita* fingerlings fed with control feed showed normal glomerulus, distal tubes and inter-renal cells. Kidney tissue is available from formulated, experimental (Exp-I, II and III) fingerlings showed no structural damage. Normal glomerulus, distal tubes and inter-renal cells were also noted. However, the formulated, experimental fingerlings of probiotic fed challenged with probiotic blend showing in the normal architecture, as saw in kidney tissue of control. The kidney is one of the vital organs to be affected by contaminants in the water[45]. In the present study, kidney showed normal architecture in the fingerlings fed with a probiotic blend (formulated feed and experiments (Exp-I, II and III). Fingerlings showed normal glomerulus and distal tubule and No necrosis was noted in the kidney.

**Plate No. 1. Histopathological analysis of the fish *Labeo rohita* fingerlings**




**Muscle**

Muscles of *Labeo rohita* fingerlings (control feed) showed the normal arrangements of muscle fibres and muscle bundles. Muscles tissue is obtained from formulated, experimental (Exp-I, II and III) fingerlings

showed no deformities. Normal arrangements of muscle fibres and muscle bundles were also pointed out. However the experiment fingerlings (probiotic blend) did not show any marked deformities and not infected in the muscles. Histology of formulated and probiotic fed muscles showed the normal architecture as saw in the control. The muscle of experimental (Exp-I, II and III) fish *Labeo rohita* fingerlings challenged with probiotic blend and did not show any deformities in the muscle. The muscle of the experimental *Labeo rohita* fingerlings showed the normal architecture as saw in the control.

**Gill**

Histological observations on gills of *Labeo rohita* fingerlings fed with a control fed showed the normal architecture of gill structure such as primary gill lamellae, secondary gill lamellae and the central axis. The gills of formulated and experimental (Exp-I, II and III) *Labeo rohita* fingerlings showed the normal architecture of gill filaments. No infection and no proliferation were found in gill lamellae, inter gill lamellae and primary gill lamellae filaments. However, the formulated, experiment (Exp-I, II and III) fingerlings challenged with probiotic blend and did not show any deformities in the gills. Histology of the gill showed the normal architecture as saw in the control, formulated and probiotic fed (probiotic blend) fingerlings. The histological observation of gills on control and probiotic supplemented (Exp-I, II and III) feeds with fingerlings showed normal architecture of primary gill and secondary lamellae. No infection and no proliferation were found in gill lamellae; inter gill lamellae and primary gill lamellae filaments.

## CONCLUSION

In conclusion, the result of the present study showed that supplementary feeds improved growth performance and histopathological parameters of Indian common carp *Labeo rohita* fingerlings that are can be associated with improving health status and physiological response, to elevate growth rate and histopathological observation ability of *Labeo rohita* fingerlings the present study is carried out. From the results obtained in this study, it was obvious that feeding probiotic blend to *Labeo rohita* fingerlings resulted in increased in the value of growth parameter and this is an indication that probiotic blend has a positive role to play in the maintenance of fish health especially *Labeo rohita* fingerlings, which is helpful in the reduction of input costs. These results suggest that dietary probiotic should be taken into consideration when a long-term oral administration is conducted.

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# STUDIES ON THE COMBINED EFFECT OF SEAGRASS *THALASSIA HEMPRICHII (*EHRB.) ASCHER'S EXTRACT AND PLANT GROWTH REGULATORS ON CHLOROPHYLL, NITRATE REDUCTASE ACTIVITY AND SUGAR CONTENT IN BLACK GRAM (*VIGNA MUNGO*).

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## ABSTRACT

The present study was conducted at Matha College of Arts and Science, Manamadurai during rabi season 2016-2017 and seaweeds collected at Thondi coastal waters. The black gram (*Vigna mungo*) is a plant species in the legume family native to the Indian subcontinent. Foliar applications of different levels of seagrass extract and plant growth regulators were studied on the black gram (*Vigna mungo*) to check their biochemical parameters like chlorophyll, nitrate reductase activity and sugar contents. Treatments were arranged in a randomized block design with on black clay soil. The data were analyzed in F and t-test was p= 0.5. Critical different values were calculated at 5% level of significance in F test. Foliar applications of gibberellic acid +naphthlene acetic acid + seagrass extract (*Thalassia hemprichii*) 50ppm were found to be superior compared to other treatments. This study has been able to show the phytochemical contents and the effects of hormones and seagrass extract on the plant growth, chlorophyll contents, nitrate reductase activity and total sugar in black gram. According to the study, the presence of gibberellic acid, naphthalene acetic acid and seagrass extract at 50ppm affects positively and the growth and biochemical parameters increased.

**KEY WORDS**: Plant, *Thalassia hemprichii*, chlorophyll, nitrate, sugar

## INTRODUCTION

The black gram (*Vigna mungo*) is a plant species in the legume family native to the Indian subcontinent. Black gram is the most commonly grown pulses crop in the hill districts of Assam and it contributes 12% of the total pulse production of the country. The extracts of seaweed and seagrass as fertilizer to provide beneficial effects of the plants[1]. The effect of seaweed and seagrasses liquid fertilizers on the chlorophyll content of zeamays[2]. *Thalassia hemprichii* was recorded from Krusadai and Rameshwaram Island[3]. The importance of seagrasses were reporte[4,5 &6] and plant growth regulators play an important role in plant life. Plant growth[7] regulators are used to modify and control the growth and development of vegetable crops. This study has been able to show the phytochemical contents and the effects of hormones and seagrass extract on the plant growth, chlorophyll contents, nitrate reductase activity and total sugar in black gram.

## MATERIAL & METHODS

Gibberellic acid and Naphthalene acetic acid were required chemicals purchased from Hariyali industries with high purity. Seagrass was collected at Thondi coastal area.

## EXPERIMENTAL PROCEDURES

The present study was conducted at Matha College of Arts and Science, Manamadurai during rabi season 2016-2017 and seaweeds collected at Thondi coastal waters, to find out the influence of seagrass extract and plant growth regulators on chlorophyll content, nitrate reductase activity and sugar content in black gram (*Vigna mungo*). The experiment consists of nine treatments having two plant growth regulators viz.,

gibberellic acid (50 and 100 ppm), naphthalene acetic acid (50 and 100 ppm) and seagrass extract (50 & 100 ppm). The experiment was laid out in a randomized block design with on black clay soil. The various biochemical parameters were studied at different stages. The chlorophyll content and nitrate reductase activity were estimated at 35, 55 and 75 DAS using DMSO method[8 &9]. The level of significance used in F and t-test was p=0.5. Critical difference values were calculated at 5% level of significance in F test.


**Figure 1. Seagrass Collected at Thondi Area**

## RESULTS

The observation regarding the growth and biochemical parameters of black gram in table 1 to 4. The growth attributes viz., plant height (at 35, 55, and 75 DAS) and a number of branches per plant at 55 DAS were significantly influenced due to various treatments (Figure 2-4). The similar results were also recorded by authors[11&12] in black gram. The data on chlorophyll content presented in table 2 indicated that there was an increase in chlorophyll up to 55 DAS and thereafter it decreased. There was no significant difference in chlorophyll a, b and total chlorophyll at 35 DAS. 50 ppm of $GA_3$ + NAA +SGE was recorded higher chlorophyll a ( 2.348 ) at 55 DAS followed by 100 ppm. Significantly chlorophyll b was also increased at 50 ppm followed by 100 ppm. The total chlorophyll maximum increased in the treatment of $GA_3$+NAA + SGE at 50 ppm showing maximum total chlorophyll (2.5308) followed by $GA_3$ + NAA + SGE at 100 ppm. At 75 DAS, GA3 + NAA + SGE at 50 ppm continued to show maximum chlorophyll 'a', 'b' and total chlorophyll among all the treatments. Control recorded minimum chlorophyll a, b and total chlorophyll at all stages. The increase in photosynthetic rate due to GA3 was reported by Arteca and Donga (1981).

**Table 1. Influence of plant growth regulators and SGE on plant height (cm) and number of branches per plant**

| Treatment | Plant height (cm) | | | Number of branches per plant |
|---|---|---|---|---|
| | 35 DAS | 55 DAS | 75 DAS | |
| $T_1$ - $GA_3$(50ppm) | 24.82 | 46.72 | 38.20 | 13.33 |
| $T_2$- $GA_3$(100ppm) | 22.81 | 46.20 | 38.60 | 11.88 |
| $T_3$- NAA(50ppm) | 25.21 | 47.01 | 42.11 | 15.35 |
| $T_4$- NAA(100ppm) | 22.64 | 46.16 | 37.14 | 10.72 |
| $T_5$ - SGE (50ppm) | 25.21 | 47.65 | 41.38 | 14.60 |
| $T_6$- SGE (100ppm) | 22.41 | 45.41 | 38.52 | 13.06 |
| $T_7$ - $GA_3$+NAA+SGE (50ppm) | 26.23 | 48.16 | 42.80 | 16.52 |
| $T_8$ -$GA_3$+NAA+SGE (100ppm) | 25.03 | 46.97 | 40.86 | 15.18 |

| | | | | |
|---|---|---|---|---|
| $T_9$ - Control | 22.62 | 45.20 | 36.50 | 10.58 |
| S.Em± | 0.76 | 1.00 | 1.20 | 0.26 |
| CD (5%) | NS | 2.22 | 3.53 | 0.76 |
| *Values are the averages of three replicates are found 5% level of significance between treatments and control.* | | | | |

**Table 2 Influence of plant growth regulators and SGE on chlorophyll a, b and total chlorophyll (mg/g fresh wt-1) in leave at different stages in black gram**

| Treatments | Days after sowing (DAS) | | | Days after sowing (DAS) | | | Days after sowing (DAS) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 35 | 55 | 75 | 35 | 55 | 75 | 35 | 55 | 75 |
| T1 - GA3(50ppm) | 0.771 | 2.278 | 0.857 | 0.169 | 1.281 | 0.228 | 0.940 | 3.559 | 1.085 |
| T2- GA3(100ppm) | 0.772 | 2.315 | 0.875 | 0.182 | 1.294 | 0.221 | 0.954 | 3.609 | 1.096 |
| T3- NAA(50ppm) | 0.769 | 2.181 | 0.822 | 0.165 | 1.274 | 0.204 | 0.934 | 3.455 | 1.026 |
| T4- NAA(100ppm) | 0.767 | 2.199 | 0.839 | 0.155 | 1.254 | 0.186 | 0.922 | 3.453 | 1.025 |
| T5 - SGE (50ppm) | 0.771 | 2.239 | 0.850 | 0.186 | 1.276 | 0.230 | 0.957 | 3.515 | 1.080 |
| T6- SGE (100ppm) | 0.772 | 2.242 | 0.838 | 0.190 | 1.260 | 0.206 | 0.962 | 3.502 | 1.044 |
| T7 - GA3+NAA+ SGE (50ppm) | 0.774 | 2.348 | 0.952 | 0.192 | 1.296 | 0.239 | 0.966 | 3.644 | 1.191 |
| T8 -GA3+NAA+ SGE (100ppm) | 0.772 | 2.282 | 0.937 | 0.165 | 1.285 | 0.221 | 0.937 | 3.567 | 1.158 |
| T9 – Control | 0.768 | 2.159 | 0.815 | 0.153 | 1.239 | 0.193 | 0.921 | 3.398 | 0.998 |
| S.Em± | 0.002 | 0.022 | 0.026 | 0.002 | 0.002 | 0.003 | 0.003 | 0.020 | 0.020 |
| CD (5%) | NS | 0.068 | 0.078 | NS | 0.008 | 0.009 | NS | 0.060 | 0.070 |

**Table 3 Influence of plant growth regulators and SGE on nitrate reductase activity (nmol No2 g. fr.wt.-1 hr-1 hr-1) in leaves at different stages in black gram**

| Treatment | Days after sowing (DAS) | | |
|---|---|---|---|
| | 35 | 55 | 75 |
| $T_1$ - $GA_3$ (50ppm) | 98.44 | 125.40 | 87.39 |
| $T_2$- $GA_3$ (100ppm) | 98.36 | 127.02 | 86.37 |
| $T_3$- NAA (50ppm) | 98.03 | 128.26 | 87.24 |
| $T_4$- NAA (100ppm) | 97.51 | 126.69 | 87.38 |
| $T_5$ - SGE (50ppm) | 102.94 | 122.90 | 88.88 |
| $T_6$- SGE (100ppm) | 105.98 | 129.16 | 89.02 |
| $T_7$ - $GA_3$+NAA+SGE (50ppm) | 101.80 | 135.28 | 89.41 |
| $T_8$ .$GA_3$+NAA+SGE (100ppm) | 101.62 | 131.76 | 87.53 |
| $T_9$ – Control | 94.85 | 114.62 | 85.41 |
| S. Em ± | 0.58 | 1.41 | 0.82 |
| CD (5%) | NS | 4.25 | 2.46 |

**Table 4. Influence of plant growth regulators and SGE on reducing sugars, non reducing sugars and total sugars (mg g fresh wt-1) in black gram at harvest**

| Treatments | Reducing sugars | Non reducing sugars | Total sugars |
|---|---|---|---|
| $T_1$ - $GA_3$(50ppm) | 0.67 | 4.41 | 5.08 |
| $T_2$- $GA_3$(100ppm) | 0.71 | 4.51 | 5.22 |
| $T_3$- NAA(50ppm) | 0.68 | 4.56 | 5.24 |
| $T_4$- NAA(100ppm) | 0.63 | 4.52 | 5.15 |

| | | | |
|---|---|---|---|
| $T_5$ - SGE (50ppm) | 0.71 | 4.53 | 5.24 |
| $T_6$- SGE (100ppm) | 0.73 | 4.54 | 5.27 |
| $T_7$ - $GA_3$+NAA+SGE (50ppm) | 0.73 | 4.64 | 5.37 |
| $T_8$ .$GA_3$+NAA+SGE (100ppm) | 0.65 | 4.59 | 5.28 |
| $T_9$ – Control | 0.61 | 4.29 | 4.90 |
| S.Em± | 0.03 | 0.19 | 0.19 |
| CD (5%) | 0.05 | 0.54 | 0.54 |

**Figure 2 Seed germination of *Vigna mungo***

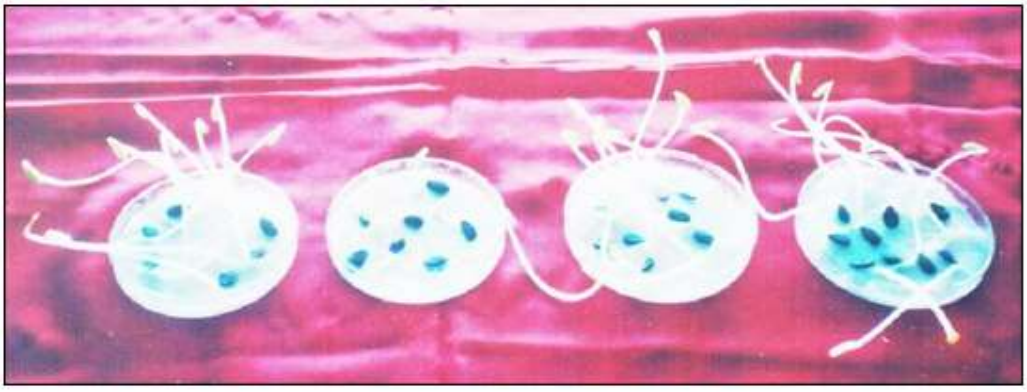

**Figure 3 Seed germination of *Vigna mungo***

**Figure 4 Growth of *Vigna mungo***

## DISCUSSION

A combination of plant growth regulators and seagrass extract exhibited a significant difference in nitrate reductase activity (NRA) in leaves (Table.3). However, at 35 DAS, there was no significant difference among the treatments. The present study revealed that NRA was maximum at 55 DAS and increased significantly with the foliar application of $GA_3$ + NAA + SGE at 50 ppm followed by 100 ppm as

compared to control. At 75 DAS, $GA_3$ + NAA + SGE at 50 ppm continued to show maximum NRA (89.41) followed by SGE at 100 ppm and SGE at 50 ppm. Authors[13] suggested that photosynthesis is associated with an increase in the enzyme activity and nucleic acid metabolism. Foliar application of plant growth regulators results in the enhanced nitrate uptake by plants[14]. The data on sugar content in black gram presented in table 4 indicated significant differences among the treatments. The maximum reducing sugar (0.73) was recorded in $GA_3$ + NAA+SGE at 50 ppm and SGE at 100 ppm. The minimum reducing sugar (0.61) was recorded in control. Significantly higher non reducing sugar (4.64) was also recorded in $GA_3$ + NAA + SGEat 50 ppm followed by $GA_3$+ NAA + SGE at 100 ppm compared to all the treatments. The total sugar also recorded maximum in GA3 + NAA + SGE at 50 ppm followed by $GA_3$ + NAA + SGE at 100 ppm and control showing significantly very low total sugar.

## CONCLUSION

This study has been able to show the phytochemical contents and the effects of hormones and seagrass extract on the plant growth, chlorophyll contents, nitrate reductase activity and total sugar in black gram. According to the study, the presence of gibberellic acid, naphthalene acetic acid and seagrass extract at 50 ppm affects positively and the growth and biochemical parameters increased. However, these effects must be taken into cognizance for optimal yield. Foliar applications of $GA_3$ + NAA + SGE at 50 ppm concentration increased yield of black gram.

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# FATTY ACID ANALYSIS IN LEAVES EXTRACTS OF *SYZYGIUM CUMINI* (INDIAN JAMUN) – AN INVITRO ANALYSIS

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## ABSTRACT

A *Syzygium cumini* (Indian Jamun) leaf comprised lipid fractions by the least percentage of dry weight and consistently blended in plastids. *Syzygium cumini* (Indian Jamun) leaf extract has done by Steam distillation (SD) extraction was evaluated and widely investigated for the first time. Using Steam distillation methods, methanol, and chloroform are used as the solvent for seclusion the plant lipids from Jamun leaves. Fatty acids (FAs) were standardized by using the robust technique and compared to Jamun leaf extracts were analyzed with corresponding standard fatty methyl esters (FAMEs) by gas chromatography The results, shows on GC analysis is Lauric acid (C12:0), Myristic acid (C14:0), palmitic acid (C16:0); Oleic acid (C18:1 Cis (n9), Linoleic acid (C18:2 Cis (n6), Linolenic acid (C18:3 (n6), Arachidonic acid (C20:0), Lignoceric acid (C24:0), Nervonic acid (C24:1). Fatty acid composition showed *S. Cumini* leaves were rich in antioxidants and essential fatty acids, specifically linolenic acid (18:3) whose percentage is detected as 100%. Further, these studies show that fatty acids ester's analysis has the dual role of metabolic or physiological changes which bring out in support of therapeutics antidiabetic, anti-inflammatory, hypolipidemic and antinephrotics.

**KEYWORDS:** GC-FID, *Syzygium cumini* (Indian Jamun) leaves, antioxidants

## INTRODUCTION

*Syzygium cumini* (Family: Myrtaceae) is also known as "Jamun or Indian blackberry" is an important medicinal plant in various traditional and alternative systems of herbal medicine[1]. It is more valuable in the cure of diabetes, inflammation, gastric ulcers, and biochemical studies have also exposed it to have the properties of chemopreventive, radioprotective and anticancer[2]. The leaves of the plant are rich in compounds containing flavanoids, anthocyanins, Cardiac glycoside, fats, steroids, isoquercetin, kaempferol, and myricetin. The seeds are contending to include terpenoids, alkaloid, jambosine, and glycoside, which stop the progress of the diastatic transfer of starch into sugar. Jamun is most often predictable as an adjuvant treatment in T2D. Because, in seeds of Jamun contains rich in anthocyanins, dark-purple fleshy pulp is used for in-vitro studies for treating T2D[3]. Plant lipids have a considerable influence on global wealth and human nutrition[1]. Plants metabolize a huge amount of essential fatty acids only a few are and other bioactive worldwide essential substances as palmitate, oleate, and linoleate[4]. In animal cell systems, fatty acids and triacylglycerol are essential for the catabolic biogenesis of cell membranes as signaling molecules as a source of carbon and energy. Plants have the source of carbon energy and oxygen power (reduced or nonreduced) changes are consequential for fat biosynthesis derived from chloroplast[5]. The catabolic pathway of lipids can be synthesized fatty acid in plants particularly on chloroplast of green cells and in the plastids of devoid of photosynthetic tissues and not takes place in the

cytosol as in the animal cell. Even if, oxidation of fatty acid synthesis is sited in the stroma cells, mitochondria of plants are limited which are not synthesized by fatty acids[6-7]. The membrane of plastid composed essentially galactolipids, whereas, phospholipids occur in extrachlorplast membranes of the animal cells[7]. In cell membranes, Fatty acids consist largely of polyunsaturated fatty acids and saturated fatty acids. A higher plant contains trienoic fatty acids which can occur in photosynthetic tissues and plastids[8]. Free fatty acid substrate Carbon 18:3 in plants are generally present in medicinal plants and advance angiosperm families like spinach and pea whose position is Super nuclear family-2 of the galactolipids is esterified obviously by PUFA with 18-20 carbon atoms. Brassica family plant contains the carbon family is 16:3 which are less evolved families in angiosperms whose position is Super nuclear family-2 of the galactolipids is esterified evidently through Polyunsaturated fatty acids with C-20 atoms[9]. Lipid structure of fatty acids present is atypical in plants; the membrane lipids also contain double bonds present in the fatty acids are Cis type. Understanding of Linoleate (sn-2) and palmitate (sn-1) require to prop up among plastids and endoplasmic reticulum for the growth of glycerophospholipids in chloroplasts[7-9]. Jamun is commonly known for its antidiabetic activity as it has been proved to be the most promising therapeutics for renal complications shows natural antioxidants[10]. The existence of bioactive compounds has the putative role of pharmacological effects with antioxidants, antimicrobial, antidiabetic, brain ischemia, anti-inflammatory, antiallergic, hepatoprotective respectively.

## MATERIAL AND METHODS

Plant materials Leaves of *S. cumini* (Indian Blackberry) were collected in June-July 2018-2019 from Agriculture Farm - Dhanalakshmi Srinivasan Agriculture College, Perambalur of Tamil Nadu, India from a single tree. The *S. cumini* leaves were identified and authenticated in the Department of Plant Biology and Plant Biotechnology, St. Joseph College (Autonomous), Trichy, Tamil Nadu, India.

### Preparation of extracts

The S. cumini leaves were first washed well several times with distilled water to remove the traces of debris. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with Methanol and Chloroform to remove lipids[12]. It was then filtered and the filtrate was discarded. The residue was successively extracted with methanol using a steam distillation method. Using Steam distillation methods, methanol, and chloroform are used as the solvent for seclusion the plant lipids from Jamun leaves. Fatty acids (FAs) were standardized and compared to Jamun leaf extracts were investigate through corresponding standard fatty methyl esters (FAMEs) by gas chromatography. The percentage yields were 12.36% in methanol. The phytochemical screening gave optimistic results for triterpenoids, flavonoids, saponins, and tannins[12-13].

## GC-FID METHOD

### Prepare the Sample

Transfer 5 mg of *S. cumini* leaves sample into a test tube. If the sample is in powder form, dilute directly with 1 ml of methanol. If the sample is in methanol and chloroform, evaporate the solvent with nitrogen and add 1 ml of methanol. Add 50 µl of isopropanol to the sample in methanol. Vortex mix thoroughly. Keep warm the sample at ambient temperature for 5 minutes. Centrifuge the sample at 3000 rpm for 10 minutes. The supernatant of *S.Cumini*.L was used for Fatty acid analysis through GC-FID detection[14-15].

### Instrument Conditions

| Injector temp | Detector temp | Oven temp | Column flow | Split flow | Run time |
|---|---|---|---|---|---|
| 225°C | 200°C | 200°C | 1 ml/min | 20:1 | 30 min. |

### Run the Standard

To run the program the sequence desk to run FAME standardization in first available position followed by NIST Library reference. Next, we should add the methanol solvent blank in the final lid and purge. Isopropanol was used as the diluents for saturated and unsaturated fatty acids due to their solubility. A fatty acid stock solution contains Oleic acid and related were prepared for FAME analysis in Jamun leaves extraction by sonication. Fatty acid methyl esters were used as the reference standard - Oleic acid (C18:1)

and individual fatty acids, including Lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C22:0), tricyclic acid (C23:0) and lignoceric acid (C24:0), were purchased from Sigma Aldrich Co. (India). Isopropanol (IPA) was of GC grade from Thermo Fisher Scientific (India). Milli-Q water was obtained from a Millipore Direct-Qultra-pure water system (Sigma Aldrich Co. (India). All other reagents were of analytical grade as customary for FAME analysis. Automatic calibration of retention times and response factors are determined through calibrators' data[15.]

**Sample Run**

To run the sample program the samples of Jamun leaves extract kept into successive accessible positions and start the sequence. Data for each injection will be detected.

**Limit of Detection**

The limit of detection is 0.03% by weight of the original sample. The limit of quantitation for the method is 0.1% by weight of the original sample. No impurity or fraction should be reported that is is less than 0.1%.

**Fatty acid methyl ester mixture solution**

A fatty acid methyl ester mixture solution was used for analysis by adding 8 mL of each of the individual fatty acid stock solutions into a 100 mL of the standard flask, without oleic acid, and dilute to volume with sample solution[14].

**Method of system suitability samples**

The system suitability samples were newly prepared by adding an aliquot of 10 mL of oleic acid stock solution and add 1 mL each of palmitic acid, stearic acid, linoleic acid linolenic acid, and Arachidic acid stock solutions into a 100 mL standard flask, mix thoroughly and diluted to volume with sample solution[15].

**Accuracy and Linearity Limit**

The linearity and range samples were prepared in triplicate by serial dilution in isopropanol, covering the ranges of quantitation limit[16].

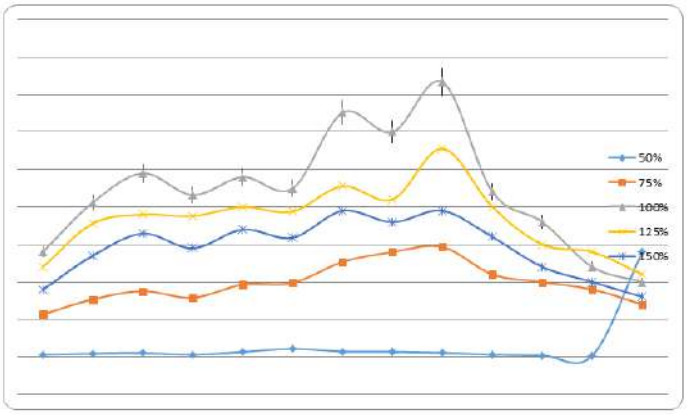
**Table.1 Fatty acid Profile in S. cumini leaves analyzed by GC-FID (Gas chromatography–flame ionization detection)**

| Fatty acid – GC-FID analysis | Molecular Weight | Structure | Bioactive principles |
|---|---|---|---|
| Lauric acid | 200 | 12:0 | Antifungal Activity |
| Myristic acid | 228 | 14:0 | Lipid attach in biomembranes |
| Palmitic acid | 256 | 16:0 | Antioxidants |
| Palmitoleic acid | 254 | 16.1 | Antibacterial & algal activity |
| Stearic acid | 284 | 18:0 | Drug and Cosmetic industry |
| Oleic acid | 282 | 18:1 | MUFA – Rich Antioxidants – Antibacterial and Antifungal activity |
| Linoleic acid | 280 | 18:2 | Prostaglandins biosynthesis and cell membranes. Anticardiac activity |
| Linolenic acid | 278 | 18:3 | Antidiabetic, Anti-inflammatory and vasoconstriction, Rich in Plant oils |
| Arachidic acid | 312 | 20:0 | Anti-inflammatory, Leucocytes - eicosanoid inflammatory mediators |
| Eicosenoic acid | 310 | 20:1 | Antibacterial and Antifungal activity |
| Behenic acid | 340 | 22:0 | Antibacterial and Antifungal activity, Lubricants |
| Erucic acid | 338 | 22:1 | Anticancer, Anticardiac and degenerative diseases in the brain. |
| Lignoceric acid | 368 | 24:0 | Neurodegenerative diseases - Cerebrosides |

**Table.2 & Fig.1 represent the Accuracy and Linearity limit of detection – Fatty acids profiles in Jamun leaves extracts**

| Conc range (µg/ml) | OA | LA | MA | PA | PAM | SA | LA ($n_3$) | LA ($n_6$) | AA | COV (r) |
|---|---|---|---|---|---|---|---|---|---|---|
| 50 % | 1.10 | 0.30 | 0.46 | 0.53 | 0.32 | 0.67 | 0.72 | 0.70 | 0.56 | 0.9998 |
| 75% | 9.2 | 5.67 | 7.67 | 8.78 | 7.89 | 9.67 | 12.67 | 14 | 14.67 | 0.9998 |
| 100% | 22.5 | 14 | 20.6 | 24.5 | 21.6 | 24 | 38.4 | 30 | 36.6 | 0.9998 |
| 125% | 19.0 | 12 | 17.8 | 19 | 18.8 | 20 | 22.8 | 21 | 27.8 | 0.9994 |
| 150% | 15.0 | 8.98 | 13.5 | 16.45 | 14.5 | 16.98 | 19.5 | 18 | 19.5 | 0.9990 |
| LD | 1.20 | 0.98 | 0.99 | 0.97 | 0.97 | 1.01 | 1.10 | 1.15 | 1.18 | |

FAME esters LA – Lauric acid ; MA- Myristic acid; PA – Palmitic acid; PAM- Palmitoleic acid; SA – Stearic acid; LA – Linoleic acid (n-3); LA – Linolenic acid (n-6); AA – Arachidic acid; LD- Limit of Detection; COV (r) – 0.9998.




## RESULTS AND DISCUSSION

The fatty acid composition of the Jamun leaves of *S. cumini* was decisive as their methyl esters are determined in Table. 1 and Table 2. Mostly, saturated, mono-unsaturated, and poly-unsaturated fatty acids were reported Lauric acid - C12:0; Myristic acid C14:0; Palmitic acid C16:0; Palmitoleic acid C16.1; Stearic acid C18:0; Oleic acid C18:1; Linoleic acid C18:2; Linolenic acid C18:3; Arachidic acid C20:0; Eicosenoic acid C20:1; Behenic acid C22:0; Erucic acid C22:1 and Lignoceric acid C24:0. Furthermore, Palmitic acid C16:0; Palmitoleic acid C16.1; Stearic acid C18:0 mono-unsaturated fatty acids. Linoleic acid C18:2; Linolenic acid C18:3; Arachidic acid C20:0 were the poly-unsaturated fatty acids reported. In this study, unsaturated fatty acids were observed as the essential components for therapeutic diabetics and renal related complications used as drug molecules. Also, other fatty acids, including polyunsaturated cyclic fatty acids, were found in less amount such as by GC-FID shows Eicosenoic acid C20:1; Behenic acid C22:0; Erucic acid C22:1 and Lignoceric acid C24:0 (Table.1 and 2). From the study, it was shown that most of the mono- and poly-unsaturated fatty acids were found in the seeds, as compared to the flesh and pulp. As a result, mono and poly-unsaturated fatty acids determined in this study to explore Indian blackberry it can be used as dietary importance to human health, whilst consumed as a component of their diets[14-15]. Fatty acid composition analysis showed that it's *S. Cumini* leaves were abundant in unsaturated fatty acids, specifically linolenic acid (18:3) whose percentage is about 100%[16]. Higher glucose and crude protein along with higher nitrogen to sulfur ratio supplements the nutritive value of this plant to prevent renal diseases. Phytochemicals such as total phenol, flavonoids, biochemical substrate antioxidants scavenging activity and configuration of DNA protection showed that this Jamun leaves and seed containing mostly antioxidant properties. Endogenous free Fatty acids are the primary metabolite which constitutes the biomolecules, macronutrients, and n3 α-linolenic and n6 linoleic acid are vital nutrients[16]. Plants have evolved extremely detoxification which includes xenobiotics metabolic enzymes possess drugs and toxins—which do not occur extensively in humans. With no exception, endogenous fatty acids compounds are handled by specific metabolic mechanisms that have to transform to standardize the concentrations and distribution, which have essential roles in human wellbeing. Approximately the globe, a large-scale analysis of n–3 and n–6 fatty acids depicts that ~95% of study apprehensive based on biochemical assays such as total lipids (TLs), Free fatty acids (FFA), phospholipids (PLs), and conjugation

of blood cells (RBCs)[13]. The present investigation focus here is on fatty acids profile that has been of primary interest in therapeutic studies measuring fatty acids methyl esters with chain lengths from 14 to 24 carbons which include the saturated, unsaturated, polyunsaturated fatty acids and long-chain PUFAs.Essential fatty acids are obligatory for the cells and high-quality for health, and they have to be obsessive necessary a diet, as the human body is not proficient to generate them[13-16]. Due to the altering way of life and elevated consumer difficulty for precise nutritious foods, more investigation into functional foods is desired, in particular, those of natural sources. Demand for plant essential oils has risen as a consequence of consumers searching for cheaper, more "natural" alternatives to disease-fighting medications[17-19]. Fatty acids constitute the key lipid units and are required in human nutrition as a source of energy, and for physiological and structural functions. Dietary fats provide essential fatty acids and facilitate the assimilation of fat-soluble vitamins, with saturated, mono- and polyunsaturated fatty acids being the most common. Previously study has revealed the positive roles involved as a result of leaves and fruits in the disruption of oxidative stress, eventually ailment impediment, and this consequence has been linked with the existence of polyunsaturated fatty acids, fibers, minerals, and vitamins[21-22]. Unusual mechanisms are expressing the positive effects of secondary metabolites reveals the metabolic synthesis of glucose and fatty acid, Secretion of Insulin, stimulation of β cells[23], signaling pathway of NF-*k*B in mice model[24], gluconeogenic enzymes inhibition, and stress enzymes protective action [25-26] are all determined. In this study, to authenticate the analytical method permissible the real-time quantification of two main components of fatty acids present in *S. cumini*.L, consent to the direct observance of these components in biochemical evaluation in vitro and in vivo.

## CONCLUSION

During the analysis of phytochemicals, it shows the responsible for the antidiabetic effects have stepped forward in the last few decades. The antidiabetic effect of plants is attributed to the mixture of phytochemicals or single components of the plant extracts. Phytochemicals painstaking for antidiabetic properties primarily are alkaloids, phenolic acids, flavonoids, glycosides, saponins, polysaccharides, stilbenes, coumarin, and tannins. In the present study, the fatty acid composition shows Oleic acid, Linolenic acids, Arachidic acid, and linoleic acids were more abundant whose percentage is about 100%. The percentage inhibition of polyunsaturated fatty acids reveals from 85.7-100% assessed by the GC-FID limit of detection. Moreover, phytochemical plant composition is highly dependent on several endogenous and exogenous factors, including genetic traits; plant organs used, and the growing, drying and storing conditions. Therefore, more efficient clinical studies are necessary for further validation. On the other hand, efforts should be made to characterize antidiabetic active principles from antidiabetic plants. Prognosis of diabetics with plant-derived compounds, which are easily reached and do not entail painstaking drug analysis, seem to be extremely therapeutics.

## ACKNOWLEDGMENTS

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SP-34

# EFFECTS OF MONOCROTOPHOS ON SOME ENZYMOLOGICAL PARAMETERS IN FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*(TILAPIA)

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## ABSTRACT

The toxic effect of Monocrotophos on some enzymological parameters such as AST, ALT, ALP and ACP in freshwater fish Oreochromis mossambicus (Tilapia) experimented. The fish O.mossambicus exposed for 30 days to various sublethal concentration (1/10, 1/20 and 1/30) of Monocrotophos. After completion of the 30$^{th}$ day treatment period the fish O.mossambicus sacrificed, and tissue samples of gill, liver, kidney analyzed. Increased value of AST, ALT and decreased value of ALP and ACP observed in all the (1/10$^{th}$, 1/20$^{th,}$ and 1/30$^{t}$ ) exposure concentration of Monocrotophos at 30 days of the exposure period while comparing with the control group. The liver shows a high variation of AST and ALT at 1/10$^{th}$ concentration of monocrotophos, and elevated variation of ALP and ACP observed in the liver at 1/10$^{th}$ concentration of sublethal level for 30 days exposure. While compare with control group the effects of Monocrotophos on fish found during this experiment showed that the level of AST, ALT in various tissues (gill, liver and kidney) of O. mossambicus increased and while compare with control group the effects of Monocrotophos on fish found during this experiment showed that the level of ACP and ALP in various tissues of gill, kidney and liver of O. mossambicus decreased.

**KEYWORDS**: *Monocrotophos, AST, ALT, ALP and ACP, Tilapia*

## INTRODUCTION

Organisms and their environment are two integral and inseparable, entities of an ecosystem. A significant problem is an environmental pollution in both developed and undeveloped countries[1]. The treated or without treatment of agricultural and industrial wastes discharged into surface water. Fish is highly susceptible to environmental problems and respond significantly to pollution. The fish exposed various stressors, such as water PH fluctuations in water temperature and oxygen[2]. The man uses pesticides to kill pests and insects. Pesticides usage became an indispensable and important integral part of agriculture in the world. Organophosphorous insecticides are used to control agricultural and domestic insect pests throughout the world. The contaminated water cause damages to aquatic life, especially to fish which are very sensitives to a wide range of toxicant in the water. The persistence of organophosphorus pesticides is very low in the environment[3]. Pesticides affect the nutritional value and growth of fish. Pesticides are toxic to many non-target organisms such as fish and macrophytes[4,5.] Organophosphorus insecticides monocrotophos is mainly used in animal husbandry and agriculture[6]. The colour of the monocrotophos is brownish yellow and the smell of monocrotophos irritates the skin and eyes. 223.2 is the molecular weight of monocrotophos. Enzymes play a significant role in food utilization and metabolism. The proteolytic enzymes break down protein molecules into amino acids to give energy for body function. Acid phosphatase plays an important role in the autolytic process of tissues and is a lysosomalenzyme[7]. The present experiment is to investigate the effect of monocrotophos on some enzyme activity in Tilapia Fish (*O. mossambicus*) that may use as environmental, biological indicators of pollution.

## MATERIAL AND METHODS

In the present experiment, fish *O.mossambica* was exposed to various concentrations (1/10,1/20 and 1/30) of an insecticide monocrotophos for 30 days, and the enzymological constituents like ACP, ALP,AST and ALT of the fish were studied. Organophosphorus insecticide, monocrotophos purchased from a local shop at Krishnagiri, Tamilnadu, India. The fish was acclimatized in a fish tank before they were used for the treatment. Fish tankswashed with potassium permanganate solution to avoid the fungal infection. The disinfected fish were maintained for two weeks in aerated tap water. Stress, physical damage and dead fish were removed.*O.mossambicus,* commonly called African Mouth breeder, is widely distributed in Indian freshwater media. Fish *O.mossambica*(The weight of the fish is 15 gm to 17gm and the length of the fish is 13cm to 16 cm ) was acquired from the Krishnagiri Reservoir Project Dam at Krishnagiri, Tamilnadu and brought to the laboratory of a government arts college, Krishnagiri with well-aerated bags and accommodate to the laboratory condition in the large tank. During four weeks of acclimation, fish were fed with rice flour mixed oil cake every day. The median lethal level (LC 50) of monocrotophos value was determined as 4.9 mg/l. of the freshwater fish *O.mossambicus.* Healthy fish were selected and divided into four groups. Each group contained ten fishes, treated with monocrotophos (35% Ec.) of lower Sublethal Concentration (1/30), median Sublethal Concentration (1/20) and higher Sublethal Concentration (1/10) and control groups in 30 days. After the exposure of 30 days the fish were sacrificed and gill, kidney, liver of both control and treated fish were dissected and removed. The removed tissues were homogenized and centrifuged at 3500rpm, prepared supernatant used for enzymological analysis (AST, ALT, ALP and ACP). Analysis of AST and ALT in the sample was done following methods of Reitman and frankle[8]. The level of ALP (alkaline phosphatase) and ACP (Acid phosphatase) in tissues of *Oreochromis mossambicus was* determined by the method of Tenniswood[9]. The values in both the cases expressed µmole/Protein/hr.

## RESULT

In the present experiment, the value of LC50 for monocrotophos to *Oreochromis mossambicus* was determined as 4.9 mg/l. Enzymes like ALT, AST was increased in all the exposure (1/10, 1/20 and 1/30) for 30 days observation in gill, kidney and liver of monocrotophos experimental fish *Oreochromis mossambicus* when it is compared with a control group. The enzymes like ACP and ALP were decreased significantly in all the exposure concentrations of monocrotophos (1/10, 1/20 and 1/30) for 30 days observation in tissues of gill, kidney and liver of *Oreochromismossambicus* when compared with the control group. Level of enzymes AST and ALT showed a maximum increase in the liver after 30 days of exposure of monocrotophos pesticide. The level of ACP and ALP showed a maximum decrease in the liver after 30 days of monocrotophos exposure.

**Table – 1Changes in the aspartate aminotransferase(µmole/mg protein/hr) in different tissues of *Oreochromis mossambicus* exposed to the various sublethal level of Monocrotophos**

| Expt. Group | Exposure Duration | Gill | Liver | Kidney |
|---|---|---|---|---|
| **Control** | **30 days** | $0.201 \pm 0.05^{a}$ | $0.410 \pm 0.03^{a}$ | $0.278 \pm 0.07^{a}$ |
| **LSC (1/30)** | **30 days** | $0.223 \pm 0.08^{b}$ | $0.437 \pm 0.06^{b}$ | $0.312 \pm 0.11^{b}$ |
| **MSC (1/20)** | **30 days** | $0.248 \pm 0.04^{c}$ | $0.476 \pm 0.08^{c}$ | $0.372 \pm 0.09^{c}$ |
| **HSC (1/10)** | **30 days** | $0.279 \pm 0.07^{d}$ | $0.518 \pm 0.04^{d}$ | $0.393 \pm 0.12^{d}$ |

Values are mean ± S.D., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p<0.05$) level between exposure groups.

**Table – 2Changes in the alanine aminotransferase(µmole/mg protein/hr) in different tissues of *Oreochromis mossambicus* exposed to the various sublethal level of Monocrotophos**

| Expt. Group | Exposure Duration | Gill | Liver | Kidney |
|---|---|---|---|---|
| **Control** | **30 days** | $0.440 \pm 0.07^{a}$ | $0.751 \pm 0.08^{a}$ | $0.410 \pm 0.03^{a}$ |

| | | | | |
|---|---|---|---|---|
| **LSC (1/30)** | **30 days** | $0.508 \pm 0.05^{b}$ | $0.808 \pm 0.07^{b}$ | $0.457 \pm 0.02^{b}$ |
| **MSC (1/20)** | **30 days** | $0.546 \pm 0.09^{c}$ | $0.857 \pm 0.05^{c}$ | $0.486 \pm 0.07^{c}$ |
| **HSC (1/10)** | **30 days** | $0.596 \pm 0.03^{d}$ | $0.904 \pm 0.06^{d}$ | $0.521 \pm 0.05^{d}$ |

Values are mean ± S.D., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p<0.05$) level between exposure groups.

**Table – 3Changes in the acid phosphatase (µmole/mg protein/hr) in various tissues of *Oreochromis mossambicus* treated to various sublethal concentration of Monocrotophos**

| Expt. Group | Exposure Duration | Gill | Liver | Kidney |
|---|---|---|---|---|
| **Control** | **30 days** | $0.351 \pm 0.03^{a}$ | $0.739 \pm 0.01^{a}$ | $0.482 \pm 0.05^{a}$ |
| **LSC (1/30)** | **30 days** | $0.412 \pm 0.06^{b}$ | $0.823 \pm 0.07^{b}$ | $0.527 \pm 0.04^{b}$ |
| **MSC (1/20)** | **30 days** | $0.449 \pm 0.05^{c}$ | $0.892 \pm 0.03^{c}$ | $0.601 \pm 0.06^{c}$ |
| **HSC (1/10)** | **30 days** | $0.514 \pm 0.02^{d}$ | $0.946 \pm 0.05^{d}$ | $0.628 \pm 0.01^{d}$ |

Values are mean ± S.D., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p<0.05$) level between exposure groups.

**Table – 4Changes in the alkaline phosphatase (µmole/mg protein/hr) in various tissues of*Oreochromis mossambicus*treated to various sublethal concentration ofMonocrotophos**

| Expt. Group | Exposure Duration | Gill | Liver | Kidney |
|---|---|---|---|---|
| **Control** | **30 days** | $0.337 \pm 0.03^{a}$ | $0.826 \pm 0.02^{a}$ | $0.457 \pm 0.04^{a}$ |
| **LSC (1/30)** | **30 days** | $0.423 \pm 0.05^{b}$ | $0.900 \pm 0.06^{b}$ | $0.568 \pm 0.05^{b}$ |
| **MSC (1/20)** | **30 days** | $0.467 \pm 0.06^{c}$ | $0.948 \pm 0.05^{c}$ | $0.627 \pm 0.03^{c}$ |
| **HSC (1/10)** | **30 days** | $0.523 \pm 0.04^{d}$ | $1.018 \pm 0.07^{d}$ | $0.703 \pm 0.06^{d}$ |

Values are mean ± S.D., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p<0.05$) level between exposure groups.

**Fig. 1Changes in the aspartate aminotransferase(µmole/mg protein/hr) in different tissues of *Oreochromis mossambicus* exposed to the various sublethal level of Monocrotophos**

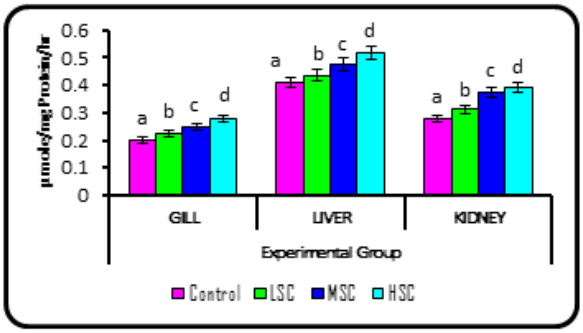



**Fig. 2 Changes in the alanine aminotransferase(µmole/mg protein/hr) in different tissues of *Oreochromis mossambicus* exposed to various sublethal level of Monocrotophos**

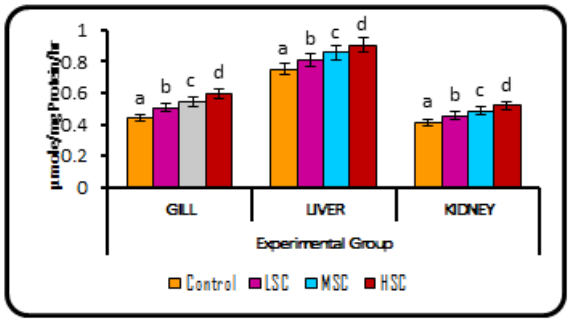


**Fig. 3Changes in the acid phosphatase (µmole/mg protein/hr) in different tissues of *Oreochromis mossambicus* exposed to various sublethal concentration of Monocrotophos**

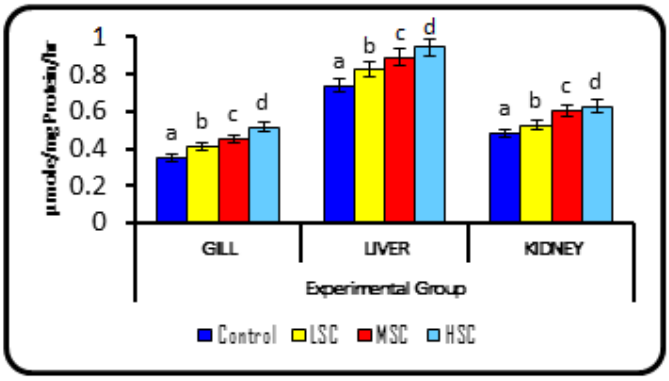
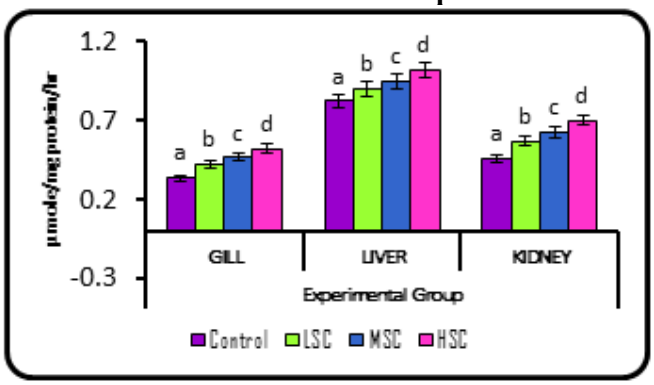



**Fig. 4Changes in the alkaline phosphatase (µmole/mg protein/hr) in different tissues of *Oreochromis mossambicus* exposed to various sublethal concentration monocrotophos**




## DISCUSSION

The level of ALP, ACP in gill, liver and kidney at the various sublethal concentration (1/10,1/20 and 1/30) of Monocrotophos, declined during the experimental period of 30 days and AST, ALT level increased. Endosulfan exposed freshwater fish *Channastriatus* showed a decreased level of ALP, ACP in muscle and brain[10]. Bhatnagar and Tyagi made the same observation[11]. Higher accumulation of pesticides, tissue damage and decline of metabolic activity may increase the level of ALT and AST activity[12].ACP and ALP indicated decreased activity because of the disturbance of cell organelles.Shakoori[13] reported that a decrease of ACP and ALP activities are due to increased necrosis in the tissues like hepatocytes in the fish. Velisek[14] reported the reduced serum alkaline phosphatase value in rainbow trout, as a result of stress-induced by low doses of insecticide. Decreased level of ACP observed in *Labeorohita* exposure to Arsenic[15]. Rahman[16] reported that cellular necrosis decline the level of ACP, ALP in various tissues. The present study concludes that monocrotophos has an influence on the enzymatical analysis and monocrotophos is highly toxic to aquatic organisms like fish when it's used beyond the safe level. The results insinuate a healthier understanding of the toxicological destination of the particular pesticides and provide useful knowledge on safe levels of pesticide usage.

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SP-35

# AWARENESS ON PREVENTION OF CANCER IN INDIAN WOMEN – A REVIEW

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## ABSTRACT

In India, 50% of the female deaths are due to cervix and breast cancer. Gall bladder cancer in Delhi women is one of the highest (9%) in the world. The consumption of red meat is the main cause of several cancers including gastrointestinal tract and colorectal, prostate, bladder, breast, gastric and oral cancers. Various activities of Govt, NGO`s and funding schemes of various projects and implementation of prevention and control measures and its results are discussed in the present study.

**KEYWORDS:** India, Cancer, Women, Mortality, Prevention

## INTRODUCTION

This article states that availability and thus the analysis of research articles due to search engines. Cancer imposes a heavy societal burden worldwide. The causes for cancers can be both either internal factors like, inherited mutations, hormones, and immune conditions or environmental and societal factors. In India, the annual burden for new cancers is approximately one million, and the mortality rate is 67.2 per 100,000[1]. In 2018 affected cancer 11, 57,294. Recently new cancer cases7, 84 821 death male - 4, 13519; Female 3, 71302.22, 58, 208 people living with cancer risk of male 9.81% & Female 9.42%[2]. 1881 to 1920, Denmark 25, 598 pairs of twins were birth. Data on the occurrence of cancer among them were obtained by questionnaire sent at intervals to the twins, from cancer registry unrelated disease was aggravated by the physiological effects of pregnancy and 103 were quite fortuitous in that women who died in road accidents or from viral encephalitis or certain forms of malignancy happened to be pregnant. During 1976-1978,11 deaths were attributed to this cause, but probably several others put down to postpartum hemorrhage associated with a blood coagulation disorder arose directly from the passage of amniotic fluid into the maternal bloodstream and therapy development of disseminated intravascular coagulation and defibrination.[3&4]. Since 1973 the reports have accepted the diagnosis of death from amniotic fluid embolism only when histological examination of the lungs has confirmed the diagnosis. This attempt at precision may be masking the extent of this cause of maternal death, for the quantity of amniotic fluid that has to pass before it can be identified in the lung is unknown. Presumably lesser quantities of amniotic fluid (insufficient to be seen in histological sections of the lung)[5] can result in fatal blood coagulation defects. Amniotic fluid syndrome might be a better term, for it could include death from embolism as well as those from hemorrhage after disseminated intravascular coagulation[6].

### Origin of Cancer in India

In India regions, poor and moderate living standards are inadequate medical facilities available. Asians have fewer incidences of prostate and Breast cancer compare to western countries (due to simple lifestyle and safe sexual practice). Improper diet & imbalanced diet. Other external factors such as tobacco, alcohol and radiation.T he finally environmental risk factors one of the most common 90% cancer due[7]. 10 million new cases diagnosed globally. In India cancer cases lower than in other countries. Lung cancer 14% in worldwide but in India 6.8%. these data are a good example for India are developing countries compare to other countries in medicinal or medical field[8].

## Cancer Mortality Profile in India

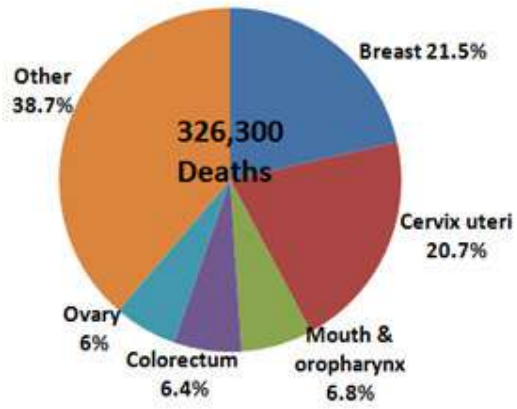
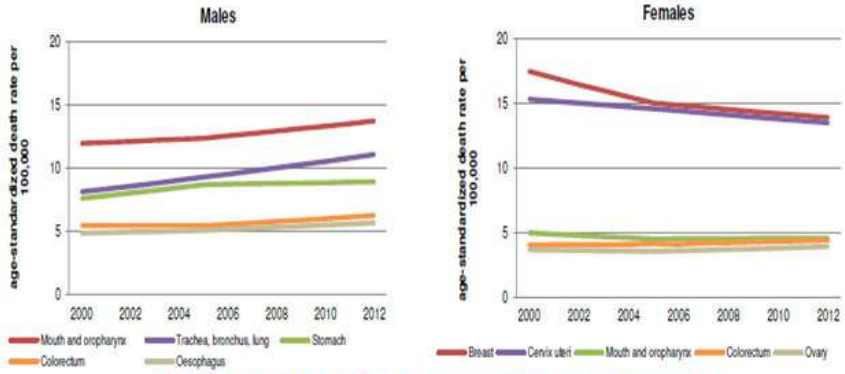
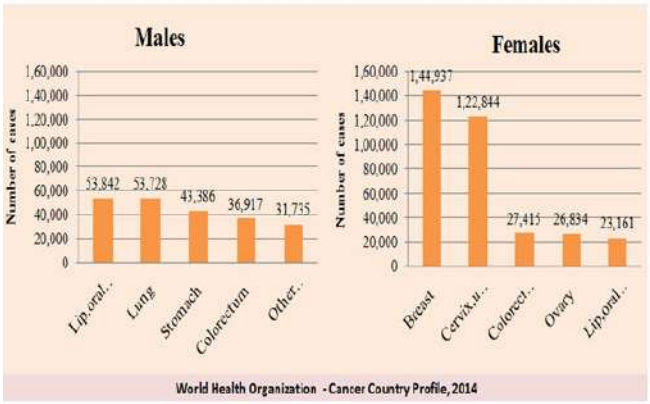



**Fig:1: World Health Organization - Cancer Country Profile, 2014 Age-Standardized Cancer Mortality Trends**




World Health Organization - Cancer Country Profile, 2014

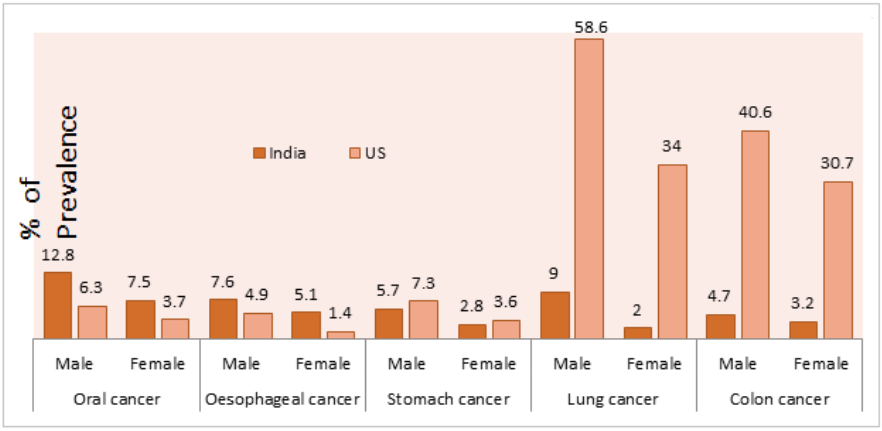
## Cancer Incidence




## Risk Factors

| | | |
|---|---|---|
| Current tobacco smoking (2011) | 25% | 3.9% |
| Total alcohol per capita consumption, in litters of pure alcohol (2010) | 8.0% | 0.5 |
| Physical inactivity (2010) | 9.2% | 15.1% |
| Obesity (2014) | 3.1% | 6.5% |

**World Health Organization - Cancer Country Profile, 2014**
**Comparison of India – America**




**Prevention**[9&10]

NCCP: National cancer control program led to the RCC, oncology wing in medical colleges, NCCP – RCC –DCCPandadult education program. Tobacco control can reduce 30% cancer and Ban public Liquor shop. Nuclear power plants should be constructed with a high shield to avoid radiation. Eco-Friendly organic farming is needed. ETP for treating sewage. Synthetic products, fabric dressing, cell phone uses should be minimized.

**Cancer awareness & Associate Activities in India**

Cancer scan software package for early detection of Breast cancer.1 lake pap smear kits for early detection of cervix cancer costing around 75,000 USD[11]. Trainers of a Training program on awareness, prevention, early detection & Treatment in TATA Memorial Hospital, Mumbai.Orientation program and workshops for cytopathologists for a pap smear test.Health education in PrasarBharathi by providing Kalyani Tele magazine once in a week.Health meals in various parts of country[12]. In India Cancer awareness programs from governmental and non-governmental organizations have evolved in the past few years. The National Cancer Control Programme in India used media campaigns to educate people about cancer and to encourage them to undergo screenin.[13]

**Advanced Diagnostic Techniques**[14]

Current techniques for diagnoses of mammography is X-ray mammography, ultrasound, MRI, and positron emission tomography (PET). These cause side effects to the patients due to a long time exposed to irradiation. The latest research on Recent advances in microwave imaging for Breast Cancer Detection is appreciated now to minimize side effect[15].

**Funding to be needed for cancer research**

In India, there is no direct funding for cancer research. Most of these studies are probably indirectly funded by the core grant to the institutions from the parent funding body (eg, Department of Atomic Energy, ICMR, Department of Science and Technology, and University Grants Commission)[16&17]. More funding for cancer research from the central government and states is also needed. Current research not as much for cancer research as for other areas. Pharmaceutical industries must allocate some percentage of funding for cancer research is mandatory. The government advises the public health department to establish separate advanced diagnostic and treatment facility in all the government hospitals[18&19].

**CONCLUSION**

A special attending will need to control the threatening disease cancer in India. The people should have the awareness through Govt& NGO`s, program. Every year the growing cancer detection should be organized from the children to adult. The stem cell research might the establish in research & development level for curing the cancer through gene therapy of low cost.

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SP-36

# TOXIC EFFECT OF CYPERMETHRIN ON SOME BLOOD PARAMETERS IN FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS* (TILAPIA)

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## ABSTRACT

Industrial and agriculture run-off affects the normal activities of the aquatic organisms in the nearby ecosystems, which result in the contamination of water. Sublethal effect of cypermethrin (25% EC) on some hematological parameters such as RBC, WBC and Hb values studied in O.mossambicus. The fishes are exposed to $1/10^{th}$,$1/20^{th}$ and $1/30^{th}$ sublethal concentration of cypermethrin for 10 days, 20 days and 30 days. The blood samples analyzed every $10^{th}$, $20^{th,}$ and $30^{th}$ day of the exposure period. A decreased value in RBC, Hb and increased amount of WBC observed in $1/10^{th}$, $1/20^{th}$ and $1/30^{th}$ sublethal concentration of cypermethrin at all the exposure periods. The high variation observed in $1/10^{th}$ sublethal level of $30^{th}$-day exposure.

**KEYWORDS**: *Cypermethrin, Haemoglobin, RBC, WBC, Tilapia, Oreochromis mossambicus*

## I. INTRODUCTION

Toxic chemicals reach the aquatic environment either directly or indirectly. Among insecticides, pyrethroids are considered to be the most harmful ones.[1] In India, agricultural practices involve a wide range of usage of pesticides to fulfill the food need for the increasing population.[2]. Non-degradable properties of pesticides make their persistence for long days in the environment, and they concentrated in the bodies of animals.[3] Water bodies are polluted by the indiscriminate usage of pesticides threatening aquatic life.[4] Organochloride compounds are replaced by synthetic pyrethroids nowadays in agriculture. Pyrethroids obtained from ornamental plant *chrysanthemum cinerariifolium* used to control pest in agriculture for the past two decades.[5] 30% of the pyrethroid insecticide is used in the world to control pest of household and agriculture importance.[6] Cotton plants are mainly treated by synthetic pyrethroids, which constitute about 80% in the world.[7] Pyrethroids usage is increasing in day-to-day agriculture practices. Pesticide poisoning in fish is increasing recently, which creates a hazard for human consumption. Fish enzymes are inefficient to hydrolyze pyrethroids.[8] The insecticide-treated fishes showed a decreased level of enzyme in different organs of treated fishes.[9] The organization of the liver structure disturbed when fish *Oreochromis mossambicus* were exposed to a sublethal concentration of quinalphos.[10] Pyrethroids exert their influence mainly on the central nervous system, and the sodium channel is an active site of the nervous membrane.[11] Cypermethrin inclusive products are represented as restricted-use pesticides. The lipophylic nature of cypermethrin increases their absorption by the gills in a more accessible manner.[12] Hematological parameters determine fish health an considered an easy tool to manifest the relationship between fish and environment.[13] Stress hormones increase the consequent changes at the physiological, organismal and population levels.[14] Environmental changes modify the physicochemical nature of fish blood. RBC, WBC and Hb assess the changes in fish health.[15] Haematological parameters act as a bioindicator to determine the pathophysiological changes in the animals before any disease symptoms persist to manifest the healthy condition of the fish. Blood indices vary with different environmental conditions and chemicals.[16] In fishery management fish blood is analyzed first to monitor the pathophysiological status of fish. Histopathological alteration to the surface of the gill by insecticide cypermethrin is attributed to its accumulation in organs of gills. Irritation in the gill due to elevated mucus secretion and decreased oxygen uptake efficiency of a gill.[17] Gills are directly exposed to the aquatic

environment and undesirable changes in the aquatic media reflect in the blood parameters through gills.[18] A decrease in the blood indices value in fishes against toxicants studied by many authors.[19,20] Thus hematological parameters provide a necessary tool to investigate fish health. The present research was undertaken to test the effect of cypermethrin on blood parameters of fish *O.mossambicus* (Tilapia).

## MATERIALS AND METHODS

Live and healthy *O.mossambicus* (Tilapia) fish procured from local fishermen in KRP dam in Krishnagiri district. Fish with an average length of 14 -18 cm and weight of 17 to 20 selected for the study. The fish was immersed in 0.1% of the kmno4 solution and acclimated in a large cement tank for 30 days. The pesticide cypermethrin (25% EC), a commercial-grade was used for the present experiment. The sub Lethal value of cypermethrin was analyzed using the method of Finneys Probit analysis.[21] The value of 4.5 mg/l was found to be a 96hr Lc50. Fishes are divided into four groups, and each divided group consists of 10 fish (*Oreochromis mossambicus*).

Group 1: Control group - Fishes with pesticide-free water.
Group 2: Fishes treated in $1/10^{th}$ sublethal the concentration of cypermethrin.
Group 3: Fishes treated in $1/20^{th}$ sublethal the concentration of cypermethrin.
Group 4: Fishes treated in $1/30^{th}$ sublethal the concentration of cypermethrin.

The duration of the experimental period is for 30 days. The experiments are carried in plastic aquaria of (20 x 35cm) holding 35 liter capacity of water. Physiochemical parameters of water recorded by the method of APHA.[22] The fish fed with groundnut oil cake and rice bran at the ratio of 1:1. The feed was calculated at a rate of 5% bodyweight of the fish. Feeding and application of a cypermethrin done every day after changing the water regularly. At the end of every experimental duration period of 10, 20, and 30 days, fishes from each experimental tubs comprising a sublethal concentration of $1/10^{th}$,$1/20^{th}$,$1/30^{th}$ sacrificed for the test study. Blood samples of fish were collected by puncturing caudal peduncle using a micropipette and collected in Appendroff tubes with EDTA anticoagulant.[23]

## HAEMATOLOGICAL STUDY

Total count of RBC done with Neubauer hemocytometer and the total count expressed as $10^6 mm^{-3}$.[24] Total count of White blood corpuscles done with Newbauer hemocytometer and it was calculated in $mm^3 x10^3$.[25,26] Hb Estimation was done by using Sahli's method.[27] The experimental analysis done by ANOVA (one-way analysis of variance) test and the probability level is 0.05%. The differences were computed by using the Tukey test.[28] Statistical data were analyzed using SPSS 20.0 software.

## RESULT

Fish exposed to sublethal concentration of $1/10^{th}$, $1/20^{th}$ and $1/30^{th}$ of cypermethrin (25%EC) elicited a decreased level of RBC , WBC and increased level of Hb at the end of the $30^{th}$ day of the treatment.

### RBC

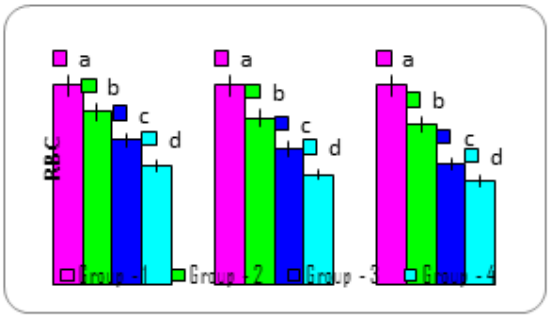
The mean values of RBC were 2.34 $x10^6 mm^{-3}$ in $1/10^{th}$ sub-lethal concentration of cypermethrin, 2.86 x $10^6 mm^{-3}$ in $1/20^{th}$ and 3.42 x $10^6 mm^{-3}$ in $1/30^{th}$ sub-lethal concentration level of cypermethrin for the experimental period of 10 days. Fish exposed at $1/10^{th}$ sublethal concentration level of cypermethrin for 20 days showed RBC valves of $2.18x10^6 mm^{-3}$ and 2.69 $x10^6 mm^{-3}$ in $1/20^{th}$ sublethal, $3.30x10^6 mm^{-3}$ in $1/30^{th}$ sublethal of cypermethrin. Fish exposed to a sublethal concentration of cypermethrin for 30 days showed RBC valves of $2.05x10^6 mm^{-3}$ in $1/10^{th}$, 2.09 $x10^6 mm^{-3}$ in $1/20^{th}$ and 3.18 x $10^6 mm^{-3}$ in $1/30^{th}$ sublethal of cypermethrin.

**Table 1. Haematological (RBC) changes of *O.mossambicus* exposed to different sublethal concentrations of Cypermethrin.**

| TREATMENT | 10 Days | 20 Days | 30 Days |
|---|---|---|---|
| RBC ( cu.mm ) | | | |
| Group – 1 | 3.95±0.51[a] | 3.95±0.51[a] | 3.95±0.51[a] |
| Group – 2 | 3.42±0.65[b] | 3.30±0.45[b] | 3.18±0.57[b] |
| Group – 3 | 2.86±0.35[c] | 2.69±0.55[c] | 2.39±0.45[c] |
| Group – 4 | 2.34±0.42[d] | 2.18±0.75[d] | 2.05±0.65[d] |

Values are mean ± SD., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p < 0.05$) level between treatment groups.

**Fig.1 Changes in the RBCs count of *O. mossambicus* exposed to different concentration of cypermethrin**




**WBC**

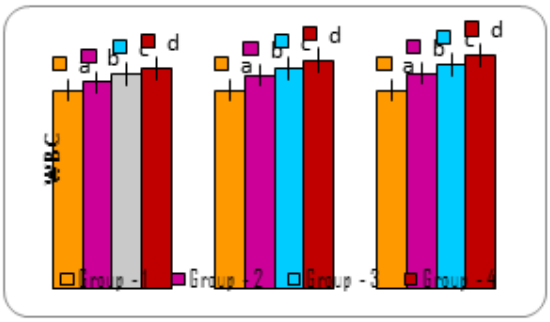
WBC total count showed an increased value in all the three exposure periods of 10,20,30 days in all the sub-lethal concentration of 1/10,1/20,1/30 of cypermethrin. The 10th day exposed fishes showed values of 21.54 $mm^3x10^3$ in 1/10th sublethal of cypermethrin, 20.98 $mm^3x10^3$WBC in 1/20th sub-lethal level and 20.15$mm^3x10^3$ WBC in 1/30th sub-lethal concentration level of cypermethrin. In 20th-day cypermethrin exposed fishes valves were 22.35$mm^3x10^3$ in 1/10th sublethal of cypermethrin, 21.56 $mm^3x10^3$ WBC in 1/20th sub-lethal concentration and 20.85 $mm^3x10^3$ WBC in 1/30th sub-lethal concentration level of cypermethrin. The 30th-day cypermethrin exposed fishes showed 22.80 $mm^3x10^3$ values of WBC at 1/10th sublethal, 21.98 x $mm^3$ x $10^3$WBC in 1/20th sub-lethal concentration and 21.02$mm^3x10^3$ WBC in 1/30th sub-lethal concentration level of cypermethrin.

**Table 2. Haematological (WBC) changes of *O.mossambicus* exposed to different sublethal concentrations of Cypermethrin.**

| TREATMENT | 10 Days | 20 Days | 30 Days |
|---|---|---|---|
| WBC ( cu.mm ) | | | |
| Group – 1 | 19.39±0.09[a] | 19.39±0.09[a] | 19.39±0.09[a] |
| Group – 2 | 20.15±0.12[b] | 20.85±0.15[b] | 21.02±0.18[b] |
| Group – 3 | 20.98±0.25[c] | 21.56±0.24[c] | 21.98±0.27[c] |
| Group – 4 | 21.54±0.42[d] | 22.35±0.52[d] | 22.80±0.24[d] |

Values are mean ± SD., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p < 0.05$) level between treatment groups.

**Fig.1 Changes in the WBCs count of *O. mossambicus* exposed to different concentration of cypermethrin**




## HAEMOGLOBIN

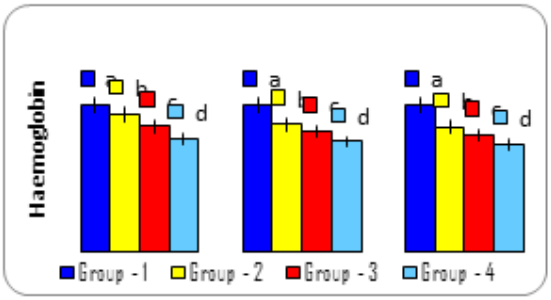
Hemoglobin content showed a decreased values in all the three exposure period of 10,20,30 days in all the sub-lethal concentration of 1/10,1/20,1/30 of cypermethrin. The 10th day exposed fishes showed values of 7.23 g/ml Hb in 1/10th sub-lethal concentration of cypermethrin, 8.01 g/ml Hb in 1/20th sub-lethal concentration of cypermethrin and 8.01 g/ml Hb in 1/30th sub-lethal concentration level of cypermethrin. In 20th-day cypermethrin exposed fishes valves were 7.02 g/ml Hb in 1/10th sub-lethal concentration of cypermethrin. 7.67 g/ml Hb in 1/20th sub-lethal concentration of cypermethrin and 8.12 g/ml Hb in 1/30th sub-lethal level of cypermethrin. Highly decreased values observed in the 30th day exposure period, 6.85 g/ml Hb in 1/10th sub-lethal concentration of cypermethrin, 7.42 g/ml Hb in 1/20th sub-lethal concentration of cypermethrin and 7.98 g/ml Hb in 1/30th sub-lethal concentration of cypermethrin.

**Table 3. Haematological (Hb) changes of *O.mossambicus* exposed to different sublethal concentrations of Cypermethrin.**

| TREATMENT | 10 Days | 20 Days | 30 Days |
|---|---|---|---|
| HAEMOGLOBIN (%) | | | |
| Group – 1 | 9.35±0.17$^{a}$ | 9.35±0.17$^{a}$ | 9.35±0.17$^{a}$ |
| Group – 2 | 8.76±0.19$^{b}$ | 8.12±0.15$^{b}$ | 7.98±0.13$^{b}$ |
| Group – 3 | 8.01±0.24$^{c}$ | 7.67±0.19$^{c}$ | 7.42±0.21$^{c}$ |
| Group – 4 | 7.23±0.32$^{d}$ | 7.02±0.21$^{d}$ | 6.85±0.27$^{d}$ |

Values are mean ± SD., Sample Size (N) = 6. Different letter designations denote significant at 5% ($p < 0.05$) level between treatment groups.

**Fig.3 Changes in the Hb level of *O. mossambicus* exposed to different concentration of cypermethrin**




## DISCUSSION

Fish Haematological parameters reveal the pathophysiological condition of the animal. Diagnosing blood parameters intoxicant exposed fishes helps to understand the present status of the fish.[29] Erythrocyte formation is depressed due to the non-availability of Hb content. Cypermethrin exposed fishes showed a drastic reduction in RBC's total count. Erythropoietic tissue damage caused by cypermethrin resulted in decreasing RBC levels.[30] The decreased level of Hb may be due to increased destruction or lesser synthesis of Hb. M.Atamanalp reported a reduction in the WBC level in O.*mykiss* fishes treated to cypermethrin[31].

This result correlated with the present study. Similar changes are reported in *Cyprinion watsoni* fishes exposed to malathion.[32] Reduced RBC value was reported in *Cyprinus carpio* after 48 hours of treatment to cypermethrin by Reddy and Bashmohideen.[33] All the results are in support of the present work. In *Labeorohita* fish treated to sublethal concentration of cypermethrin for 28 days, a significant reduction in RBC, Hb levels, elevation in TLC observed by Adhikari *et al.*. [34] Increased level of WBC observed in *Cyprinus carpio* fishes treated to Cypermethrin for seven days duration.[35]. Results similarities were observed in the present study also. Pathophysiological states resulted in increased leucocytes value due to stimulation of the hemopoietic tissues and acted as a defensive tool to produce antibodies against lethal infections.[36]

## CONCLUSION

The present study evaluates the changes in the blood indices caused by cypermethrin and the disturbances in blood profile parameters may reduce the survival ability in fishes due to decreased RBC's count and hemoglobin content, and enhanced WBC count suggests the defense mechanism developed in fishes to overcome the toxic effect of cypermethrin.

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# *IN-SILICO* ANALYSIS ON THE NEW HAIR LOSS GENE DSG4 *(HOMO SAPIENS)*

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## ABSTRACT

Hair loss is the main problem that is being faced by more than half of the population. This way, each cell to become the right type of hair cell receives the right signals. Mutation in $DSG_4$ gene at A153T, T267P and I534T position disrupt the function of the gene resulting in disorganization and separation of cells in the hair follicle. *In-silico* docking studies were performed for the mutated protein and the binding pocket was exactly found at the region of mutation. This mutated structure was compared with the normal structure to understand the difference in the conformation of the protein. The study of this gene could have roles in gene therapy, to help for hair loss problems both permanent and temporary solutions.

**KEYWORDS:** DSG4, Desmoglein, Docking. Mutation and *In-silico.*

## INTRODUCTION

Hair is one of the defining characteristics of mammalians[1]. Hair often refers to two distinct structures: first is the part beneath the skin, called the hair follicle, or when pulled from the skin, called the bulb. The bulb maintains stem cells and it is located in the dermis, which is not only re-grow the hair after it falls out but also is recruited to regrow skin after a wound[2], and second is the shaft, which is the hard filamentous part that extends above the skin surface. The visible is the hair shaft, which exhibits no biochemical activity and is considered "dead". The base of the root is called the bulb, which contains the cells that produce the hair shaft[3]. The diameter of human hair varies from 17 to 180 μm[4&5]. Some hairs, such as eyelashes, are especially sensitive to the presence of potentially harmful matter[6]. A study traced the origins of hair to the common ancestor of mammals, birds, and lizards that lived 310 million years ago in 2008 by the Medical University of Vienna and found humans chickens and lizards were possessed a similar set of genes that were involved in the production of alpha keratin. The α-keratin was found in their claws of chickens and lizards, but in mammals, it was used to produce hair. The scientists involved continued searching for the mechanisms that allowed mammals to use the keratins of animal claws to produce hair. Health and youth are represented by healthy hair (important in evolutionary biology). Hair texture and colour can be a symbol of ethnic ancestry. The facial hair is a symbol of puberty in men. White hair is an indication of age or genetics, which may be concealed with hair dye. Male pattern baldness is a notable sign of age, which may be concealed with a toupee, hats, or religious and cultural adornments. Many drugs and medical procedures are existing for the treatment of baldness, many balding men simply shave their heads. Hair whorls are associated with brain development too[7].

### Hair loss

Many things can cause excessive hair loss. For example, major surgery or about 2 to 3 months after an illness,We may suddenly lose a large amount of hair. The stress of illness may cause hair loss and is temporary. Hormonal problems may cause hair loss. If the thyroid gland is underactive or overactive, the hair may fall out. if male or female hormones (androgens and estrogens) are out of balance, hair loss may occur. Correcting all these hormonal imbalances may result to stop hair loss. Women may undergo hair loss after 3 months to deliver a baby. This loss is also related to hormones, high levels of some hormones cause the body to keep hair that would normally fall out during pregnancy. The hair falls out and the normal cycle of growth appears when hormones return to pre-pregnancy levels. Hair loss happens in children due to the fungal infections of the scalp. The infection is easily treated with antifungal medicines. Finally, as part of an underlying disease hair loss may occur, like lupus or diabetes. Since hair loss may be an early symptom of a disease, it is important to find the causes to treat.

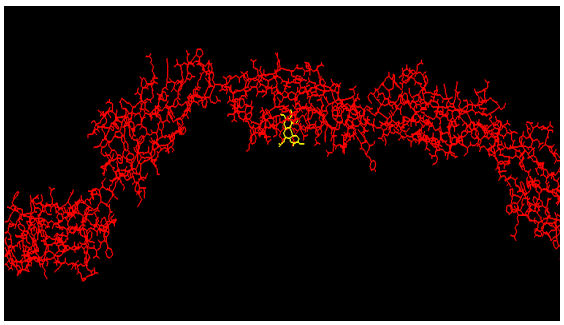
**DSG4 Gene**

The intermediate filament cytoskeleton of the trichocytes formed by hair keratins, which are linked to abundant cell-cell adhesion junctions, called desmosomes. The hair keratins are highly expressed when Desmoglein-4 (*DSG4*) desmosomal cadherin expressed in the hair shaft cortex in humans, mutations affecting either the hair keratins or *DSG4* lead to beaded hair phenotypes, which is postulated that the regulatory pathways governing the expression of *DSG4 and* hair shaft keratins. Tissue integrity of stratified epithelia and their appendages may happen by desmosomes. The highly specific expression pattern of *DSG4* combined with the phenotype of human patients and rodent models with desmoglein-4 mutations in the integrity of the hair shaft underscores the importance of this adhesion molecule.

**Software and Tools**

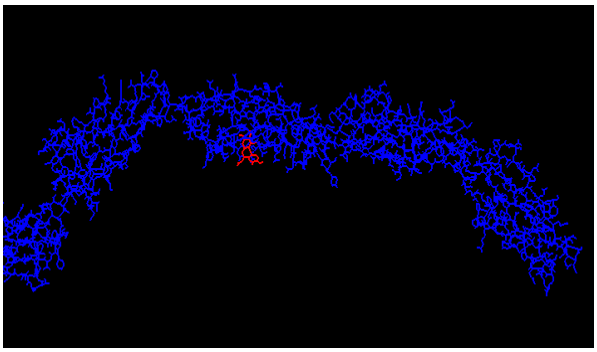
| | | |
|---|---|---|
| Sequence Retrieval | : | SWISSPROT |
| Structural Retrieval | : | PDB |
| Mutating the sequence | : | SPDBV |
| Functional domain identification | : | PRODOM |
| Drug Retrieval | : | PubChem |
| Drug Drawing | : | CHEMSKETCH |

**RESULTS**



**Evalue -217.07**

***Fig.1: Docking result of normal Desmoglein-4 protein with colchicine drug-using hex tool***



**Evalue-219.09**

***Fig. 2: Docking result of mutated Desmoglein-4 protein with colchicine drug using hex tool***

**DISCUSSION**

This research was initiated to analyze the gene *DSG4* using online Bioinformatics tools to find mutational changes that occur and compare the normal and mutated gene coding proteins based on protein structures. Finally, analyze the proteins with docking studies against available drugs and to that off validating this drug like how much it will bind to Desmoglein-4 protein and to check the pharmaceutical parameters of that particular drug. First, the sequences of the Normal *DSG4* gene, mutated *DSG4* gene and Desmogline-4 protein were retrieved from the SWISSPROT database. The 3D structure of *DSG4* gene was downloaded from the NCBI database. Mutation of *DSG4* gene causes hair loss in *Homo sapiens*. This mutation information was found by the MUTDB tool. The Domain region was identified using the PRODOM tool. Finally, the Normal Desmogline-4 protein and mutated Desmogline-4 proteins were docked against the

COLCHICINE drug-using HEX tool. Figure number 1 shows the docking result of COLCHICINE drug with normal Desmoglein-4 protein and it shows the E .Value of -217.07. Figure number 2 shows the docking result of mutated Desmoglein-4 protein against COLCHICINE drug with the E .Value of -219.09. This hair loss occurs due to the mutation of this *DSG4* gene. Its codes for a mutated desmoglein-4 protein. It is responsible for hair loss in human beings. Finally, these computational studies in the new hair loss gene suggest that colchicine is maybe a suitable drug for hair loss. The result of docking also confirmed the same.

## CONCLUSION

The hair loss gene DS4 has been studied and analyzed using various tools and databases. Thus, it has brought about the comparative difference between the normal and mutated gene both structurally and functionally and it is finally proved using the docking studies, where the docking exactly occurs at the binding site for the mutated gene. The docking studies are performed using the inhibitor colchicines.

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# ENZYMOLOGICAL CHANGES INDUCED BY ALGAE SPIRULINA AND AZOLLA ON FRESH WATER FISH, *OREOCHROMIS MOSSAMBICUS* (TILAPIA)

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## ABSTRACT

The future development of aquaculture depends on alternative feed ingredients. Azolla and Spirulina are considered as a feed supplement that has various health benefits for humans and other animals like fishes. The present study is to assess the enzyme AST,ALT, ACP and ALP level in Gill, liver, kidney and muscle of the freshwater fish *Oreochromis mossambicus* exposed to different concentrations of Azolla with formulated feed and spirulina with formulated feed, spirulina and Azolla with formulated feed. In the first group(control) 10 fishes were exposed to 20 gram of formulated feed, in the second group E1 (Experiment 1) 10 fishes exposed into 12 gram of Azolla with 8 gram of formulated feed and in the third group E2 (Experiment 2) 10 fishes exposed into 12 gram of spirulina with 8 gram of formulated feed and in the fourth group E3( Experiment 3) 10 fishes exposed into 6 gram of Azolla, 6 gram of spirulina and 8 gram of formulated feed for 60 days. In the present investigation, gill, liver, kidney and muscle of AST,ALT,ALP and ACP levels decreased when *Oreochromis mossambicus* exposed to different concentrations of Azolla and spirulina with formulated feed. The decrease in the enzyme level in the present study indicates that the tissue protein does not undergo proteolysis. The current research suggests a high enzyme level in group-4 (fishes treated with 6-gram Azolla and 6 gram of spirulina with 8 grams of formulated feed). This present study indicates that there was no toxic effect influenced by spirulina on freshwater fish *Oreochromis mossambicus* and ultimately spirulina decreased the level of enzyme AST,ALT,ACP andALP.

**KEYWORDS:** *Oreochromis mossambicus*, Azolla, Spirulina, AST,ALT,ACP,ALP

## INTRODUCTION

The future development of aquaculture dramatically depends on the development of alternative feed ingredients. Spirulina and Azolla produced better growth rates and biochemical performance in Tilapia. Spirulina and Azolla become one of the most commonly used microalgae in the aquafeed as it is a rich source of minerals, essential fatty acids, essential amino acids, protein, vitamins and pigments such as carotenoids that have potent antioxidant and anti-inflammatory activities.[1] *Azollapinnata* is one of the aquatic plants found in lakes, paddy fields, freshwater pond areas, rivers and irrigation channels around the year.[2] The cost of production of aqua feed reduced using this eco-friendly and sustainable resource. *Oreochromis niloticus* fry fed rations containing up to 42% *of A. pinnata* outperformed fish fed a fishmeal-based control diet.[3] Some studies indicate that catalase (CAT) and alkaline phosphatase (ALP) enzyme activities are strictly relevant to immune competence in fish.[4]ALT is purely cytoplasmic, catalyzing the transamination reaction. Any liver cell injury can reasonably increase ALT levels. Aspartate transaminase, (AST) is one enzyme that is of vital importance to fishes. The serum ALP activity is mainly from the liver, with 50% contributed by bone.[5] *Spirulina platensis* is a free-floating filamentous microalga with spiral characteristics of its filaments. Fish is an essential dietary animal protein source in human nutrition. Consumption of Spirulina alga and Azolla also increases the ability to absorb nutrients. When Spirulina alga is used as feed for young prawns and fingerlings, the fish exhibit good coloring, as well as maintain a low death rate and a high growth rate.[6] Spirulina and Azolla considered as an excellent source of useful nutrients as well as an excellent energy source.[7] The present study aimed to elucidate the effect of Spirulina and Azolla on enzymological changes in a freshwater fish *Oreochromis mossambicus (Tilapia)*. Freshwater fish *O. mossambicus* inhabiting rivers, ponds, shallow streams and lakes. They have a mild,

white flesh that is appealing to consumers. Spirulina and Azolla have studied more than others have due to their costly components, positive effects, and being a nontoxic supplement. The present study is to assess the enzyme AST, ALT, ALP and ACP content in Gill, Liver, Kidney and Muscle of the freshwater fish *Oreochromis mossambicus* exposed to different concentration of spirulina, and Azolla combined with formulated feed for 60 days

## MATERIALS AND METHODS

### Procurement and Rearing of Experimental fishes

*Oreochromis mossambicus,* commonly called Tilapia, is widely distributed in the freshwater of India. *O. mossambicus* (Tilapia) collected from the KRP Dam located at Krishnagiri, Tamilnadu, India, 10 km away from the Govt. Arts. College (men) campus, Krishnagiri. The collected fishes without the least disturbance transported in polythene bags filled half with water. About 20 fishes put into each bag with well-aerated water. This proved successful since there was no mortality in all consignments throughout this study.

### Maintenance of Experimental fish in Animal House

The fishes brought to the laboratory were acclimatized in the fish tank for a fortnight before they used for the experiment. The fish tanks were kept free from the fungal infection. The fish were disinfected with 0.1% of Potassium permanganate solution and maintained for three weeks in well-aerated tap water. Test fishes critically screened for the signs of disease, stress, and physical damage. The abnormal and dead individuals were removed. Feeding discontinued two days before the experiments to reduce the additive effects of animal excreta in the test trough.[8] The fish were exposed to different concentrations of spirulina and Azolla with formulated feed and control (pure formulated feed) for 60 days. The test water was renewed every day. The fish were sacrificed from both the experimental and control group at 60$^{th}$ days of exposure periods.

### Analytical Procedure

Fish of particular size and weight were used irrespective of their sex for the experiments (length is 15 ± 1 cm and weight is 26 ± 5 g). Only healthy individuals were collected from the same body of water and employed in all tests. The test solutions were renewed every 24 hr. While conducting the experiments, care was taken not to deviate from the modified main principles of bioassay techniques outlined by Sprague[9] and recommended by APHA.[10]

### Experimental Procedure

| | |
|---|---|
| **Group – I** | The first group of Fishes (10 number) maintained in the regular formulated feed (20 gram) and free from Spirulina and Azolla for 60 days, served as the control |
| **Group – II** | The second group of Fishes(10 number) were maintained in the regular formulated feed (8 gram) with Azolla (12 gram) for 60 days, served as the Experiment 1 (E1) |
| **Group – III** | The third group of Fishes (10 number) were maintained in the regular formulated feed (8 gram) with Spirulina (12 gram) for 60 days, served as Experiment 2 (E2). |
| **Group - IV** | The fourth group of Fishes(10 number) were maintained in the regular formulated feed (8 gram) with Azolla (6 gram) and Spirulina (6 gram) for 60 days, served as Experiment 3 (E3). |

### Steps in Feed Preparation

**Feed 1**: The combination of feed containing 100% formulated feed (20 gram) (control)
**Feed 2**: The combination of feed containing 8 gram of formulated feed and 12 gram of dried Azolla powder (E1)
**Feed3**: The combination of feed containing 8 gram of formulated feed and 12 gram of dried Spirulina powder (E2)
**Feed4**: The combination of feed containing 8 gram of formulated feed and 6 gram of

dried Azolla and 6 gram of Spirulina powder (E3)

## STATISTICAL ANALYSIS

The values are expressed as mean ± SD. SPSS 20.0 software used for Data analysis (ANOVA) along with Duncan's Multiple Range Test (DMRT)[11] which was applied to find out the significant difference between various treatment means and control means for the observed parameters.

## RESULT

## ENZYMOLOGICAL CHANGES

### AST and ALT

The present results showed that there is no significant variation of AST, ALT in gill, liver, kidney and muscle of *Oreochromis mossambicus* (tilapia) exposed to different concentration of spirulina and Azolla with formulated feed (12 gram Azolla with 8 gram of formulated feed (E1), 12 gram of spirulina with 8 gram of formulated feed (E2), 6 gram of Azolla and 6 gram of spirulina with 8 gram of formulated feed (E3) for 60 days while compared with the control group (Table – 1, Table – 2).

### ALP and ACP

The present results revealed that spirulina and Azolla experimental groups shown significant variation in ALP and ACP levels. The results showed no significant elevation of ALP and ACP in gill, liver, kidney and muscle of *Oreochromis mossambica* (tilapia) exposed to different concentration of spirulina with formulated feed (12 gram Azolla with 8 gram of formulated feed (E1), 12 gram of spirulina with 8 gram of formulated feed (E2), 6 gram of Azolla and 6 gram of spirulina with 8 gram of formulated feed (E3) for 60 days while compared with the control group (Table – 3, Table – 4).

**Table – 1 AST level in various organs of O. *mossambicus*exposed to different concentration of *spirulina, Azolla and formulated feed*for 60 days of exposure**

| Experimental Group | Gill [mg/g/hr] | Liver [mg/g/hr] | Kidney [mg/g/hr] | Muscle [mg/g/hr] |
|---|---|---|---|---|
| **Control (T1)** | $38.98 \pm 0.12^{a}$ | $44.68 \pm 0.12^{a}$ | $42.48 \pm 0.14^{a}$ | $41.15 \pm 0.32^{a}$ |
| **Treatement (T2)** | $38.15 \pm 0.18^{b}$ | $43.89 \pm 0.27^{b}$ | $41.71 \pm 0.28^{b}$ | $40.84 \pm 0.36^{b}$ |
| **Treatement (T3)** | $37.14 \pm 0.21^{c}$ | $43.01 \pm 0.52^{c}$ | $40.83 \pm 0.32^{c}$ | $39.79 \pm 0.11^{c}$ |
| **Treatement (T4)** | $36.11 \pm 0.31^{d}$ | $42.02 \pm 0.34^{d}$ | $40.03 \pm 0.21^{d}$ | $39.02 \pm 042^{d}$ |

Values are mean ± S.D. and Sample Size (N) = 6.
Values that are not sharing common superscript differ significantly at 5% ($p < 0.05$) Duncan multiple range test (DMRT).

**Table – 2 ALT level in various organs of O. *mossambicus* exposed to different concentration of *spirulina, Azolla and formulated feed* for 60 days of exposure**

| Experimental Group | Gill [mg/g/hr] | Liver [mg/g/hr] | Kidney [mg/g/hr] | Muscle [mg/g/hr] |
|---|---|---|---|---|
| **Control (T1)** | $27.73 \pm 0.15^{a}$ | $33.65 \pm 0.32^{a}$ | $31.81 \pm 0.12^{a}$ | $29.56 \pm 0.16^{a}$ |
| **Treatement (T2)** | $26.88 \pm 0.31^{b}$ | $33.02 \pm 0.11^{b}$ | $30.94 \pm 0.21^{b}$ | $29.01 \pm 0.18^{b}$ |
| **Treatement (T3)** | $25.97 \pm 03.19^{c}$ | $32.89 \pm 0.39^{c}$ | $30.08 \pm 0.43^{c}$ | $28.53 \pm 0.54^{c}$ |
| **Treatement (T4)** | $25.14 \pm 07.01^{d}$ | $31.11 \pm 01.20^{d}$ | $29.34 \pm 0.51^{d}$ | $27.76 \pm 0.23^{d}$ |

Values are mean ± S.D. and Sample Size (N) = 6.
Values that are not sharing common superscript differ significantly at 5% ($p < 0.05$) Duncan multiple range test (DMRT).

**Table – 3 ALP level in various organs of O. *mossambicus* exposed to different concentration of *spirulina, Azolla and formulated feed*for 60 days of exposure**

| Experimental Group | Gill [µmole/mg/hr] | Liver [µmole/mg/hr] | Kidney [µmole/mg/hr] | Muscle [µmole/mg/hr] |
|---|---|---|---|---|
| **Control (T1)** | $39.62 \pm 0.12^{a}$ | $45.07 \pm 0.31^{a}$ | $42.01 \pm 0.13^{a}$ | $40.17 \pm 0.19^{a}$ |
| **Treatement - 2** | $38.91 \pm 0.14^{b}$ | $44.02 \pm 0.22^{b}$ | $41.06 \pm 0.30^{b}$ | $45.30 \pm 0.19^{b}$ |
| **Treatement - 3** | $38.02 \pm 0.24^{c}$ | $43.82 \pm 0.34^{c}$ | $40.12 \pm 0.23^{c}$ | $45.01 \pm 0.25^{c}$ |
| **Treatement - 4** | $37.06 \pm 0.65^{d}$ | $43.01 \pm 0.87^{d}$ | $40.32 \pm 0.82^{d}$ | $44.09 \pm 0.43^{d}$ |

Values are mean ± S.D. and Sample Size (N) = 6.
Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$) Duncan multiple range test (DMRT).

**Table – 4 ACP level in various organs of O. *mossambicus* exposed to different concentration of *spirulina, Azolla and formulated feed* for 60 days of exposure**

| Experimental Group | Gill [µmole/mg/hr] | Liver [µmole/mg/hr] | Kidney [µmole/mg/hr] | Muscle [µmole/mg/hr] |
|---|---|---|---|---|
| **Control (T1)** | $37.88 \pm 0.09^{a}$ | $41.15 \pm 0.21^{a}$ | $39.93 \pm 0.12^{a}$ | $40.35 \pm 0.18^{a}$ |
| **Treatement – 2** | $37.02 \pm 0.13^{b}$ | $40.94 \pm 0.18^{b}$ | $38.58 \pm 0.32^{b}$ | $39.91 \pm 0.23^{b}$ |
| **Treatement - 3** | $36.93 \pm 0.17^{c}$ | $40.03 \pm 0.21^{c}$ | $38.09 \pm 0.76^{c}$ | $38.76 \pm 0.34^{c}$ |
| **Treatement - 4** | $36.23 \pm 0.98^{d}$ | $39.82 \pm 078^{d}$ | $37.98 \pm 0.98^{d}$ | $38.01 \pm 025^{d}$ |

Values are mean ± S.D. and Sample Size (N) = 6
Values which are not sharing common superscript differ significantly at 5% ($p < 0.05$) Duncan multiple range test (DMRT).

## DISCUSSION

### ENZYMOLOGICAL CHANGES

### AST and ALT

The significant activity of transaminase in the liver, any condition leading to large-scale degeneration of cells would result in the liberation of these enzymes into the circulating bloodstream.[12] Spirulina and Azolla might have increased the metabolic activity of liver cells, which might have synthesized and degraded more of dispensable amino acids leading to the higher concentration of AST enzymes in the serum. There is no significant variation of AST, ALT in gill, liver, kidney and muscle of *Oreochromis mossambicus* (tilapia) exposed to different concentration of spirulina with formulated feed (12 gram Azolla with 8 gram of formulated feed (E1), 12 gram of spirulina with 8 gram of formulated feed (E2), 6 gram of Azolla and 6 gram of spirulina with 8 gram of formulated feed (E3) for 60 days while compared with the control group. This result agrees with Abdel-Tawab *et al*. [13] who investigated the use of commercial baker yeast as growth for Nile Tilapia. ALT and AST are enzymes found mainly in the liver, but also found in red blood cells, heart cells, musculatures tissue and other organs, such as the pancreas and kidneys. ALT or AST levels are a valuable aid primarily in the diagnosis of liver disease. Biochemical indices in fish are affected by many endogenous and exogenous factors. Liver enzymes (ALT and AST) are the variables

most suitable as indicators of the toxicity of cyanobacteria after intraperitoneal or oral biomass application in fish. Administration of Spirulina to feed-limited catfish resulted in the reduction of AST and ALT in the same line with that occurred in deltamethrin intoxicated Nile tilapia.[14]

**ALP and ACP**

Alkaline phosphatases a 'marker' enzyme for the plasma membrane and endoplasmic reticulum.[15] Any alteration in the activity of the enzyme in the tissue would indicate likely damages to the external boundary of the cell.[16] The present results revealed that spirulina and Azolla experimental groups not shown significant variation in ALP and ACP levels. Therefore, the non-significant changes in the gill, liver, kidney and muscle ACP and ALP activities in this study following the supplementation of *S. platensis* and Azolla attributed to either control of the enzyme activity at the cellular level and this could indicate the regular transportation of ions or molecules across the membrane.[17] Acid phosphatase is an indicator of damage to the lysosomal layer.[18] The nonsignificant of an effect by the supplemented diet on the ACP activity of the tissues of the fish in this study may be an indication that the lysosomal membrane was not adversely affected. Spirulina helps to lower the cholesterol level, facilitate minerals absorption and protect the immune system. Spirulina reduced hepatic damage.[19] Spirulina is rich in protein, chlorophyll and nucleic acid. The present results showed that there is no significant variation of AST, ALT in gill, liver, kidney and muscle of *Oreochromis mossambicus* exposed to various concentrations of spirulina and Azolla with formulated feed.

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SP-39

# STUDIES ON THE BIOCONVERCTION OF FISH AND WATERMELON PEEL WASTES (*Citrullus lanatus*) INTO VERMICOMPOST BY *EUDRILUS EUGENIAE*

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## ABSTRACT

The present work has been carried out to understand the level of nutrients present in the various types of wastes such as fish waste and watermelon waste obtained from the local market. The macronutrients such N, P and K were analyzed on the 15$^{th}$ day as well as on the 30th day. The different values indicated the heterogeneous levels of the nutrients. The animal waste when subjected to vermicompost, showed an increase of macronutrients when compared to control. Similarly, Micronutrients such as Zn and Cu also revealed a slight increase in their levels in both the wastes. Copper showed 0.95ppm and 0.84 ppm of zinc were found in the watermelon peel waste. Among the various micronutrients, Fe showed a very low level in both the wastes when compared to control. Histology studies revealed slight changes in the anatomy of the earthworm. Besides FT-IR showed the different types of functional groups. Physico-chemical characters such as EC and pH were found to increase in the compost of fish waste and watermelon peel waste. The compost material obtained from this study is also used to assess its efficiency in the growth of *Vigna mungo*. The values in all the treatments were statistically insignificant at the P<0.01 level.

**KEYWORDS:** *Eudrilus Eugenie, FT-IR, Vermicompost, fish waste and watermelon peel waste.*

## 1. INTRODUCTION

Increasing civilization and urbanization have led to an everlasting generation of wastes and disposal of such wastes is becoming a serious global problem. Hence, intensive attention has been paid in recent years to develop efficient low-input technologies to convert nutrient-rich organic solid wastes into value-added products for sustainable land practices[1-2]. Vermicomposting is a simple biotechnological process in which certain species of earthworms are used to enhance the process of waste decomposition to produce a better end product[3]. The use of earthworms for the biodegradation of organic wastes has been reported to be a positive development in the biological waste management[4]. Vermicompost mesophilic process which enhances the levels of plant-nutrients of organic waste [5]. During the process, the nutrients locked up in the organic waste are changed in worm's gut to simple and more readily available and absorbable forms[6-7]. *Eudrilus Eugenie,* worms have been considered as a key agent for organic waste management through the process of vermicomposting[8]. Effective sanitization and pathogen control could be done by the thermophilic composting. Furthermore, composts have been reported to suppress plant pathogens. Reported that composts were suppressive to soil-borne plant diseases. Composts have also been reported to enhance population development of beneficial nematodes[9]. Fish farms produce large quantities of organic waste. This material can accumulate in the pool, as well as be suspended in the water bodies. Its composition is determined according to several parameters, such as the no consumed scraps of feeding stuff and excrements, or other organic droppings from fish. Waste management strategy must consider several issues, including requirements for the storage and disposal of wastes in an environmentally safe manner. It is well known that the fish wastes have been used as organic fertilizer and nutrients for both agricultural purposes and rehabilitation of degraded areas [10-11]. Similarly, fruit waste is also dumped in an unauthorized place and raises the issues of disposal of fruit waste particularly watermelon peel waste which is also used in the present study to find out the nutrient levels. Since earthworms are considered as bioindicators as well as an important agent for compost, the present study is aimed to study the efficiency of earthworms in the bioconversion of fish and watermelon peel wastes by analyzing micro and

macronutrients of the compost and also attempted to understand the plant growth in vermicompost soil.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Earthworm

*Eudrilus eugeniae*, the elusion earthworm species were collected from Chithathur, Thiruvannamalai District, Tamil Nadu. The collected earthworms were brought to the laboratory for acclimatization.

### 2.2. Experimental procedure

Vermi bed was prepared as per the standard procedure. The Earthworms were kept in an earthen pot, which was half-filled with a mixture of loamy and humus soil. The earthworms were kept in an earthen pot for a minimum of 30 days, to allow them to adapt to laboratory conditions. During the period of acclimatization, the worms were fed with air-dried cow manure. Earthworms of similar sizes were carefully selected from the earthen pots for further studies. The selected fish and watermelon peel wastes were collected from the Cheyyar fish market and fruit shop respectively and mixed thoroughly with 1kg of soil. This setup was maintained for 30 days. Various Physico-chemical parameters such as pH, Electrical Conductivity (EC), and the macro and micronutrients such as Total Nitrogen (TN), Total Phosphorus (TP), Total Potassium (TK), Total Manganese (TMn), Total Zinc (TZn), Total Copper (TCu), and Total Iron (TFe) were estimated by the standard procedures and also assessed the plant growth of *Vigna mungo*. Also, histological studies of the earthworm and FTIR study were undertaken.

### 2.3. Histological Studies

The histology of earthworm was studied by adopting the paraffin method. The control and experimental animals were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of the anterior and clitellar region of the desired size and fixed in Bruin's fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24hr, after which the tissues were transferred to 70% alcohol. Several changes of 70% alcohol were given until the yellow color disappeared from the tissues. The tissues were then dehydrated by passing through ascending graded of alcohol, cleared in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax.Tissue section of the 5-µm thick of cross-sections was obtained using a rotary microtome. The section, thus obtained, was stained in harris hematoxylin and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a Qasmo research microscope.

### 2.4. Statistical Analysis

The data obtained were subjected to statistical analysis to understand the significance of the result by using the student 't' test. Differences were considered to be significant at a level of $P<0.5$.

## 3. RESULTS AND DISCUSSION

Natural materials such as Earthworm are responsible for reducing the pollution caused by environmental pollution by improving the process of decomposition of the complex. It is important to use the correct types of Earthworms for waste management. The importance of the severity of environmental symptoms back when they first recognized the role of global bees in agriculture and called themselves a farmer of the land[12]. These Vermin resources have a large and varied potential for bio-fertilizer production, land reclamation, natural biodiversity, and food sources. Biofertilizers can absorb chemical fertilizers to some extent and create a better environment for plant growth. Vermitechnology has gained momentum in recent years due to its recurring benefits. Eco-friendly and inexpensive technology works to use Earthworms as bioreactors to convert organic matter into valuable fertilizer. According to

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

the cost of crop production may be reduced significantly if vermicompost is integrated into nutrient management in the agricultural field[13]. Research on vermicomposting by using various wastes has been successfully going on throughout the world. In these lines of research, the present work has been carried out The analysis was carried out to understand the level of nutrients present in the various types of wastes such as fish waste and watermelon peel wastes obtained from the local market and macronutrients such as Nitrogen, Phosphorous, and Potassium was analyzed on 15$^{th}$ day as well as on 30$^{th}$ day (fig 1-4). The different values indicated the heterogeneous trend of the nutrients. The animal waste when subjected to vermicompost showed a 49.4 % increase when compared to control. An Increase in the nitrogen content in the experiments was due to the presence of nitrogen-fixing bacteria. The decomposition of organic material by earthworms accelerates the nitrogen mineralization process and changes the nitrogen profile of the substrate.

**Fig: 1. Analysis of N, P and K in the Vermicompost of Fish and Watermelon peel wastes**

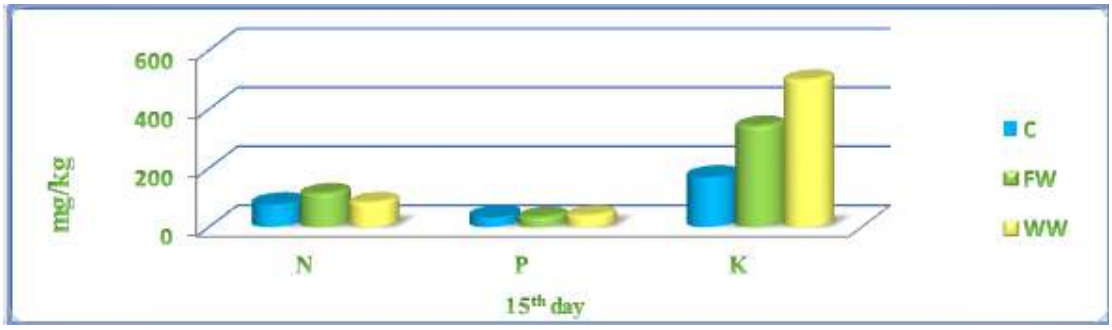



**Fig: 2. Analysis of Fe, Mn, Zn, Cu, EC and pH in the Vermicompost of Fish and Watermelon peel wastes**

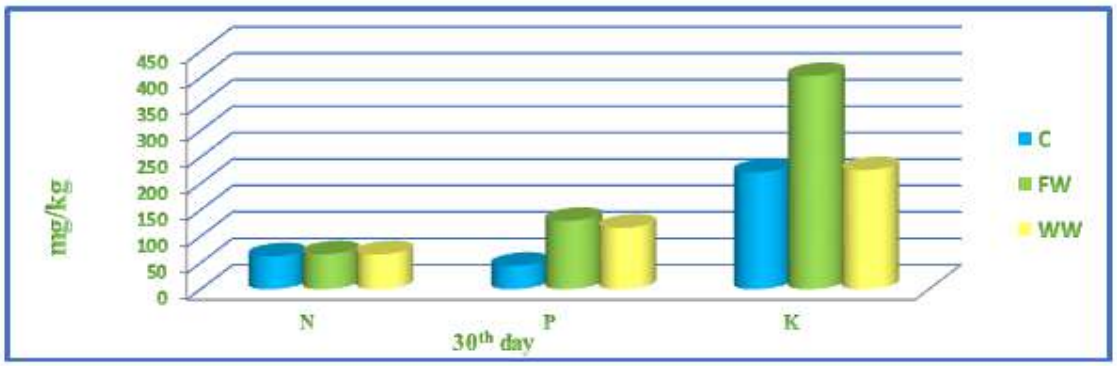



**Fig: 3. Analysis of N, P and K in the Vermicompost of Fish and Watermelon peel wastes**

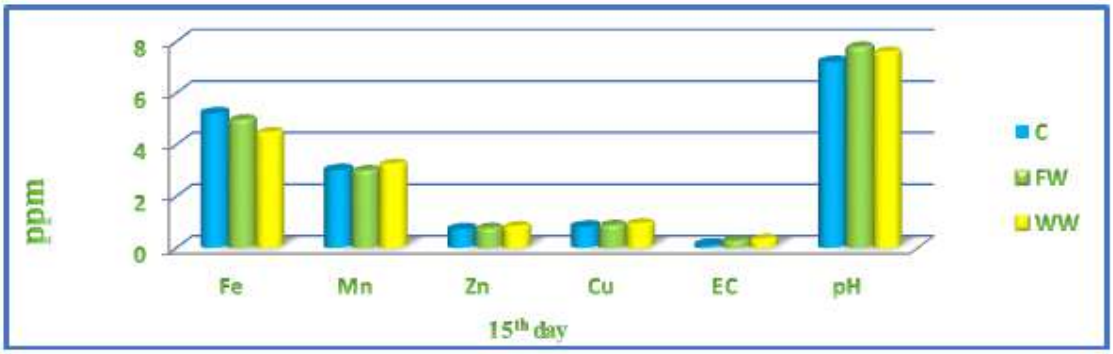
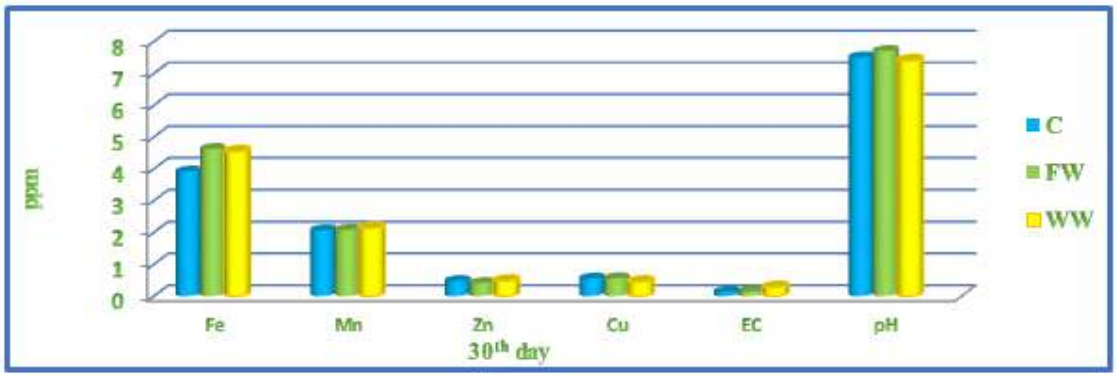


**Fig: 4. Analysis of Fe, Mn, Zn, Cu, EC and pH in the Vermicompost of Fish and Watermelon peel wastes**




Control, FW- Fish Waste, WW- Watermelon peel Waste.

Potassium increase might be due to changes in the distribution of potassium between non-exchangeable and exchangeable ion forms. Earthworms processed waste material containing a high concentration of exchangeable potassium which consequently enhances the rate of mineralization[13]. Micronutrients like iron, copper, calcium, zinc, and manganese also increased in traces. Earthworms convert calcium oxalate crystals into calcium bicarbonate which is then egested in the cast materials the value was statistically significant at $P<0.05$ whereas the vermicompost obtained from the watermelon waste showed only 195.08% of increase when compared to control. Nitrogen also showed variations among the vermicompost. However, the phosphorus level was not much increased at the end of 15$^{th}$day and found only 15% of the increase in phosphorus level. The other important nutrient 'K` was increased nearly by 104% and this was further increased in the waste of watermelon peel. The watermelon compost revealed a high level of phosphorus. The statistical analysis also showed significance at $P<0.05$ level. Among the two wastes, Nitrogen showed a maximum increase only in the animal waste whereas K showed a maximum of nearly 195% in the watermelon peel wastes. The enhanced phosphorous level suggests the mineralization during the vermicomposting process. This suggests that the passage of organic matter through the gut of earthworm results in the conversions of phosphorous which are easily available to the plants[14]. Further release of phosphorous might be attributed to the phosphorous solubilizing microorganisms present in the compost[15]. Also reported the enhanced potassium content in the vermicompost[13]. The results obtained in this study are in support of demonstrated that the higher potassium concentration in the compost obtained from sewage sludge[16]. Micronutrients such as Zn and Cu revealed a slight increase in the levels in both the wastes. Copper was 0.95ppm level in the watermelon peel waste, whereas 0.84 ppm of Zinc was found in the same compost. The increase of copper content was due to the presence of several coppers containing oxidizing enzymes. Mn was reduced in the compost of watermelon peel when compared to the values of Zn and Cu. Among the various micronutrients, Fe showed a very low level when compared to the control of both the wastes. In the 30$^{th}$ day study, Nitrogen value was increased significantly whereas Phosphorus showed a maximum of 116 ppm in the watermelon peel waste and 129 ppm in the fish waste compost. Such type of increase was also observed in the Potassium. Among the macronutrients, animal waste compost consists of the maximum value of P and K. In contrast Zinc was shown the same level as that of the control whereas Fe and Mn showed a marginal increase in the fish and watermelon peel waste compost. Cu level was decreased in the watermelon peel waste when compared to control. It may be due to the low level of enzymes and co-factor in the earthworm gut and the decrease of copper content may be due to the low level of several coppers containing oxidizing enzymes.

### 3.1. PHYSICO-CHEMICAL PROPERTIES

Physico-chemical properties of the soil mixed with wastes were analyzed on the 15$^{th}$ and 30$^{th}$ day along with control and experiment. There was no change in the electrical conductivity (EC) in the Fish and Watermelon peel waste compost. The values in all the treatments were statistically insignificant at the $P<0.01$ level. On the 15$^{th}$ day, pH value was increased in all the treatments. pH is an important factor that limits the distribution and number of species[17]. Several researchers have also stated that most species of earthworm prefer a pH of about 7.0[18]. The increase of pH in experiments might be due to the participation of microbes in the decomposition process. In contrast to the analysis on the 15$^{th}$ day, the value was normal

in the treatments after the 30th day when compared to the control value. In this study, a reduction in the pH value was recorded at the end of the 30th-day experiment. The variability in pH could be due to the production of organic acids during the organic waste decomposition. In the present study, organic residues may be produced that could lower the pH level at the end of the experiment. The low level of pH in control may be due to the presence of anaerobic bacteria in the sample.

### 3.2. HISTOLOGY OF THE EARTHWORM

In the control animal, the epidermis of the *Eudrilus eugeniae* consists of an epidermal epithelium and an overlying fibrous cuticle. Below the epidermis, circular and longitudinal muscles were intact and form the body wall. (Plat 1 & 2). The coelom was seen. Along with this, lumen and blood vessels were seen clearly. Below the epidermis, the pigment cells were numerous. The mixed waste earthworm subjected to the treatment showed less change in the clitellum when compared to control. The cuticle, epidermis and circular muscles were not changed in the experiment. The supposed to be the pigment cells was reduced to a greater extent. Similarly in the fish and watermelon peel wastes treated clitellar region showed very little changes in the lumen, blood vessels, and pigment cells. Inter vascular space was much reduced. The majority of the anatomical details were intact except few changes like the size of the lumen, number of the pigment cells and the size of the coelom. Only in certain regions, the thickness of the body wall was reduced. Histology of the anterior region reveals that the cuticle is not much damaged. Not much spoilage in the area of circular muscles but some part of the longitudinal muscle showed the signs of injury. A detachment of peritoneum from longitudinal muscles can also be seen in some of the areas. The ventral nerve cord is damaged to a lesser extent. The dorsal and ventral blood vessels were ruined largely. The control animal of the clitellar region showed an intact structure of internal organs. The coelom and Blood vessels are well marked. In the experimental animal, the clitellar region showed a slight modification of the anatomy. The pigment cells are prominent in the fish and watermelon peel waste treatments. In some places, the circular muscles are disorganized. The cuticle is slightly damaged in several areas. The epidermis is also damaged largely. Even the circular muscles have shown signs of damage in several areas. The longitudinal muscle has shown the signs of crakes in major parts of the regions. The chromogen cells have been damaged. The ventral nerve cord is faintly visible, whereas the dorsal and ventral blood vessel and the nephridia are seen faintly.

### 3.3. FT-IR SPECTROSCOPY

Structural determination and functional group confirmation have been made using IR spectroscopy in the mid-IR region. The presence and absence of functional groups give us to predict the structure of certain bio-molecules. Mid-infrared (IR) spectroscopy is a reliable and well-known method of fingerprinting. Many items can be displayed, identified and disconnected. One of the most powerful IR- spectroscopy techniques is as an analytical technique for detecting spectra in a very wide range of solids, liquids, and gases respectively (Table-1 and Fig.5, 6&7).

| **S. No** | **Control** wave number ($cm^{-1}$) | **Fish waste** wave number ($cm^{-1}$) | **Watermelon peel waste** wave number ($cm^{-1}$) | **Band Assignment** | **Functional group** |
|---|---|---|---|---|---|
| 1 | 3620(m) | 3621(m) | 3621(m) | O-H (St) Free hydroxyl | Alcohols, phenols |
| 2 | 3396(m) | 3430(m) | 3430(m) | O-H (St) H-bonded | Alkanes |
| 3 | 2921(m) | 2923(m) | 2923(m) | C-H (St) | Alkanes |
| 4 | 2853(w) | 2852(w) | 2853(w) | C-H (St) | Alkanes |
| 5 | 1723(s) | 1663(s) | 1635(s) | C=O (St) | α,β-unsaturated-esters |
| 6 | 1462(s) | 1383(s) | 1383(s) | C- H bend | Alkanes |
| 7 | 1078(m) | 1033(m) | 1033(m) | C-N(St) | Aliphatic amines |
| 8 | 913(m) | 912(m) | 912(m) | O-H bend | Carboxylic acid |
| 9 | 778(m) | 778(m) | 777(m) | C-CL | Alkyl halides |
| 10 | 534(m) | 535(m) | 535(m) | C-Br (St) | Alkyl halides |

**TABLE -1 FT-IR-Band Assignment**

- **m** – Medium, **w** - weak**, s** - strong, **St** - Stretching,

In FT-IR study it is noted that fish waste and watermelon peel waste absorb more water content in soil due to the blueshifts of –OH stretching band at 3430 cm-[1] as well as C -O-H in-plane and out of plane bend have been red-shifted and the band observed at 1870; 1869 cm-1 and 1033; 1033 respectively. The corresponding shift in the peaks is in good agreement with the literature value.

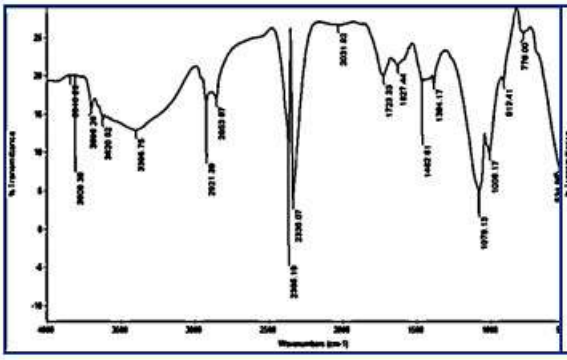


**Fig: 5. FTIR Spectrum of Control**

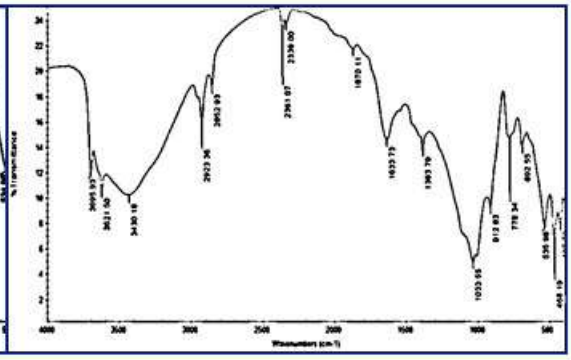
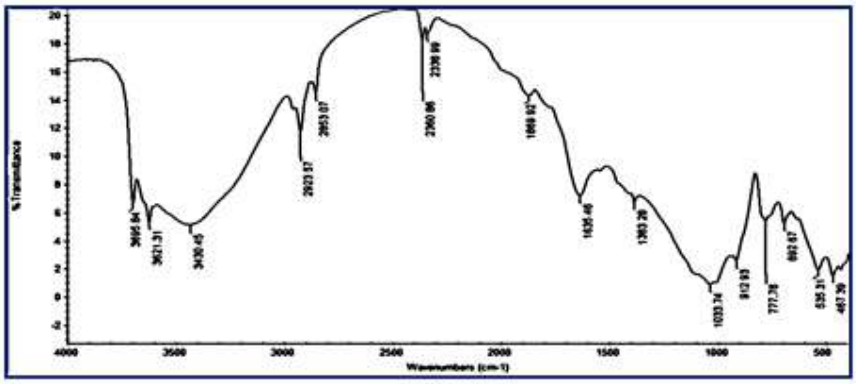


**Fig: 6. FTIR Spectrum of Vermicompost of Fish waste**

**Fig: 7. FTIR Spectrum of Vermicompost of watermelon peel waste**



### 3.4. PLANT GROWTH STUDIES

Effect of vermicompost on the growth of plant *Vigna mungo* (Black gram) was studied at two different intervals. In the present study, the length of stem, root, leaf, and petiole was measured on the 5$^{th}$ and 10$^{th}$ day. Initially, the stem length was increased to 54% in the vermicompost obtained from the fish waste and it was still increased on the 10$^{th}$ day by 60%. The increases were statistically significant, whereas when the watermelon peel waste was used, the height increased more than 100% (Table-2 and fig 8&9).

**Fig: 8. EFFECT OF VERMICOMPOST ON THE GROWTH *Vigna mungo***

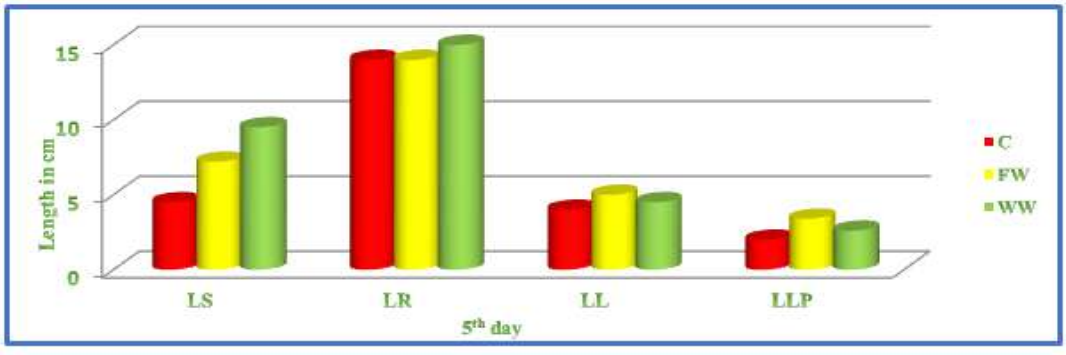
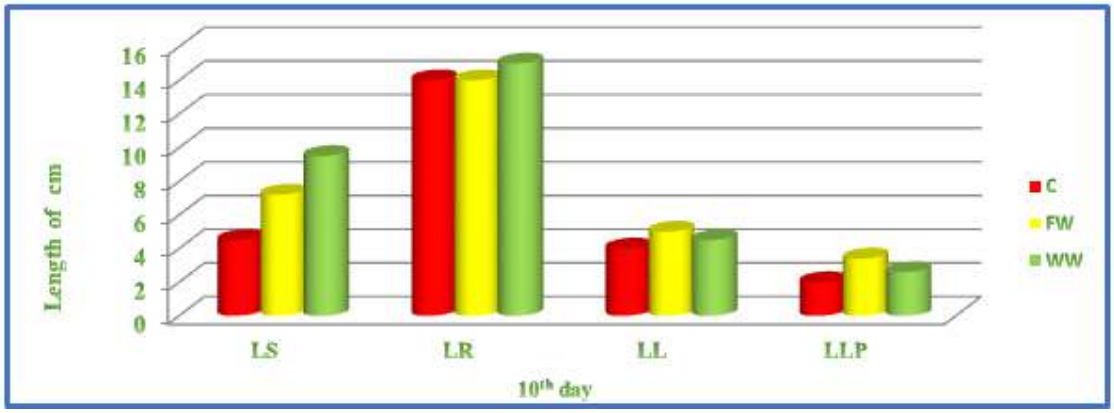


**Fig: 9. EFFECT OF VERMICOMPOST ON THE GROWTH OF *Vigna mungo***




**C**- **C**ontrol, **FW**- **F**ish **W**aste, **WW**- **W**atermelon peel **W**aste, **LS**- **L**ength of **S**tem, **LR**- **L**ength of **R**oot, **LL**- **L**ength of **L**eaf, **LLP** - **Length** of **L**eaf Petiole

**TABLE-2: Effect Of Vermicompost On The Growth Of Plant *Vigna mungo* On 5th and 10th Days.**

| TREATMENT | LENGTH OF STEM | LENGTH OF ROOT | LENGTH OF LEAF | LENGTH OF LEAF PETIOLE | NUMBER OF DAYS |
|---|---|---|---|---|---|
| CONTROL | 3.5 ± 1.0 | 7.8 ± 1.5 | 2.5 ± 0.9 | 1.2 ± 0.5 | 5 |
| FISH WASTE | 5.4 ± 1.2 | 11.5 ± 2.0 | 4.2 ± 0.2 | 2.2 ± 0.9 | |
| WATERMELON PEEL WASTE | 4.6 ± 1.1 | 9.2 ± 2.0 | 3.5 ± 0.8 | 1.5 ± 0.6 | |
| CONTROL | 4.5 ± 1.0 | 14 ± 1.2 | 4 ± 1.0 | 2 ± 0.3 | 10 |
| FISH WASTE | 7.2 ± 1.8 | 14 ± 0.5 | 5 ± 0.8 | 3.4 ± 1.2 | |
| WATERMELON PEEL WASTE | 9.5 ± 1.5 | 15 ± 2.0 | 4.5 ± 0.9 | 2.6 ± 0.8 | |

The root length was increased to a maximum of 17% on the 5th day and slowed down on the 10th day by 7%. Among the stem and root, the stem showed increasing in length within a short period of 10days.

## 4. CONCLUSION

Vermicompost contains macro and micronutrients which are necessary for the growth of the plants. The available nutrients can be easily assimilated for growth and development. Fertilizer comprises some of the larvae and their associated microbes that act as promoters for growth and other nutrients. Due to the presence of all these essential substances, vermicompost has many effects on plant influence. Investigations revealed the potential of selected earthworm *Eudrilus eugeniae* in the degradation of different wastes. Root and shoot length, number of leaves were greater in the plant treated with vermicompost than the plant treated with cow dung compost. Thus it is concluded that vermicompost is more efficient in plant growth. Vermicompost plays a significant role in protecting the environment as it uses waste as a raw material in building up soil fertility and improving the soil quality for sustainable agriculture.

## ACKNOWLEDGMENTS

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SP-40

# ISOLATION OF *LACTOBACILLUS SP* FROM CURD AND IT'S APPLICATION IN ICE CREAM

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## ABSTRACT

Ice cream with lyophilized powder contains probiotic *Lactobacillus sp* and thus making it probiotic. It has been found that ice cream masses are suitable matrices for the inclusion of probiotic bacteria. The concentration of viable cells of probiotic bacteria decreased in the course of storage of ice cream, to carry out its inherent preventive role in the consumption of this type of functional food. This probiotic ice cream should have antibacterial activity.

**KEYWORDS -** Probiotics, *Lactobacillus sp*, Lyophilization, Probiotic ice cream

## 1.INTRODUCTION

The term probiotic comes from the Greek word pro bios which mean for life[1]. The acid[2] producing bacteria in fermented milk products could prevent fouling in the large intestine and if consumed regularly it leads to a long and healthier life. According to him, Bulgarians are hypothesized as healthy and long-lived because of the consumption offer fermented milk products containing rod shaped bacteria (*Lactobacillus spp).* These bacteria affect the gut microflora positively and decrease the microbial toxic activity. *Lactobacillus* is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped non-spore-forming bacteria[3]. They are a major part of the lactic acid bacteria group (i.e., they convert sugars to lactic acid). In humans, they constitute a significant component of the microbiota at several body sites, such as the digestive system, urinary system and genital system. In women of European ancestry, *Lactobacillus* species are normally a major part of the vaginal microbiota. *Lactobacillus* forms biofilms in the vaginal and gut microbiota, allowing them to persist during harsh environmental conditions. Ice cream is an ideal vehicle for the delivery of these organisms in the human diet[4]. *Lactobacillus* and *Bifidobacterium* are the most common species of lactic acid bacteria used as probiotics for fermented dairy products.

## 2. MATERIALS AND METHODS

### *2.1 Sample Collection*

Curd sample was collected from the local market at Mannargudi in Thiruvarur District. The samples were collected and brought to the laboratory in an icebox under aseptic conditions.

### *2.2 Serial Dilution*[5].

1ml of curd sample was suspended in 9ml distilled water to make $10^{-1}$ dilution. From this, 1 ml sample was mixed with 9 ml distilled water to make $10^{-2}$ dilution and these samples were diluted up to $10^{-9}$dilution. From these 9 dilutions, $10^{-4}$, $10^{-5}$, and $10^{-6}$ dilution were selected. It was spread nutrient agar. Few colonies of *E. coli*, *Pseudomonas* were identified. The nutrient agar not support the growth of *Lactobacillus.* Then, $10^{-4}$, $10^{-5,}$ and $10^{-6}$ dilutions were spread and streaked over MRS agar. The plates were incubated in an incubator at 37°C for 24-48 hours. After a period of incubation, colonies were grown on Petri plates. The isolated colonies formed on MRS agar plates were identified using Gram staining and biochemical tests[6]. The identification was performed according to Bergey's manual of bacteriology. The culture was kept in MRS agar slant and stored at 4°C for future use.

### *2.3 Gram Staining was done by using standard procedure followed*[7]

### *2.4 MOTILITY TEST*[8]

Petroleum jelly was applied around the cavity slide. A drop of culture was placed at the center of the cavity slide. Then the coverslip was placed over it. The slide was observed under a microscope with a magnification power of 40X and 100X.

### *2.5 Biochemical test was done by using standard procedure followed by Cappuccino and Shermann, 1998.*

### *2.6 Bile Salt Tolerance Test*[9]

The bile salt tolerance test was assessed by investigating the ability of a strain to grow in the presence of bile. The fresh cultures of isolated *Lactobacillus* were grown at 37°C for 24 h in MRS broth without bile salt and 100µl of culture of appropriate dilution were spread on sterile containing 2% bile salt and incubated anaerobically.After 48 hours of incubation, the bacterial growth was evaluated, to select bile tolerant *Lactobacillus spp*. Bacterial growth was expressed as colony forming units.

### *2.7 Acid pH Test*

Pure *Lactobacillus* was grown in MRS broth at 37°C for 24 h incubation. The cells were harvested and washed twice with sterile phosphate buffer saline (pH 7) by centrifugation at 10000 rpm for 5 minutes[10]. The cell pellets were suspended in sterile MRS broth and adjusted the pH 1, 2 and 3 using 1M HCL and incubated at 37°C.

### 2.*8 ANTAGONISTIC ACTIVITY OF PROBIOTIC CULTURE*

The antagonistic activity of the probioticated culture was studied against certain pathogenic species. The antagonistic activities of the selected isolates were evaluated against *E. coli, Pseudomonas and S. aureus.* The three pathogenic test organisms were collected from the stock collection of laboratories of Dept. of Microbiology, S.T.E.T Women's College, Mannargudi. Muller –Hinton Agar media was uniformly seeded with test organisms. A 1 cm wide ditch was cut in the agar plates. Then, 100µl of *Lactobacillus* culture was poured into the well. The plates were first incubated at 4°C for 60 minutes to allow the test material to diffuse into the agar and then incubated at 37°C for 24 hr. After incubation, the diameter of the clear zone was measured[11].

### *2.9 Lyophilization*[12]

One colony was taken from the streak plate and inoculated in 500ml of MRS broth and it was freeze-dried (powdery form) using lyophilization techniques.

### *2.10 Preparation of Probiotic Ice Cream*[13]

The method for the preparation of ice cream was a slight modification of the method as suggested[13]. 1L of milk was heated well, the heating procedure continues until its level reaches up to half liters. Three egg yolk were taken and added 150g of sugar in to the egg yolk. Then it was mixed well. This mixture was added to the pasteurized milk and mixed well. It again heated up to 5-6 minutes. One spoon of vanilla essence was added. When it reaches normal temperature, the freeze-dried bacteria were added. Finally, it was stored in the freezer at a low temperature.

### *2.11 Shelf Life Study*

The safety and quality of ice cream were determined by significant total bacterial count. After a week of storage, 10 g of melted probiotic ice cream was diluted in distilled water. The 0.1ml inoculum of $10^{-4}$ dilution was placed on the surface of the respective medium. Finally, the MRS agar plates were incubated at 37°C for 24- 48 hr. The viability of probioticated ice cream was determined and expressed as colony-forming units (CFU).

### *2.12 Statistical Analysis*[15]

All the experiments were carried out in the mean values and standard deviation was calculated. Those data were presented as mean for each sample by using the formula given below,

$$\text{Mean} = \overline{X} = \frac{\Sigma X}{\overline{N}}$$

Where,
Σ = sum of all the values of variables
N = Number of observation

## 3. RESULT

### *3.1 Collection of Sample*

**The present study was done with** *Lactobacillus sp* isolated from curd samples & the curd was collected from the local market at Mannargudi in Thiruvarur Dt. The *Lactobacillus spp* were identified using culture medium and based on morphological and biochemical characteristics.

### *3.2 Isolation of Bacteria from Curd Using Nutrient Agar*

***E. coli* and *Pseudomonas***

Serial dilution and plating methods were used for the isolation of bacterial colonies. Nutrient agar was used for the isolation of organisms from curd. The solated organisms II and III showed gram-negative. The bacterial isolate II showed negative for catalase and oxidase. The bacterial isolate II showed positive for indole test, MR – VP test and Carbohydrate fermentation test. Then, the bacterial isolate I showed positive for oxidase and catalase test. These organism, showed negative results for the Indole test, MR – VP test, Citrate Utilization test and Carbohydrate fermentation test. According to the Biochemical test the isolated organisms II and III were identified as *E. coli* and *Pseudomonas* and the isolate I indicated that *Lactobacillus sp*.

### *3.3 Identification of Lactobacillus Spp*

Serial dilution and plating methods were used for the isolation of bacterial colonies. MRS agar was used for the isolation of *Lactobacillus spp*. The serially diluted curd samples were streaked over the surface of MRS agar plates and incubated at 37°C for 24-48 hours. After incubation, the white and pale white colour colonies were observed.

### *3.4 Morphological and Biochemical Characteristics Of Bacteria*

The morphological and biochemical characteristics of the isolates on MRS agar plates were incubated at 37°C for 24-48 hours. The isolated bacteria were observed by a compound microscope.

### *3.5 Gram Staining*

It is clear that the bacteria were gram-positive, (appear as purple) long rod-shaped organisms were identified by using gram staining. The gram staining results indicated that the isolated bacteria was *Lactobacillus sp.*

### *3.6 Motility Test*

The organism was observed under a microscope by using the hanging drop method. The isolated organism showed non- motile under the microscopic observation.

### *3.7 Biochemical Test*

**Indole Test**

Absence of cherry red ring formation was observed on the surface of the tubes, it indicated the isolated *Lactobacillus* showed indole negative

**Methyl Red Test**

In the methyl red test, the development of red colour was observed at the surface of the tubes. It indicates the isolated *Lactobacillus* was MR positive.

**Voges- Proskauer Test**

In VP test, the medium was observed for the colour change. The development of deep pink colour was observed on the surface of the tubes. So, the isolated *Lactobacillus* showed VP negative.

**Citrate Utilization Test**

In the citrate utilization test, Simmons's Citrate Agar was observed for the colour change. No colour change was observed on the tube. It indicated the isolated *Lactobacillus* showed citrate negative.

**Urease Test**

In the urease test, no colour change was observed at the surface of the tubes. No colour formation indicated

*Lactobacillus* showed a negative result for the urease test.

**Oxidase Test**

In the oxidase test, no purple colour formation was observed within 30 seconds, it indicated the isolated *Lactobacillus* showed oxidase negative.

**Catalase Test**

In catalse test, the slide was observed for gas bubble formation within one hour. No gas bubble formation was observed on the surface of the slide, within one hour. It indicated, the isolated *Lactobacillus* showed a negative result for the catalase test.

**Carbohydrate Fermentation Test**

In the carbohydrate fermentation test, the isolated *Lactobacillus* fermented carbohydrate and produced acid and gas. It showed positive results for the carbohydrate fermentation test.

**Hydrogen Sulfide Production Test**

The SIM agar plates were observed for the colour change. No colour change was observed after 48 hours of incubation. It indicated the isolated *Lactobacillus* showed $H_2S$ negative.

### *3.8 Antagonistic Activity*

The *Lactobacillus* was well performed for the antagonistic activity of tested pathogens like *E. coli, Pseudomonas* and *S. aureus*. *Lactobacillus sp* showed better antagonistic activity against *E. coli* (20mm)*, Pseudomonas* (19 mm) followed by *S. aureus* (19 mm).

### *3.9 Acid pH Test*

The results of our study showed that the isolated *Lactobacillus sp* was tolerable to pH 1, 2 and 3.The viability of *Lactobacillus* decreased after incubation of 1, 2 and 4 hours by using pH 1, 2and 3. At the pH one, and the incubation time 2 and 4 hours no growth appeared on Petri plates.

### *3.10 Bile Salt Tolerance Test*

Although the bile concentration of the human gastrointestinal tract varies was believed to be 0.3% and the staying time suggested to be 4h. In our study, the isolated *Lactobacillus was* tolerated up to 1% - 4% of bile concentration after 24 hours of incubation. There was no growth observed at 5% of bile. Finally, the isolated *Lactobacillus* exhibited better tolerance to 2% bile salt compared to others. The ingested bacteria must be resistant to the enzymes in the oral cavity, as well as to the environment during the digestion process in the stomach and the intestine (e.g., exposure to bile).

### *3.11 Lyophilization And Probiotic Ice Cream*

Freeze-dried species was used here. This powder was added at the last step of the preparation of ice cream. The ice cream was easily assimilated and it is better to eat ice cream than medicinal tablets.

Colony forming unit/ml = No. of colonies X Dilution factor / Volume of culture plate.

**Week One**

Plate I = $152 \times 10^5$

Plate II = $145X\ 10^6$

**Week Two**

Plate I = $16O \times 10^6$

Plate II = $150 \times 10^7$

**Week Three**

Plate I = $15X10^6$

Plate II = $14 \times 10^6$

**Week Four**

Plate I = $14 \times 10^6$

Plate II = $13\ X10^6$

## 4. SAFETY AND KEEPING QUALITY OF PROBIOTIC ICE –CREAM

During storage, the counts of *Lactobacillus sp*, remained broadly stable over 28$^{th}$ days and the highest count achieved by the 14$^{th}$ day.

**Table I : Morphological and Biochemical characterization of isolated bacteria**

| S.No | Name of the test | Isolate I | Isolate II | Isolate III |
|---|---|---|---|---|
| 1 | Colony morphology | Long Rod | Rod | Rod |
| 2 | Gram staining | + | - | - |
| 3 | Motility | Nonmotile | Motile | Motile |
| 4 | Indole test | - | + | - |
| 5 | Methyl red | + | + | - |
| 6 | Voges - Proskauer test | - | + | - |
| 7 | Citrate utilization test | - | - | - |
| 8 | Oxidase test | - | - | + |
| 9 | Catalase Test | _ | + | + |
| 10 | Carbohydrate Fermentation test | + | + | - |

\+ indicates Positive
_ indicates Negative

Isolate I *Lactobacillus*
Isolate II *E. coli*
Isolate III *Pseudomonas*

**Table 2: Bile salt tolerance test (24 hours)**

| Test organism | Concentration (%) | Result |
|---|---|---|
| *Lactobacillus spp* | 1 | + |
| | 2 | + |
| | 3 | + |
| | 4 | + |
| | 5 | + |

**+ indicates Tolerant, _ indicates Non-Tolerant**

**Table 3: ACID pH TEST**

| pH values | Time (hour) | TEST | Results |
|---|---|---|---|
| pH 1 | 1 | 180 X $10^4$ | + |
| | | 165 X $1O^5$ | + |
| | 2 | ……. | _ |
| | | ……. | _ |
| | 4 | **……** | _ |
| | | **……** | _ |
| | 1 | 119 X $10^4$ | + |
| pH 2 | | 130 X $10^5$ | + |
| | 2 | 78 X $10^4$ | + |
| | | 52 X $10^5$ | + |
| | 4 | 54 X $10^4$ | + |
| | | 70 X $10^5$ | + |
| pH 3 | 1 | 184 X $10^4$ | + |
| | | 144 X $10^5$ | + |
| | 2 | 147 X $10^4$ | + |
| | | 90 X $10^5$ | + |
| | 4 | 77 X $10^4$ | + |
| | | 83 X $10^5$ | + |

**+ indicates Tolerate, _ indicates Non tolerate**

## 4. DISCUSSION

This study showed that ice cream provides good conditions for probiotic growth in large numbers and their survival during storage. Low-fat ice cream in comparison with regular one provides better conditions for the survival of *Lactobacillus acidophilus, Lactobacillus paracasei* and *Bifidobacterium lactis*[14]. Probiotics are live microorganisms, which when administrated in adequate amounts confer health benefits on host. These include bacteria, molds, yeast but most probiotics are bacteria of which, LAB are the most common type. Several health benefits have been claimed for probiotic bacteria and more than 90 probiotic products containing one or more groups of probiotic organisms are available worldwide. In general, these *Lactobacilli* are presently the most frequent "probiotic" representatives in commercial probiotic products and are followed by *Bifidobacterium* spp.

## 5. CONCLUSION

According to the biochemical test, the isolate I was identified as *Lactobacillus sp.* The *Lactobacillus sp* showed negative for Voges- Proskauer, Citrate utilization, Catalase, Oxidase, $H_2S$ production test, Indole and urease test and positive results for Methyl red. The isolated *Lactobacillus* supernatant was examined for the antagonistic activity of tested organisms. The extracted compound showed antagonistic activity against pathogenic microorganisms. The isolated bacteria tolerate up to 1% -4% of bile salt. One colony *of Lactobacillus sp* taken from streak plate and it was inoculated in 500ml of MRS broth and it was freeze-dried, using lyophilization technique. The lyophilized probiotic bacteria were added at the last step of the preparation of ice cream. The viability of these probiotic bacteria was checked by using the total bacterial count. Ice cream with lyophilized powder contains probiotic *Lactobacillus spp* and thus making it probiotic. It has been found that ice cream masses are suitable matrices for the inclusion of probiotic bacteria. The concentration of viable cells of probiotic bacteria decreased in the course of storage of ice cream, to carry out its inherent preventive role in the consumption of this type of functional food. This probiotic ice cream should have antibacterial activity. The bacterium was tested in high salt concentration and acidic pH values and antimicrobial activity against *E. coli, S. aureus* and *Pseudomonas sp*. Therefore the developed probiotic ice cream can be one of the upcoming health benefits. They may have a good commercial market and consumer's consciousness regarding their health. The growing interest of consumers in the therapeutic product has led to the incorporation of probiotic cultures into the preparation of ice cream and other probiotic products. Ice cream is good enough and the concentration of living bacteria fulfills the demand for probiotic products. Ice cream can serve as an excellent vehicle for the dietary incorporation of probiotic bacteria. Frozen storage of the product has little effect on culture survival.

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# BIOCONTROL EFFICIENCY OF SOIL FUNGI ISOLATED FROM EDAIYUR, THIRUTHURAIPOONDI TALUK AGAINST CERTAIN PLANT PATHOGENS

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## ABSTRACT

The population dynamics of soil fungi from rhizosphere soil can the measured by serial dilution technique the maximum number of propagules from $10^{-5}$, dilution factors when compared to other factors of $10^{-3}$, and $10^{-4}$, can be calculated. The some of the fungi like *A.flavus, A.niger, A.fumigatus, A.terreus, Fusarium solani, Fusarium* sp., *Penicillium* sp, and *Trichoderma* sp. was conformed from the native soil sample. Screening of potential fungi by using different commercial antibiotics *Flucouazole, Itnaconazole, Ketoconazole* and *Clotrimazole* with 10ppm concentrative was treated against *Aspergillus flavus, A.niger, A.terreus and Trichoderma sp* recorded by *invitro* method.

**KEYWORDS**: Population, soil fungi, *A.niger, A.terreus and Trichoderma sp.*

## INTRODUCTION

The soil is a reservoir for a large number of microorganisms such as bacteria, viruses, fungi and protozoa. Microbes of soil increase the soil fertility maintain ecosystem sustainability, antibiotic and enzyme production and biodegradation are the distinct beneficial effects[1]. Many of fungi occur as saprophytes in the environment and are scattered throughout the world. Although these fungi had previously been considered to be non pathogenic, are now being encountered as causes of humans and animals infection especially in hosts with impaired immune systems. Plant pathogen which is caused by fungus is becoming a major thread now a day because it is causing a serious loss to agriculture products. So overcome these problems many and more scientist are working but still there is no biocontrol resource to settle down the issue. Alternative methods need to search out to control plant disease which is cause by plant pathogen fungi as biological control or natural control resources. Here describes a different mode of actions of biocontrol active microorganisms to control fungal plant diseases, which include hyper parasitism, predation, cross protection antibiosis, nutrient and induced resistance. There are so many application of biocontrol available but no one will stand for upcoming challenges[2]. There are some common biocontrol products are applied against seed borne and soil borne fungal pathogens, including the causal agents of seed rot, damping, root rot disease. These products are mostly used as seed treatment and have been effective in protecting several major crops such as wheat, corn, rice sugar beet and cotton against fungal pathogens. Some cases biocontrol microorganism has also been used as spray application on foliar diseases. More than a few strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants. So here the research aim is to Isolation and Identification of fungi from contaminated soil to build biological resource as biocontrol activity. After this identification, the research will proceed for the Detection of Bio control activity[3].These properties have made *Trichoderma* a ubiquitous genus present in any habitat and at high population densities. Several advantages of using *Trichoderma* in managing soil borne plant pathogens are reported by different workers such as, ecofriendly[4], effective in managing diseases caused by soil borne plant pathogens which cannot be easily controlled by chemicals[5], ease and cost effective mass Culturing of antagonists[6], growth promoting effect[7] and long lasting effective disease management[8]. Several strains of *T. viride* had a significant reducing effect on plant diseases caused by pathogens such as *Rhizoctonia solani, Sclerotium rolfsii, Pythium aphanidermatium and Fusarium oxysporum.* To overcome these problems researchers look for alternative options such as the use of bicontrol agents (BCA) for disease control either alone or in an integrated approach with other chemicals for ecofriendly and sustainable methods of disease control. Currently, several biocontrol agents have been recognized and are available as bacterial agents for example as fungal agents such as *Aspergillus, Gliocladium, Trichoderma, Ampelomyces, Candida,* and *Coniothyrium*[9-10]. Among these biocontrol agents *Trichoderma* spp. is one of the most versatile biocontrol agents which have long been used for managing plant pathogenic fungi.

## MATERIALS AND METHODS

### *Soil sample collection and physicochemical analysis*

Soil sample was collected from paddy filed Edaiyar, Thirudhuraipoondi (Taluk), Thiruvarur District, Tamil Nadu. The collected soil sample were subjected to different physico-chemical parameters including pH[11], electrical conductivity[12], organic carbon[13], organic matter[14], nitrogen, phosphorus[15], potassium, calcium, magnesium, zinc, copper, iron and manganese[16].

### *Isolation of fungi from Soil Sample*

The homogenated sample was serially diluted from $10^{-1}$ to $10^{-B}$ and 0.1 ml of sample was taken from $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilution spread on PDA for fungi. One media from each of them was maintained as a control without sample. The plates were incubated at 37° C for 48 hours and the fungal colonies were calculated.

### *Identification of fungi*

The isolated species of fungi were identified based on morphological characteristics of the individual colony and cell. The fungi were cultured into PDA media and incubated at 25-28°C for 4 days. The different morphology of fungi was transferred to PDA agar. The fungi identification was performed according to the usual morphological criteria[17]. Observation and identification of the fungi cell were performed after 10 days of culture with lactophenol and observed microscopically using standard manual of soil fungi by Gilman[18], by Ellis[16] more Dematiaceous Hyphomyectes by Ellis and Ellis[16].

### *Determination of antibiotic susceptibility test[19]*

The fungal pathogens were growing on Potato Dextrose broth (PDA) at 37°C for 48 hours incubation. A sterile cotton wool swab dipped into the 48 hours old fungal suspension was spread evenly on the surface of the Potato dextrose agar plates. The inoculated plates were allowed to dry before placing the diffusion discs containing antibiotics. Susceptibility of the isolates to 12 types of antibiotics was performed using the standard Kirby – Bauer method. Commercially available discs (Hi- media) containing *Flucouazole, Itnacouazole, Ketoconazole* and *Clotrimazole* (10 ppm) and sterile disc (as a negative control) were placed on the surface of the PD agar plates and incubated at 30°C for 48 hours. The diameter of inhibition zones formed surrounding each isolate inclusive of diameter of the discs was measured. All isolates were tested duplicate for each type of antibiotic.[20].

### *Effect of fungicide of the growth of fungi*

To prepare PDA medium (potato dextrose agar) after sterilization different concentration of fungicide (20, 25, and 30µl) mixed together in PDA medium and poured in sterilized petric plate, these plate allow solidification. After solidification the potential fungi (*Aspergillus niger, Aspergillus terreus* and *Trichoderma* sp) was inoculated in centre of the PDA plate individually and there plate were kept in incubated room temperature for 7 days. After the plate were observed and measured the growth of fungi against fungicide[21].

### *Production of secondary metabolises against plant pathogen[22]*

Fungi strain Rot stop (Verdera Ltd., Esbo, Finland) was maintained on Melin Norkrans agar media (MMN) in 9 cm diameter Petri dishes at room temperature in the dark. For the production of metabolites, 500 mL Erlenmeyer flasks were used, each containing 250 mL of liquid MMN media. Five agar plugs 0.5 × 0.5 cm in size with established fungal mycelia from an actively growing colony were aseptically inoculated in each flask, and incubated on a rotary shaker at 120 rpm at room temperature for seven weeks. Cultures were filtered to obtain a mycelium-free sample for sample work-up by reversed-phase SPE.

## RESULT

### *Soil sample collection and physicochemical analysis*

Collection of soil sample from paddy field at Edaiyar, Thirudhuraipoondi (Taluk), Thiruvarur district, Tamil Nadu. Analysis of physiochemical parameters like pH,(7.2), Ec (0.20dsm%), Organic carbon (0.32), organic matter (0.28%), available nitrogen (0.35%mg/kg), available potassium (0.36mg/g), available phosphorus (0.30mg/g), zinc (0.41ppm), copper (0.26ppm), iron (0.35ppm), manganese (0.53ppm), cation, exchange capacity ((0.50C), mole, calcium (0.53km/kg), magnesium (0.37C.m/kg), and sodium

(0.43mole/kg). were recorded respectively from the soil sample.

***Isolation and identification of microorganism***

Population density of fungi was diluted from $10^{-3}$, $10^{-4}$, and $10^{-5}$, was 12, 28, and 32 total no of colonies observed from the native soil sample. In the experiments native strain improvement was better to promote the control the disease. The identification of fungi *Aspergillus flavus, A.niger, A. terreus, A.fumigatus, Fusarium solani, Fusarium* sp, and *Trichoderma* sp, were conformed with the help of standard manual of Gillman and Hyphomycetes (Table –1).

***Screening of potential fungi by using different antibiotics***

The screening of potential fungi by using different commercial antibiotics by *invitro* methods, the some of the fungi trick tolerate the fungicide like *Fluconazole, Itnaconazole, Ketoconazole,* and *Chotrimazole* were analysed from the fungi like *Aspergillus flavus, A.niger, A.terreus* and *Trichoderma* sp was screened with 1.00±3.33, 5.00±1.66, 5.66±1.66, 5.65±1.65mm zone of inhibition from 10ppm *Flucouazole* observed. In the case of *Intracouazole* was 14.3±4.66, 06.0±2.00, 11.6±3.66, 16.0±5.33mm growth inhibited from the experiments. Whereas 10ppm of *Ketocozol*e was 06.3±2.00, 13.6±4/33 and 2.66±0.66 mm zone of inhibition for *A.flavus, A.niger, A.tereus,* and *Trichoderma* sp were determined (Table -2 and plate - 1).

***Effect of fungicide on the growth of pathogen (R.solani)***

The effect of intracouazole of 20.25 and 30µl concentration treated with medium for *A.flavus, A.niger, A.terus* and *Trichoderma* sp can be conformed to the specific analysis.

***Production of secondary metabolites from fungi A.niger and their effect in plant pathogen (R.solani)***

As per the suppression activity of pathogen *A.niger* by secondary metabolites of fungi (%) treated with 2, 4, 6, 8 and 10% with 5.33 ± 1.76, 6.00 ± 2.00, 5.33 ±1.67, 5,00 ± 1.62, 5.00 ± 1.62, and 5.33 ± 1.64mm zone of inhibition recorded most of pathogen by controlling of biological methods (Table – 3).

**Table 1: Identification of fungi from native soil sample**

| S. No | Fungi |
|---|---|
| 1 | *Aspergillus flavus* |
| 2 | *Aspergillus niger* |
| 3 | *Aspergillus fumigates* |
| 4 | *Aspergillus terreus* |
| 5 | *Fusarium solani* |
| 6 | *Fusarium* sp. |
| 7 | *Penicillium* sp. |
| 8 | *Trichoderma* sp. |

**Table – 2: Screening of potential fungi by using different commercial antibiotics by *invitro* methods**

| Name of the fungi | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | ***Fluconazole*** **(10ppm)** | ***Itraconazole*** **(10ppm)** | ***Ketoconazole*** **(10ppm)** | ***Clotrimazole*** **(10ppm)** |
| *Aspergillus flavus* | 1.00 ± 3.33 | 14.3 ± 4.66 | 06.3 ± 2.00 | - |
| *A.niger* | 5.00 ± 1.66 | 06.0 ± 2.00 | 13.6 ± 4.33 | - |
| *A.terreus* | 5.66 ± 1.66 | 11.6 ± 3.66 | 2.66 ± 0.66 | - |
| *Trichoderma* sp. | 5.65 ± 1.68 | 16.0 ± 5.33 | - | 17.6 ± 5.66 |

***Standard deviation ± Standard error***

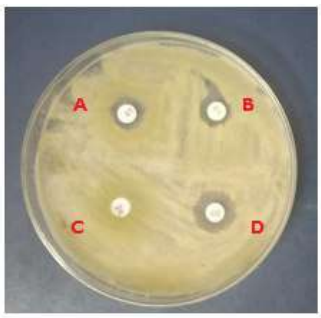
**Table 3: Production of secondary metabolites from fungi *Aspergillus niger* and their effect in plant pathogen**

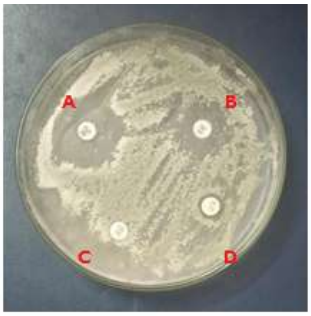
| Different concentration of Secondary Metabolites (%) | Zone of inhibition (*R.solani*) (mm) |
|---|---|
| 2 | 5.33 ± 1.76 |
| 4 | 6.00 ± 2.00 |

| | |
|---|---|
| 6 | 5.33 ± 1.67 |
| 8 | 5.00 ± 1.62 |
| 10 | 5.33 ± 1.64 |

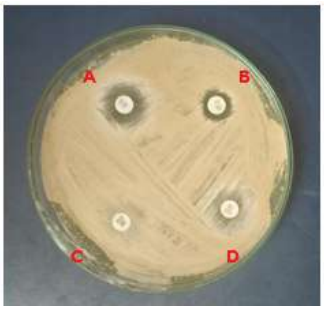
***Standard deviation ± Standard er***

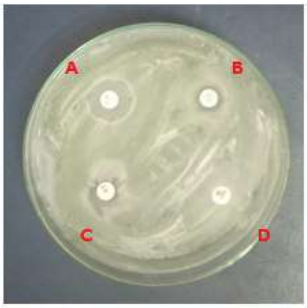
**Plate 1: Screening of potential fungi by using different commercial antibiotics by *invitro* methods**




***Aspergillus flavus*** ***A.niger***




A. ***Terreus*** ***Trichoderma* sp.**

**A - *Fluconazole,* B - *Itraconazole,* C - *Ketoconazole,* D - *Clotrimazole.***

## DISCUSSION

The fungi associated with postharvest spoilage of guava (*P. guajava* Linn.) were identified in this study as *C. gloesporioides, F. oxysporum, Mucor* sp., *R. stolonifer, A. niger, A. fumigatus* and *A. parasiticus.* Guava was also shown to have a wide range of fungal load per gramme of the fruits in Awka meteropolis. These organisms are commonly implicated in the postharvest deterioration of many fruits and vegetables and have been reported severally[23]. The biocontrol active agents are very important to find out to build the natural resources for controlling plant pathogen. This study is accomplished by the isolation and identification of the *Trichoderma*. It is not only fungal pathogens but also produce antibiotics. Meanwhile, many of the fungus have better effects on plant growth as like as yield of the agricultural product, yield of soil nutrient, increase the nitrogen fixing capacity of the plant, increases the nutrient uptake capacity of the plant as well as soil, increase the fertilizer utilization efficiency, increase the rate of seed germination and systemic resistance to plant diseases[24]. In the research study, production of secondary metabolities from potential fungi *Trichoderma viride* has more antibiotics like *Trichomycin, Trichomedin, Trichocin*, from the inhibition activity against caller rot pathogen. The biological control of plant pathogen can be depicted control of plant pathogen can be dedicated by the way of natural control measures. Secondary compounds and antibiotics produced by *Trichoderma* spp. play a vital role in antagonistic biocontrol activity [25-27] reported that *Trichoderma* spp. produced several secondary compounds, including antifungal antibiotics such as polyketides, pyrones, and terpenes. Secondary metabolites, including antibiotics, that are not directly involved in natural growth, development, or reproduction and are chemically different from natural compounds may play important roles in the defence response, symbiosis, metal transport, differentiation, and stimulating or inhibiting spore formation and germination.

## CONCLUSION

Biological control is an alternative solution to widespread usage of chemicals, which has caused a great deal of concern in recent years. There is enormous use of agrochemicals in modern agriculture, the use of environmentally friendly alternatives to chemical pesticides are absolutely required. Biological control is an attractive alternative to agrochemicals, although it is generally impossible to affect one part of the ecosystem without having indirect ecological effects.

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SP-42

# STUDY ON THE ORGANIZATION OF THE INTERNAL TISSUES AND THEIR CELLS OF GASTROPOD MOLLUSC *FICUS FICOIDES* (LAMARCK,1822)

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## ABSTRACT

Gastropod comprises the second-largest diverse group of animals. Identification of these species reported by its morphological character and report of the internal tissues and their cells are meager. The histological examination involves the evaluation of a true indication of the health of an organism. Histological organization of the selected tissues was made for a better understanding of the structural and functional integration of the different organs are foot muscle consists of gland cells, a meshwork of muscle cell bundles, epithelial cells and connective tissues. Other minor cells were pigment cells, fibroblasts, dense connective tissues. The mantle has dorsal epithelium, longitudinal muscle fibers, connective tissues, Transverse muscle fibers, Blood vessels. Gill comprises epithelium, subepithelial longitudinal muscles, connective tissues. Hepatopancreas has hepatic lobule, central hepatic venule, supporting collagenous tissue and sinusoids. Testis consist of seminiferous tubule, spermatogenic cells, Sertoli cells and Leydig cells. Ovary consist surface epithelium, oocyte, primary follicle, Secondary follicle, tertiary follicle and mature follicle mature oocyte cells characterized by the presence of a chorion. Different cells are observed in the organ tissues which show similar results of the earlier histology study of the gastropods.

**KEYWORDS**: *Ficus ficoides*, histology, organs, gastropod, mollusk

## INTRODUCTION

The fig shell *Ficus ficoides*[1] is a common, subtidal species of sandy, rocky and muddy bottoms on the southeast coast of Tamil Nadu. *Ficus* species have played a significant role as part of the nutritional requirements of rural and coastal populations. These creatures have been important to humans throughout history as a source of food, and medicinal and pharmaceutical importance[2]. Information on the histology of different organs in male and female *F.ficoides* is inadequate and so far there are no published articles in this study. This work is focussed to know whether any difference exists in the organization of the internal tissue and their cells in male and female *F.ficoides*.

## MATERIALS AND METHODS

Live specimens of *F. ficoides* collected from the study area of the Thirumullaivasal coast were brought to the laboratory maintained in aquaria for acclimatization. Tissue samples were taken from the animal body parts viz., foot, mantle, gill, hepatopancreas, testis and ovary. The tissues were fixed in 5% formalin for 12 hours. The fixed tissues were washed in running tap water overnight and then dehydrated by the usual procedure in ascending grades of alcohol series. For block making paraffin of melting point 58-60ºC was used. Sections cut at 5µ thickness were deparaffinized and stained with hematoxylin and with eosin as a counter stain. The photomicrography of various sections of the tissues was taken for microscopic observation[3].

## RESULTS AND DISCUSSIONS

Histological organization of the selected tissues was made for a better understanding of the structural and functional integration of the different organs like a foot, mantle, gill, hepatopancreas, testis and ovary.

**Foot**

The histological section of foot (Fig: 1& 2) consists of gland cells, a meshwork of smooth muscle cell bundles, epithelial cells and connective tissues. The foot surface layer was covered with single-layered epithelial cells. The foot is highly glandular. The glandular portion of the foot consists of epidermal gland cells. The gland cells are numerous and crowded with appearing as deeply staining mass in sections in which individual cells are not distinguishable. Mucus-secreting and mucus-storing cells extended deep into the subepithelial matrix. Other minor cell types observed were pigment cells, fibroblasts, dense connective tissues.

**Mantle**

The *F.ficoides* mantle tissue (Fig: 3 & 4) consists of dorsal epithelium, longitudinal muscle fibres, connective tissues, Transverse muscle fibres, Blood vessels and Blood space. The histological section of mantle reveals that the epithelium covers on either side of the mantle fold. The Interior of the mantle consists of longitudinal muscle fibres, connective tissues, Transverse muscle fibers, Blood vessels and Blood space.

**Gill**

The *F.ficoides* histology (Fig: 5 & 6) studies consist of the epithelium, subepithelial longitudinal muscles, connective tissues and Blood space. The tissues surrounding the organ have an epithelium layer and subepithelial longitudinal muscles. Different size of muscle cells covering the distal end of the gill and thin muscle cells are observed below the epithelia as a fiber.

**Hepatopancreas (HP)**

The hepatopancreas (Fig: 7 & 8) tissue have hepatic lobule, central hepatic venule, supporting collagenous tissue and sinusoids. The histology observation of light microscope reveals two types of cells namely glandular cells and secretary cells. The secretory cells which lie in the corner of the glandular tubules are less in number.The liver has a thin capsule of dense connective tissue which has clear hepatic lobules. The central hepatic venules which supporting connective tissue. Microscopic observation show sheets of hepatocytes comprise the sinusoids and the hepatocytes are lined by endothelial cells.

**Testis**

The histology of *F.ficoides* (Fig: 9) consists of a thick basal lamina enclose the seminiferous tubule, 3-4 layers of smooth muscle cells, spermatogenic cells and Sertoli cells. Spermatogenic cells lie in the cell layer luminal to the spermatogonia. Sertoli cells extend in between the spermatogenic cells. Leydig cells occur in clusters, which are variable in size

**Ovary**

The histology study of the ovary (Fig: 10) consists of surface epithelium, oocyte, primary follicle, Secondary follicle, tertiary follicle and mature follicle. Oocyte under development and the cytoplasm is homogeneous. The nucleus is centrally located. Mature oocyte cells are characterized by the presence of a chorion.

Fig 1: Foot Male

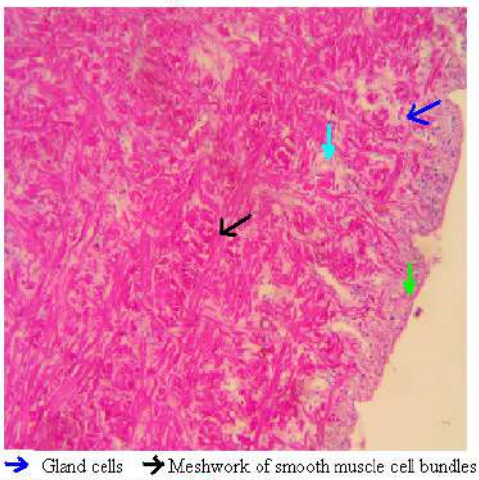



Fig 2: Foot Female

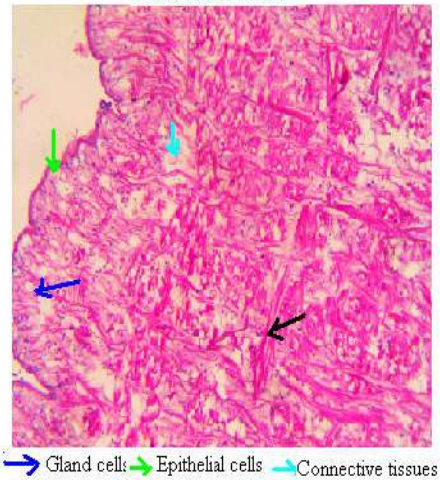
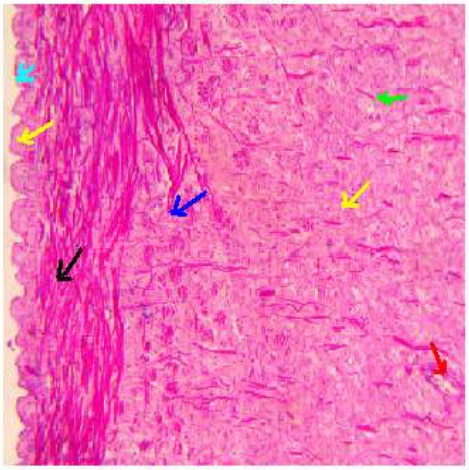
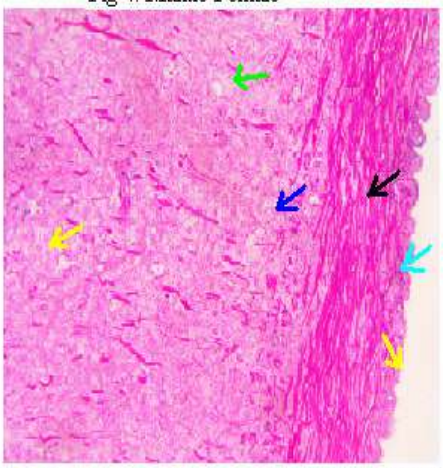


Fig 3: Mantle Male



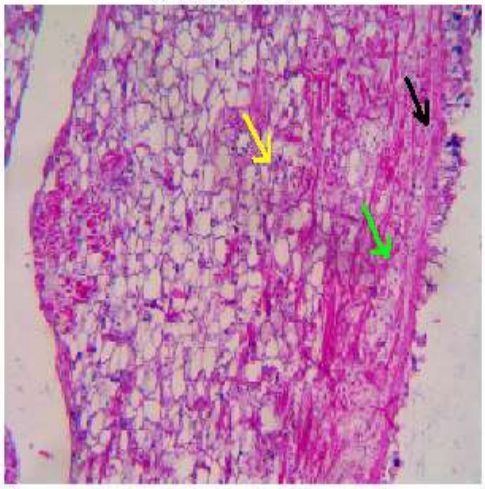
Dorsal epithelium, Longitudinal muscle fibres, Connective tissues, Blood space, Transverse muscle fibres, Blood vessels

Fig 4: Mantle Female



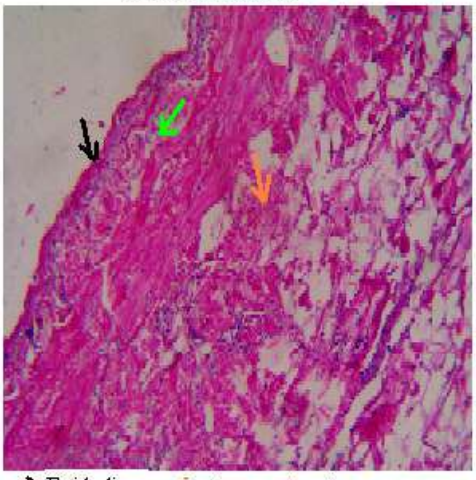
Dorsal epithelium, Longitudinal muscle fibres, Connective tissues, Blood vessels, Transverse muscle fibres

Fig 5: Gill Male



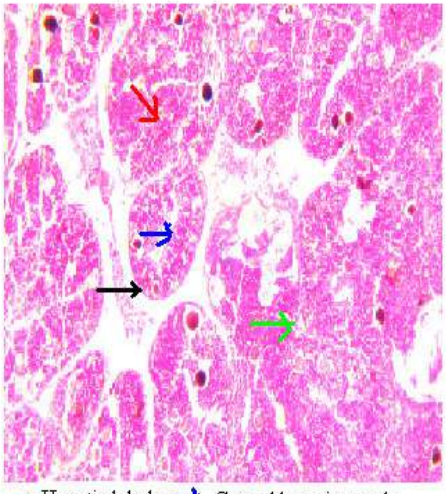
Epithelium, Subepithelial longitudinal muscles, Cornective tissues

Fig 6: Gill Female



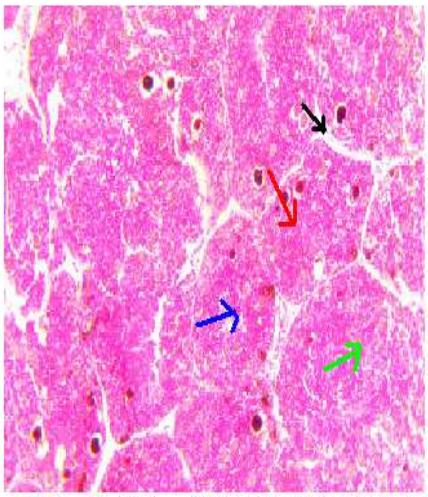
Epithelium, Connective tissues, Subepithelial longitudinal muscles

Fig 8: Hepaticpancreas Female



Hepatic lobule, Central hepatic venule, supporting collagenous tissue, Liver sinusoids

Fig 7: :Hepaticpancreas (Male)



Hepatic lobule, supporting collagenous tissue, central hepatic venule, Liver sinusoids

Fig 9: Testis

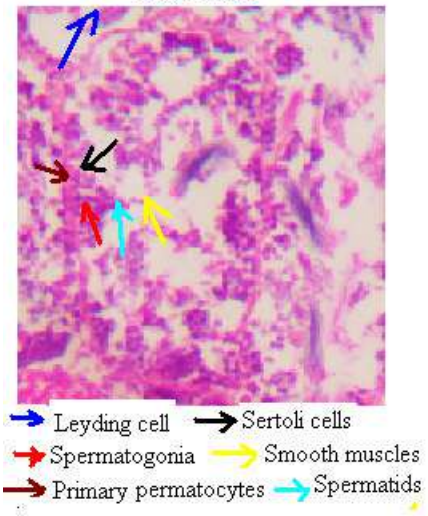
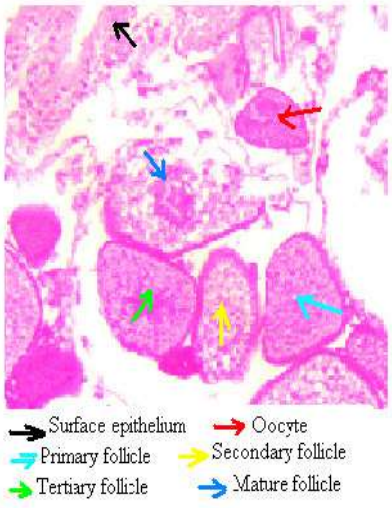



Fig 10: Ovary




## CONCLUSION

Different cells are noticed in the organ tissues of the foot, mantle, gill, hepatopancreas, testis and ovary which shows the similar results of the earlier histology study of gastropods include[4] in *Thai bufo*[5] in Gastropods *Arion rufus* and *Helix pomatia;* Jeevaselvasundari (2011)[6] in *Hemifusus pugilinus*[7] in *Chicoreus virgenius*[8] in *Pinctada radiata*[9] in *Lalemula elliptic*[10] in *Haliotis asinine*[11] in *Tegula eiseni, T. funebralis, T. aureotincta, T. gallina*, and *T. regina* [12] in the mantle of *Lymnaea luteola.*

## ACKNOWLEDGEMENT

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SP-43

# ANTIOXIDANTS AND FREE RADICALS ACTIVITIES IN *MUSA PARADISIACA* FLOWERS

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## ABSTRACT

Banana blossom is an excellent source of crude fiber in the human diet. Dietary fiber has demonstrated its benefits in health and disease prevention in medical nutrition therapy. The objective of this study is to find out antioxidant activities and free radical scavenging activity, in the flower of *Musa paradisiaca* extracts. The antioxidants may be exogenous or endogenous. The endogenous antioxidants can be classified as enzymatic and nonenzymatic. The extracts from, ethanol were investigated for the presence DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay of ethanol extract demonstrated stronger antioxidant activity than aqueous extract in which the $IC_{50}$ found to be 49.32 µg /ml, Ascorbic acid inhibited at $IC_{50}$ rate 57.43µg/ml. ABTS radical are more reactive the DPPH radicals and unlike the reactions with DPPH radical, which involve H-atom transfer, the reaction with ABTS radicals involve an electron transfer process. In concentration-dependent manner the ethanolic extract of *M.paradisiaca* was effective in neutralizing ABTS radical @ Mean $IC_{50}$ found to be 30.1 µg /ml. Potential while, ascorbic acid inhibited at $IC_{50}$ 57.43 µg /ml. Superoxide radical @ Mean $IC_{50}$ was found to be 43.14 µg /ml potential while standard, Ascorbic acid inhibited at $IC_{50}$ rate of 57.43 µg /ml. The potential of ethanolic extract was found to be equivalent to that of standard.

**KEYWORDS:** Phytochemicals, antioxidant activity, banana flower, *Musa. paradisiaca.*

## INTRODUCTION

Antioxidants are substances that may pressure cells from the damage caused by free radicals. Antioxidants interchange with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. The antioxidants may be exogenous or endogenous. The endogenous antioxidants can be classified as enzymatic and non-enzymatic. The antioxidant enzymes incorporate Superoxide dismutase SOD, Catalase CAT, glutathione peroxidase GPx, glutathione reductase GR. The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic includes glutathione, L-arginine, uric acid, bilirubin etc. While nutrient antioxidant belonging to exogenous antioxidants are compounds which cannot be produced in the body and must be provided through foods such as vitamin E, vitamin C, carotenoids, trace elements (Se, Cu, Zn, Mn)[1]. Different types of biological antioxidants include, for instance, Glutathione Vitamin C & vitamin E, cystine, etc[2]. It is believed that oxidative stress plays an important role in the development of vascular complications in diabetes particularly type 2 diabetes. ROS level elevation in diabetes may be due to a decrease in destruction and increase in the production by catalase CAT–enzymatic/non-enzymatic, superoxide dismutase SOD and glutathione peroxidase[3]. A free radical is defined as a fragment that contains one or more unpaired electrons in a single orbit. Molecular oxygen has two and nitric oxide has one Unpaired electron. Free radicals result in several human degenerative diseases affecting a wide variety of physiological functions such as atherosclerosis, diabetes, ischemia/reperfusion (l/R) injury, inflammatory diseases (rheumatoid arthritis, inflammatory small intestine and pancreatitis), cancer, neurological diseases, hyper tension[4]. The toxicity of oxygen or if its radical derivatives is often accompanied by the peroxidation of lipids. Radical

and non-radicals oxidants can convince lipid peroxidation particularly of those lipoproteins, that contain unsaturated fatty acids[5]. Free radicals convince peroxidation of membrane lipids occurs in three stages initiation, propagation, and termination beginning phase involves the removal of hydrogen atom from caused by hydroxyl radical cultivation phase under aerobic conditions. Medicinal plants are frequently used in traditional medicine to treat different diseases in different areas of the world[6]. *M.Paradisiaca* is often consumed as a vegetable[7]. *M.paradisiaca* is a popular dish. It is consumed as curry as well as a boiled or deep-fried salad with rice and wheat bread. *M.paradisiaca* is generally valued as a fiber-rich source. Dietary fiber has demonstrated its benefits in health and disease prevention in medical nutrition therapy[8]. Along with dietary fibers, proteins and unsaturated fatty acids. The present study was undertaken to investigate this free radical,ABTS, So and antioxidant potential of *M.paradisiaca*. However, isolated from *musa paradisiaca* induced diabetic rats. The work highly the effect of *M.paradisiaca* compound produce from Free radical, ABTS, So and antioxidant potential activity

## MATERIALS AND METHODS

**Materials:** Chemical and all Glassware's were obtained from the Department of Biochemistry, Government Arts College for women, Krishnagiri. All chemicals used were of analytical grade with 99 % purity. All the chemicals and reagents used for a screening test, quantitative analysis and antioxidants activity were of analytical grade obtained from various companies

**Collection of plant samples:** *The freshly and healthy of Musa paradisiaca flowers were collected from the local market of panjaliyur village, Krishnagiri (DT), Tamilnadu, in India. The flower were cut into small pieces and dried under shade for three to four weeks. The dried materials were ground in to fine powder. The entire mixture was homogenized in blander than the homogenized mixture was left at room temperature for about 48 hr.*

***Preparation of flower extract:*** 9g of *Musa. Paradisiaca* powder was extracted with 150 ml ethanol in a soxhlet apparatus for 48 hours. After extraction the solution was left out for 24 hrs was filtered and the clear filtrate was evaporated to dryness using a water bath at $40^0$C. After completion of the reaction, the entire slurry was filtered to get blossom powder extract[9]. All extracts obtained were stored in a refrigerator until required for use. Further Phytochemical screening of flower extract was analyzed by qualitatively and quantitatively.

**In vitro radical scavenging assay**
**DPPH radical scavenging activity[10].**
The DPPH radical is scavenged scavenging activity done by antioxidants through the donation of a proton forming the reduced DPPH. The effective concentration at which DPPH radicals were scavenged by 50% and were obtained by interpolation from the linear regression analysis.

**ABTS radical scavenging activity**[11].
Decolourisation assay involves the generation of the ABTS and chromophore by the oxidation of ABTS with ammonium per sulfate. The flower extracts (20-100 µg/ml) were react with 1.0ml of ABTS solution resulting in the final concentration of ABTS being 7mM.

**Hydroxyl radical scavenging assay[12]**
Generated from FeSO4 and hydrogen peroxide and detected by their ability to hydroxylate salicylate and the hydroxylated salicylate complex.

**Superoxide radical scavenging activity[13]**
The superoxide scavenging activity method was estimated at $25^0$ C by the spectrophotometric monitoring of the inhibition of pyrogallol autoxidation.

## RESULT

**Invitro antioxidant assay**
**Free radical Scavenging potential of crude extract of *M. paradisiaca* flower.**

**DPPH radical scavenging assay: T**he DPPH radical scavenging potential of *M. paradisiaca* crude extract

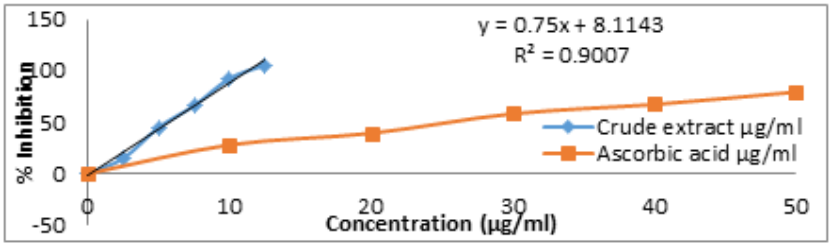


**Figure: 1 The Presents DPPH radical scavenging potential of *M. paradisiaca* crude extract**

The essence of the DPPH method is that the antioxidants react with the stable free radical i.e. α,α-Diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α,α-Diphenyl-β-picrylhydrazine with discolouration. The degree of discoloration indicates scavenging potentials of the sample antioxidant. Figure 1 Presents the free radical DPPH scavenging potential of the ethanolic extract of *M. paradisiaca* flower and ascorbic acid (ASB) For the concentration range of 2.5 -12.5µg /ml ethanolic extract of *M.paradisiaca* flower showed inhibition percentage at a range of 15-106% respectively. In concentration-dependent manner the ethanolic extract of *M.paradisiaca* flower was found to be effective in neutralizing DPPH radical @ Mean $IC_{50}$ found to be 49.32 µg /ml, Ascorbic acid inhibited at $IC_{50}$ rate 57.43µg/ml. Potential of ethanolic extract was found to be equivalent to that of standard.

**ABTS radical scavenging assay**

presents ABTS radical scavenging potential *of M.paradisiaca* flower in crude extract**.**

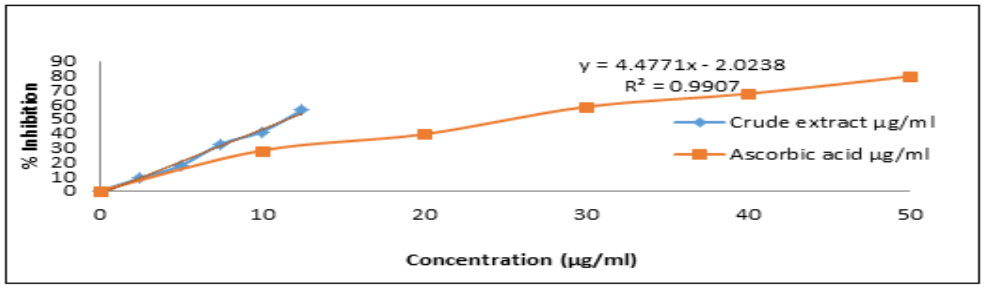


**Figure :2 Present ABTS scavenging potential of *M. Paradisiaca flower* crude extract**

Figure:2 Presents the free radical ABTS scavenging potential of the ethanolic extract of *M.paradisiaca* flower and ascorbic acid (ASB). The concentration range of 2.5 -12.5µg /ml ethanolic extract of *M.paradisiaca* showed inhibition percentage at a range of 8.75-56 % respectively. In concentration-dependent manner the ethanolic extract of *M.paradisiaca* was effective in neutralizing ABTS radical @ Mean $IC_{50}$ found to be 30.1 µg /ml. Potential while, ascorbic acid inhibited at $IC_{50}$ 57.43 µg /ml. The potential of ethanolic extract was found to be low to that of standard.

**Hydroxyl radical scavenging assay**

The presents hydroxyl radical Scavenging potential of *M.paradisiaca* flower in the crude extract.

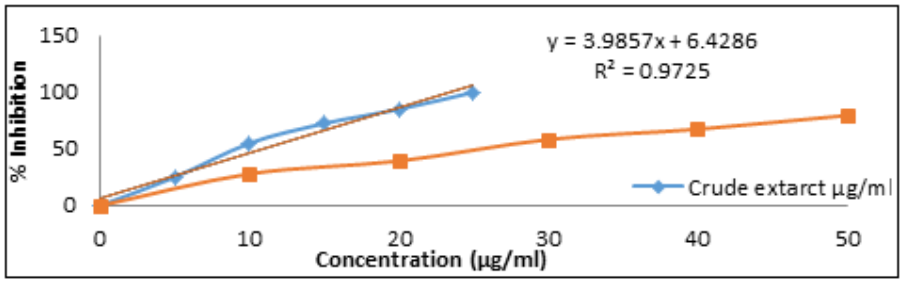


**Figure :3 Present Hydroxyl radical scavenging potential of *M.paradisiaca flower* extract.**

Figure:3 Presents the free radical Hydroxyl scavenging potential of the ethanolic extract of *M. paradisiaca* flower and ascorbic acid (ASB). For the concentration range of 5-25µg /ml, ethanolic extract of *M.paradisiaca flower* showed inhibition percentage at a range of 25-100 respectively. In concentration-dependent manner the ethanolic extract of *M.paradisiaca flower* was effective in neutralizing Hydroxyl radical @ Mean $IC_{50}$ found to be 55.23 µg /ml, While ascorbic acid inhibited at $IC_{50}$ rate of 57.43 µg/ml. The potential of ethanolic extract was found to be equivalent to that of standard.

**SO radical scavenging assay**

The Presents SO Scavenging potential of *M.paradisiaca* crude extract**.**

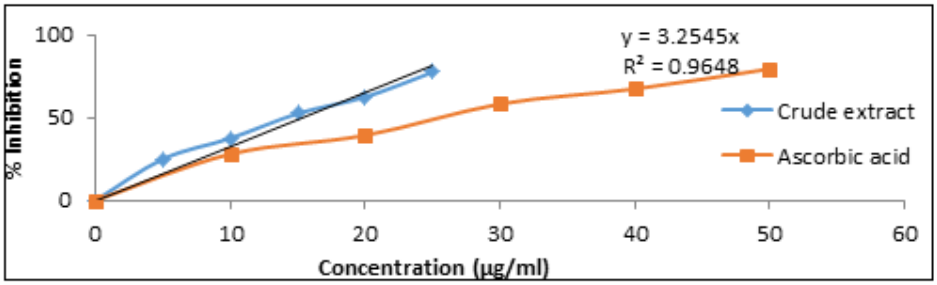


**Figure:4 Present SO radical scavenging potential of *M.paradisiaca flower in* crude extract**

Figure:4 Presents the free radical superoxide scavenging potential of the ethanolic extract of *M. paradisiaca flower* and ascorbic acid (ASB).The concentration range of 5-25µg /ml ethanolic extract of *M. paradisiaca flower* showed inhibition percentage at a range of 25 -77.5% respectively. In concentration-dependent manner the ethanolic extract of *M.paradisiaca flower* was effective in neutralizing Superoxide radical @ Mean $IC_{50}$ found to be 43.14 µg /ml potential while standard, Ascorbic acid inhibited at $IC_{50}$ rate of 57.43 µg /ml. The potential of ethanolic extract was found to be equivalent to that of standard.

**Table I. Unit Body and organ weight in g.**

| **Experimental** | **Normal** | **Diabetic control** | **Diabetic+*M. paradisiaca*** | **Normal+*M. paradisiacal*** |
|---|---|---|---|---|
| **Body weight Initial(0days)** | 210.66±10.35 | 221±13.708[a] | 218.81±11.38 | 202.37±15.73[b] |
| **Final(15days)** | 227.05±10.50 | 226±0.990[a] | 231±0.90 | 230±10.10[b] |
| **Organ weight Liver** | 7.083±0.149[a] | 4.55±0.59[b] | 7.05±0.77[c] | 5.64±0.52[a] |
| **Kidney** | 1.33±0.142[a] | 1.20±0.05[b] | 1.43±0.10[c] | 1.21±0.10[a] |

Values are mean ± SD of six samples in each group: a:comparison of Group I to Group II, b: comparison of Group II Group IV and Group I c: comparison of Group I and III. Significance: a, b, are significant at $p<0.05$, c- represent insignificantly.

Table:1 presents the changes in the bodyweight of male albino Wistar rats. Average weight of six rats was normally fed. A significant ($p<0.05$) increase in weight was observed in extract induced (group IV) rats when compared to control rats. Bodyweight was sustained in group IV (*M..paradisiaca flower* ethanolic extract administered with normal feed for fifteen days) rats. Induction with streptozotocin (group II), during treatment with *M.paradisiaca* (group III) induced rats the bodyweight of the rats, were found elevated significantly ($p<0.05$) to near in normal rats. Above body weight and organ weight significant changes infers that crude extract of *M.paradisiaca flower* with its phytochemical potential influenced the gain of body weight and recovery of disease by diabetes mellitus.

## CONCLUSION

As our knowledge of the mechanisms of animal diseases has increased particularly metabolic diseases such

as diabetes. Liver, disease and hypertension, the role played by highly reactive oxygen species such as free radicals have become increasingly relevant. The free radical scavenging activity of ethanol extract of *M. paradisiaca* flowers and also that of ascorbic acid was evaluated through its ability to quench the synthetic DPPH radical. The antioxidant activity of both natural and artificial compounds. The DPPH assay constitutes a rapid and low-cost method that has frequently been used for evaluation of the ant oxidative potential of various natural products. Therefore, in the present study, *M. paradisiaca* flower was screened for its possible antioxidant and radical scavenging activity by DPPH. The radical scavenging reaction of ascorbic acid with DPPH was essentially instantaneous; the reaction of DPPH with *M. paradisiaca* flower was also fast but slower compared to that with ascorbic acid. It is usually noticeable as discoloration of ethanol extract of plant samples from purple to yellow; hence, DPPH is widely used to evaluate the free radical scavenging capacity of antioxidants. The ABTS radical action discolorization assay can measure the relative antioxidant ability to scavenge the radical ABTS as compared an excellent tool for determining the antioxidant capacity of hydrogen-donating antioxidants. In the present study, the administration of *M. paradisiaca* flower extract to the reaction mixture significantly inhibited the hydroxyl radical activity, with a maximum inhibition of observed with the standard and *M. paradisiaca* flower, in ethanol extract respectively. Therefore, further research is needed for the isolation and identification of the active components in the extracts.

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SP-44

# THE BEHAVIOURS CHANGES AND ENZYMOLOGICAL EFFECT OF *EUPHORBIA TIRUCALLI*ON FRESH WATER FISH*OREOCHROMIS MOSSAMBICUS* (TILAPIA)

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## ABSTRACT

Aquaculture is the importance and advantages of the growth countries. Predatory undesirable's fish created great problems in the aquatic areas.The current studied of the *euphorbia tirucalli* (Family- Euphorbiaceae) latex powder was done an *Oreochromis mossambicus* fish. The tilapia fish was further subjected to sublethal concentrations 0.02, 0.04, and 0.06mg/l of the plant latex powder to the conditions as an Enzymological effect on after 28 days of studies under the laboratory conditions. After the treatments of the collection of fish organs like gill, liver, and kidney by dissection and done the procedure in the laboratory. The results of this study show the toxic effect of euphorbia tirucalli on *Oreochromis mossambicus*. The behaviors changes of Oreochromis mossambicus in 30 min fish movements in slowed but they are continuing to swim in the tank. After 45 min fish do not try to move and within 50 min the fish got loss of body equilibrium and change the reddish color in the head and enzymological changes of *Euphorbia tirucalli* latex powder significantly ($P<0.05$) decreased level of ALT, ASP, AST, LDH, ACP, and AchE in gill, liver and kidney of *Oreochromis mossambicus.* .Euphorbia tirucalli latex powder can be recommended because it is a biodegradable effect on the environment. These plants are used safely to stupefy on freshwater fish culture areas

**KEYWORDS**: *Euphorbiatirucalli, Oreochromismossambicus*, Behavioural changes, Enzymes activity

## INTRODUCTION

Aquaculture is the most important and advantages of the growth countries[1] Predatory undesirable's fish created great problems in the aquatic areas by predacious on fish's fire and commercial fish from aquatic areas are the best management to increase fish production[2] The Oreochromis mossambicus (*Mossambique tilapia*) is a tilapia child. This is native fish of southern Africa and popular aquaculture. The *Mossambique tilapia* is dull-colored and it is native habitats[3]..*Mozambique tilapia* is a stronger separation that is easy to increase and gather in making them a quality aquaculture species. Those gave birth to mild colourless muscles that are inventing to consumers. These species add up to about 4% of the whole tilapia aquaculture manufacture worldwide but are furthermore commonly hybridized with additional species[4]. This species is a very easy target for diseases such as itch and whirling diseases[5]. Mozambique tilapia are water-resistant and pollution level to wide different water quality issue for the reason that ability they have been used as bioassay organisms to generate metal toxicity data for risk estimation of local fresh water species an south Africa reverse[6] *Euphorbia tirucalli* (family Euphorbiaceae) is a common medicinal plant of India. Many values of Euphorbia tirucalli juice is carminative and useful in whooping cough gonorrhea, asthma, spleen, leprosy, dropsy, tumors, jaundice and enlargements stone in the gall bladder[7]These plants peoples have been used for the catfishes from several times. Such as the same part of plants especially stem bark, leaf, and latex, also used in tribal area people. After used the plant latex powder on the fish be taken out my hands and have been eating without any problems[8]. Stem bark and latex aqueous extracts of Euphorbia tirucalli can be used for freshwater fish channa punctuates[9] These plants chemical structure, formula, and identification of the most important of those compounds have been determined[8, 10].The intentions of the current study were inquiring the toxic effects of Euphorbia tirucalli on behavioral changes and enzyme logical parameters of fish *Oreochromis mossambicus* (tilapia).

## MATERIALS METHODS

### Collections of *Euphorbia tirucalli*

The present study was carried out on *Oreochromis mossambicus*. The latex of *Euphorbia tirucalli* was collected from Nedusalai, Krishnagiri, Tamilnadu. The collected latex was lyophilized 400 C. The wet weight of 1ml latex Euphorbia tirucalli was 1.37 grams and dry weight (lyophilized) was 0.315g.

### Identification of plant

*Euphorbia tirucalli* was identified as Dr.Ramesh, Assistant Professor, Department of Botany, GovernmentArts College for Men, Krishnagiri, Tamilnadu.

### Collection and acclimatization of *Oreochromis mossambicus*

Healthy fish of Oreochromis mossambicus (average length and weight 25.g±20g and 15g±13g) were collected from the local fish culture KRP Dam and maintained in the laboratory for 10 days after 10days experiment for acclimatization. These fish were fed with oil cake and raise the brand. Water changed every 24 hours and continuously aerated with fish tanks.

### Treatment of *Oreochromis mossambicus* for enzymes studies

The acclimatized fish was treated with different concentrations of *Euphorbia tirucalli* plant latex powder for the exposure period. Four aquariums were set up for each aquarium 8 fish in 20 liters dechlorinated tap water. After the treatment of the test animal was taken from the tubs. Control animals were normal conditions without any treatments. These animals were dissected for gill, liver and kidney tissue are regarded as measure acid phosphates, alkaline phosphates, alanine aminotransferase, aspartate aminotransferase, lactate level and acetylcholinesterase enzymes activity.

## RESULTS

### Effect on behavioral changes and poisoningsymptoms

*Oreochromis mossambicus* become hyperactive soon after exposure to different concentration of *Euphorbia tirucalli* latex powder. They are scratched their face at the bottom of glass aquaria. Required to skin irritation the natural coloring changed the fish body. Increased fish behavior of snot secretion is furthermore pronounced. After 20 min fish are jumping behaviors. Within 30 min fish movements in slowed but they are continuing to swim in the tank. After 45 min fish do not try to move and within 50 min the fish got loss of body equilibrium and change the reddish color in the head. Then the treatment level increased slowly down swimming and loss of body equilibrium increased and finally fish mortality increased. Control fish was facing such behavioral changes occurs.

**Table**: 1:**Behavioral changes of *Euphorbia tirucalli* on freshwater fish *Oreochromis mossambicus***

| TIME (MINITIES) | BEHAVIORAL CHANGES BETWEEN TIME TO TIME |
|---|---|
| **20** | Fishes jumping behaviors in the Tank |
| **30** | Fish movements in slowed but they are continually to swimming in the tank. |
| **45** | Fishes do not try to move in location. |
| **50** | Loss of body equilibrium of fishes and reddish color in head |

### Toxicity experiment

Exposure to sub-lethal concentration of 0.02, 0.04 and 0.06mg/l of 28 days of *Euphorbia tirucalli* latex powder significantly ($P<0.05$) decreased level of ALT, ASP, AST, LDH, ACP, and AchE in gill, liver and kidney of *Oreochromis mossambicus* (Table 2-7). Aspartate aminotransferase level was decreased in Gill 25.03 to 23.13mg/l; liver level 26.06 to 23.77mg/l and kidney 15.14 to 13.03, alanine aminotransferase was reduced in Gill 21.11 to 18.13;liver 18.13 to 15.37 and kidney 29.11 to21.15.alkaline phosphatase was reduced in gill 18.13 to 15.63; liver 25.39 to 23.03 and kidney 37.07 to 35.50mg/l. LDH was decreased in gill 293.15 to 203.30; liver 269.20to 263.65 and kidney 298.70 to 222.35 mg/l. AChE was reduced in gill 51.50 to 40.85; liver 47.60 to 37.85 and kidney 66.40 to 41.00mg/l of control in these tissues respectively of *Oreochromis mossambicus* after treatments with 0.02, 0.04 and 0.06mg/l of *Euphorbia tirucalli* latex

powder for 28 days of the treatment period.

**Analysis of *Oreochromis mossambicus*exposed to different sub-lethal concentrations of *Euphorbia tirucalli.***

**Table: 2 & Fig -1AST Aspartate Aminotransferase levels of fish (*Oreochromis mossambicus)* tissues Gill, Liver, and Kidney exposure to different concentrations of *Euphorbia tirucalli* latex.**

| Parameters | Control | Test 1 | Test 2 | Test 3 |
|---|---|---|---|---|
| Gill | 25.03± 0.20$^{b}$ | 24.14± 0.20$^{b}$ | 23.68± 0.15$^{b}$ | 23.13±0.15$^{b}$ |
| Liver | 26.06± 0.20$^{c}$ | 25.30± 0.20$^{c}$ | 24.13± 0.15$^{c}$ | 23.77±0.15$^{c}$ |
| Kidney | 15.14± 0.25$^{ab}$ | 14.75±0.15$^{a}$ | 13.98±0.05$^{a}$ | 13.03±0.15$^{a}$ |

Values followed by different between treatments are significantly (P<0.05) different according to the ANOVA- Tekeys honestly significantly (P<0.05) different ASD multiple comparisons.

**Fig:1 Aspartate Aminotransferase**

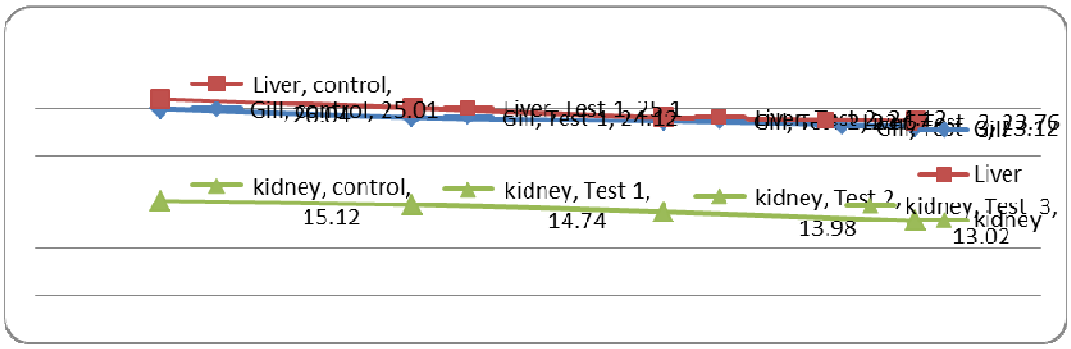



**Table: 3 & fig-2 ALT: Alanine Aminotransferase levels of fish (*Oreochromis mossambicus)* tissues Gill, Liver, and Kidney exposure to different concentrations of *Euphorbia tirucalli* latex.**

| Parameters | Control | Test 1 | Test 2 | Test 3 |
|---|---|---|---|---|
| Gill | 21.11±0.20$^{b}$ | 19.49± 0.30$^{b}$ | 19.57± 0.15$^{b}$ | 18.73±0.15$^{b}$ |
| Liver | 18.13± 0.15$^{a}$ | 18.00± 1.00$^{c}$ | 16.83± 0.05$^{a}$ | 15.37±0.15$^{a}$ |
| Kidney | 29.11± 0.15$^{c}$ | 25.13±0.15$^{a}$ | 22.48±0.10$^{c}$ | 21.15±0.05$^{c}$ |

Values followed by different between treatments are significantly (P<0.05) different according to the ANOVA- Tekeys honestly significantly different ASD multiple comparison.

**Fig:2 Alanine Aminotransferase**

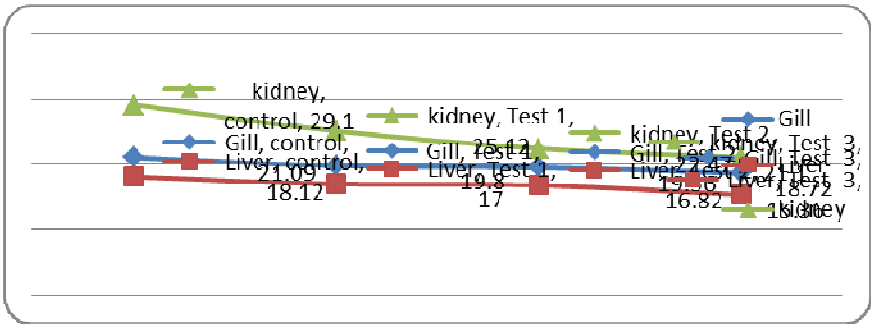


**Table: 4 & fig-3 ALP: Alkaline phosphatase levels of fish (*Oreochromis mossambicus)* tissues Gill, Liver, and Kidney exposure to different concentrations of *Euphorbia tirucalli* latex.**

| Parameters | Control | Test 1 | Test 2 | Test 3 |
|---|---|---|---|---|
| Gill | 18.13± 0.15$^{a}$ | 18.04± 0.15$^{a}$ | 17.60± 0.10$^{a}$ | 15.63±0.15$^{a}$ |
| Liver | 25.59± 0.15$^{b}$ | 24.54± 1.00$^{c}$ | 23.68± 0.05$^{b}$ | 23.03±0.15$^{b}$ |
| Kidney | 37.07± 0.15$^{c}$ | 35.98±0.05$^{b}$ | 35.85± 0.50$^{c}$ | 35.50± 0.10$^{c}$ |

Values followed by different treatments are significantly (P<0.05) different according to the ANOVA-Tekeys honestly significantly different ASD multiple comparisons.

**Fig:3 Alkaline phosphatase**

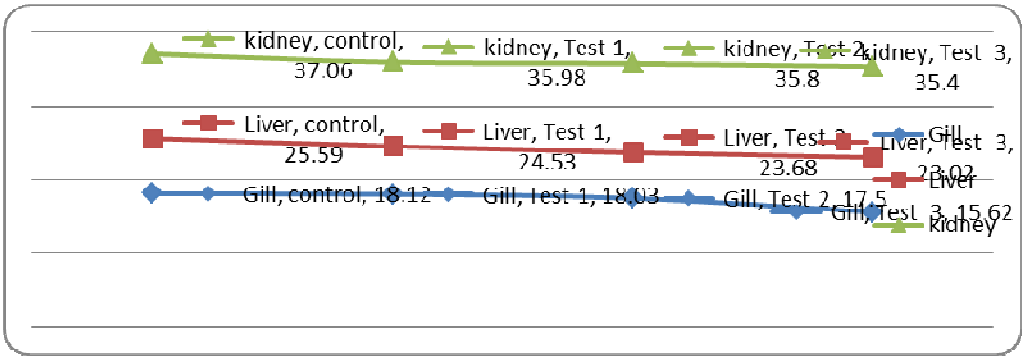



**Table: 5& fig-4 ACP: Acid phosphatase levels of fish (*Oreochromis mossambicus)* tissues Gill, Liver, and Kidney exposure to different concentrations of *Euphorbia tirucalli* latex.**

| Parameters | Control | Test 1 | Test 2 | Test 3 |
|---|---|---|---|---|
| Gill | 24.02± 0.10$^{a}$ | 22.40± 0.10$^{b}$ | 22.04± 0.10$^{b}$ | 21.63± 0.10$^{b}$ |
| Liver | 26.59± 0.05$^{b}$ | 21.54± 1.00$^{a}$ | 20.64± 0.15$^{a}$ | 19.23± 0.20$^{a}$ |
| Kidney | 46.58± 0.05$^{c}$ | 45.09±0.15$^{c}$ | 38.86± 0.15$^{c}$ | 35.19± 0.10$^{c}$ |

Values followed by different treatments are significantly (P<0.05) different according to the ANOVA-Tekeys honestly significantly different ASD multiple comparison.

**Fig:4 Acid phosphatase**

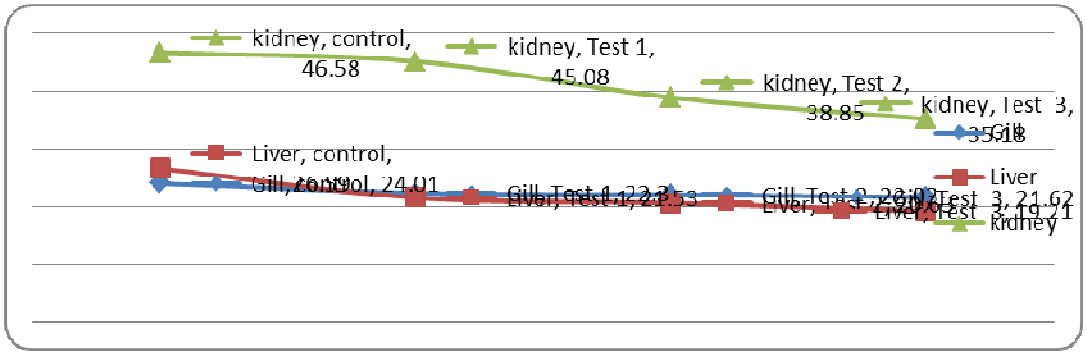



**Table: 6& fig-5 LDH (Lactate dehydrogenate) levels of fish (*Oreochromis mossambicus)* tissues Gill, Liver, and Kidney exposure to different concentrations of *Euphorbia tirucalli* latex.**

| Parameters | Control | Test 1 | Test 2 | Test 3 |
|---|---|---|---|---|
| Gill | 293.15± 0.50$^{b}$ | 254.80± 0.10$^{b}$ | 223.70± 0.10$^{b}$ | 203. 30± 0.10$^{a}$ |
| Liver | 269.20± 1.00$^{a}$ | 265.50± 0.10$^{c}$ | 264.85± 0.50$^{a}$ | 263.65±0.15$^{a}$ |
| Kidney | 298.70± 0.10$^{c}$ | 242.85±0.50$^{a}$ | 238.85± 0.50$^{c}$ | 222.35± 0.15$^{c}$ |

Values followed by different are significantly (P<0.05) different according to the ANOVA- Tekeys honestly significantly different ASD multiple comparison.

**Fig-5 Lactate dehydrogenate**

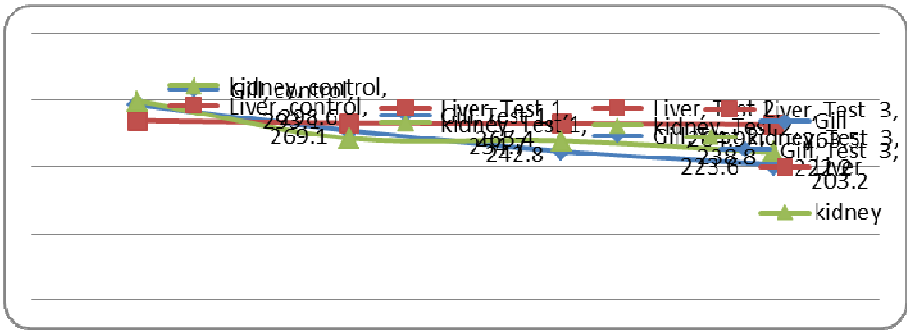
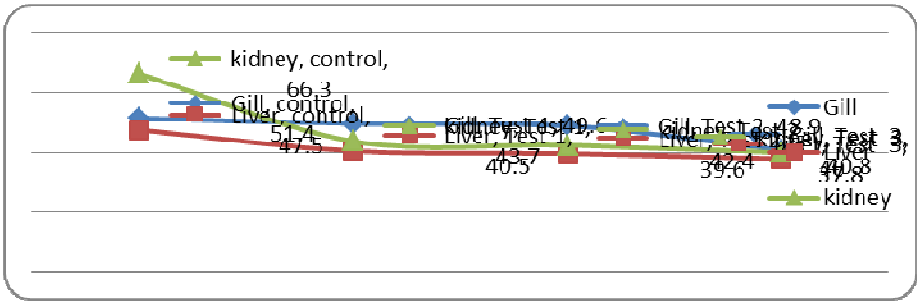



**Table: 7 & fig-6 AchE (Acetylcholine) levels of fish (*Oreochromis mossambicus)* tissues Gill, Liver, and Kidney exposure to different concentrations of *Euphorbia tirucalli* latex.**

| Parameters | Control | Test 1 | Test 2 | Test 3 |
|---|---|---|---|---|
| Gill | 51.50± 0.10$^{b}$ | 49.70± 0.10$^{c}$ | 48.90± 0.10$^{b}$ | 40.85± 0.10$^{a}$ |
| Liver | 47.60± 1.00$^{a}$ | 40.60± 0.15$^{a}$ | 39.75± 0.15$^{a}$ | 37.85± 0.05$^{a}$ |
| Kidney | 66.40± 0.10$^{c}$ | 43.80± 0.01$^{b}$ | 42.55± 0.15$^{c}$ | 41.00± 1.00$^{a}$ |

Values followed by different treatments are significantly (P<0.05) different according to the ANOVA-Tekeys honestly significantly different ASD multiple comparison.

**Fig: 6 Acetylcholine**




## DISCUSSION

The present study was conducted by the treatment of *Euphorbia tirucalli* on *Oreochromis mossambicus* were observed. The changing a lot of behavioral a reaction to something, fishes move to resist changes in belonging to their environment and in this situation decrease the harmful effect of the *Euphorbia tirucalli* latex powder. While regarded as animal behaviour on before the usual time toxicity advisable as it may provisional consolidated measure of neurotoxicity in the showing of contaminants[11] The beginning of the treatment decreasing level in the opercula movement seems to be a suggestion of the stress felt the fish exposure to *Euphorbia tirucalli* latex powder. Mucous secretion was increased in the fish seen after, the treatment is a protection, and reaction by whatever fish attempt to decrease entrance of *Euphorbia tirucalli* latex powder completed the body surface. The liberal of mucus secretion established a thin film on damaged and sensitized in gill and tissue because of this minimizing interchange of gases especially intake of oxygen[12] Neurotropically this is a regulated event in animal behavior; neurotransmitter is mediated substances[13] while studied our results, it is clear that inactive compounds in LE preventing acetylcholinesterase activity. This enzyme activity is currently in synaptic regions and arbitration transfer of impulses by breaking acetylcholine into choline and acetic acid[14] The neurotransmitter and neural area upon accumulation causes of hyperexcitability by the activity of acetylcholine[15] The alanine and aspartate amino transferee operate as a link within proteins and carbohydrate metabolism by determining the interconversion of strategic compounds disparate[16] The phosphates enzymes are a major of animal metabolites. Whatever plays and primary role in the transfer of metabolites across the membrane[17] The center of a LDH forms delicately well-balanced stability between anabolism and catabolism of

carbohydrates[18] While interconversion of LDH mediates of lactate to pyruvate be controlled on the access of NAD co enzyme[12] The reduction in lactate activity with a subsequent rise in the even levels of lactic acid recommends the prevalence of the glycolysis and anaerobic segment[12, 19] While thought the increased of production of lactic acid and pre-eminence of glycolysis as an index of physiological stress.

## CONCLUSION

It can be concluded that the current study was conducted for the toxicity of dry latex powder of Euphorbia tirucalli. These plants are used safely to stupefy on freshwater fish culture areas. Sub-lethal concentrations of latex powder were used significantly to modify the behavioral changes and enzymes both parameters effects of fish *Oreochromis mossambicus*. It was advantageous that the toxic effect of *Euphorbia tirucalli* plant latex dry powder was convertible within 28 days of treatments.

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SP-45

# PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EFFICACY OF *MUSA PARADISIACAL* FLOWER EXTRACT: AN *IN VITRO* STUDY

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## ABSTRACT

Medicinal plants are known to possess potential against endophytic microbes, due to the presence of secondary metabolites as their bioactive compounds. This study was aimed to evaluate the phytochemical composition and antibacterial efficacy of the ethanolic extract of *Musa paradisiaca* L flower extract (MFE) .The antibacterial activities of the extract of MFE were evaluated against bacterial species *such as Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and fungal strains such as *Candida albicans, Candida tropicalis, Aspergillus niger)*. Phytochmeical analysis revealed the presence of alkaloids, flavanoids, saponins,tannins,steroids and glycosides.The % yield of MFE is 8.94. MFE produced wider zones of inhibition against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*. The minimum inhibitory concentration was also evaluated for bacterial strains 18,19,8 and 9 mm respectively and for the fungal strains 13,8,5 mm respectively. The Methanolic extract of *Musa paradisiaca* has considerable antimicrobial activity against *as E. coli, S. aureus, Bacillus subtilis, P. aeruginosa* and fungal strains such as *C.albicans, C.tropicalis, A.niger.*

**KEYWORDS**: Antimicrobial activity, MFE, Endophytic pathogens, Medicinal plants, Phytochemicals

## INTRODUCTION

Many number of antibiotics have been produced nowadays to fight against various disease ailments, multidrug disease resistance(MDR) microorganisms have also increased tremendously.MDR is the multifaceted problem in the pharmaceutical industry, To felicitate this problem there is an urgent need to find out in the form of herbal remedies.

The second most widely produced fruit in the world and one of the most important food sources along with rice, wheat and corn is *Musa paradisiaca L*[1]. The different parts of Banana like fruit, flowers, leaves, stems, and fruit peels are used as a popular drug. Banana flowers known to possess antimicrobial, antihyperglycemic[2], antioxidant, anti-inflammatory, lipase inhibition [3],anti-fungal [4] and wound healing [5]. Banana fruit also studied for its antidiarrheal, antioxidant and anti-microbial [6].The present study was aimed to investigate phytochemical content and antibacterial activity of *Musa paradisiacal* flower extract [MFE].

## MATERIALS AND METHODS

*M.paradisiaca L.* flowers were collected from Mayiladuthurai, Tamil Nadu, India. The collected materials were cleaned, bracts were removed, flowers lets were shade dried and coarsely powdered.The shade dried plant materials were subjected to pulverization to get coarse powder and it was subjected to successive Soxhlet extraction using methanol (55-80%). The MFE was concentrated by distilling the solvent ina rotary flash evaporator. The yield was found to be 4.45%, The dried extract was dissolved in dimethylsulphoxideand subjected to antibacterial activity.[7]

### *Test organisms*

The bacterial strains used for the study were *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and fungal strains such as *Candida albicans, Candida tropicalis, Aspergillus niger.*The organisms wereperiodically subcultured and maintained in nutrient agarslant at 4 °C .

***Reference and Control***

Ampicillin was chosen as the reference for all bacteria species used: The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

***Antimicrobial tests***

MFE was investigated for their antimicrobial activity using the Disc Diffusion assay.

***The Disc diffusion method***

In vitro antimicrobial activity was assessed by using Disc Diffusion method . According to extract concentration and bacteria plates were labelled. An inoculum containing bacterial cells and fungal strains were applied on to Mueller Hinton agar plates. Uniformly spreading of a sterile swabbed was dipped into the bacteria culture on M-H plates and allowed to dry for 10 minutes. Four discs were placed equidistant using a sterilized tweezers on each plate,. Amoxcillin for the bacteria(100mg antibiotic/ml.) used as a reference.

***Minimum inhibitory concentration***

The minimum inhibitory concentrations of MFE was done by serial broth dilution method. Thioglycollate broth was used for MIC. In agar well diffusion, the MFE (500 µg/mL) was serially diluted as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 to bring 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.63 µg/mL, 7.81 µg/mL, 3.95 µg/ mL and 1.95 µg/mL concentrations, respectively. The tubes were then incubated for 48–72 h at 37°C. After the incubation, the MIC values were determined by visual inspection of the tubes. Turbidity in the MIC tube indicated growth of the bacteria implying that the bacteria were resistant to MFE.

***Minimum Bactericidal Concentration (MBC)***

Minimum bactericidal concentration (MBC) was determined by sub-culturing the samples having a value of lesser or equal to MIC value. The highest dilution (lesser concentration) that yielded no single bacterial colony was taken as MBC. The least concentration of the extract that showed no bacterial growth was considered as the MBC (NCCLS, 2008).

**RESULTS AND DISCUSSION**

Due to widespread, cost, nontoxic, easy availability and affordability, in the past few decades plant extracts are investigated for its various antimicrobial and antioxidant activities. By having a very old traditional knowledge and folk medicine India has been considered as medicinal garden. Due to adverse side effects caused by antibiotics there is upsurge on interest to find out the remedy in the form of herbal drugs. The present study was aimed to explore the antimicrobial efficacy of flowers of M*usa paradisiaca.*Table 1 and 2 shows the physical properties of the extract and phytochemical composition which clearly indicates the presence of alkaloids,terpenoids,saponins,phenolics. The *in vitro* antimicrobial susceptibility of MFE against Gram positive, Gram negative and fungal strains were assessed by the presence of inhibition zones and MIC. Out of seven tested microorganisms, Potent inhibitory effect on the growth of the cultures with different range of inhibition zone was shown by six cultures (Table 3). Negative control was zero was obtained as the zone of inhibition. However, larger clear zones were observed on the plates with 10µg/ml of amoxcilin when compared to the MFE. Moreover MFE was more sensitive to *B.subtilis* with inhibition zone of 21±0.1 mm whereas the range on inhibition for other microorganisms was between 10- 18mm. In the present study, *S.aureus* did not show any resistance against MFE. The MIC value ranged from 50 to 55 for the cultures tested. The extract had the highestantimicrobial activity against *E.Coli* with the lowest MIC value. The active principles present in the MFE could be responsible for the antimicrobial property. Our results were corroborated with various findings.One such study conducted using hexane, ethyl acetate and methanolic extracts of of *Musa acuminate Colla, Musa troglodytarum, Musa sapientum and Musa paradisiacal investigated* for antibacterial activity against multi-drug resistantpathogens causing nosocomial infection by agar welldiffusion method (Ponmurugan et al., 2013). Antibacterial susceptibility test,minimum inhibitory concentration and minimuminhibitory bacterial concentration were determined. Moderate antibacterial activities was exhibited by except *Musaparadisiaca* The MIC and MBC result result is shown in (Table 4).From the result it was indicated that an MIC range of (12.5µg/ml -50µg/ml) and

MBC range of (50µg/ml - 100µg/ml, 25µg/ml - 400µg/ml and 25µg/ml - 100µg/ml) . Ampicilin had MIC and MBC ranges of (6.25µg/m-25µg/m).Divya et al(2016) has conducted in vitro biological studies on banana flowers and they concluded that Musa paradisiacal known to possess anti-inflammatory and antioxidant tproperty. In addition Jawla et al(2012) studied the antimicrobial and hyperglycaemic property of *M.paradisiaca* flowers in which extracts of ethanolic and aqueous and ethanolic aqueous mixture where used. Their results reveals antimicrobial activity with minimum inhibitory concentrations.

**Table 1: Physical Properties of Extract**

| Solvent | Colour | Odour | Texture | Wt of the sample(g) | Quantity recovered | % yield |
|---|---|---|---|---|---|---|
| Methanol | Dirty green | Chemical | Slighhtly sticky | 50 | 4.3 | 8.79 |
| Aqueous | Reddish brown | Odourless | Gummy | 50 | 7.6 | 5.24 |

**Table 2. Phytochemical Composition**

| Extract | Alkaloid | Saponin | Tanin | Flavanoids | Steroids | Glycosides | Terpenoids | phenols |
|---|---|---|---|---|---|---|---|---|
| Aqueous | + | - | + | + | + | - | - | - |
| Methanol | + | + | + | + | + | - | + | + |

**+ : indicates presence, -:indicates absence**

**Table 3: Antibacterial Activity**

| Bacteria | ME (µg/ml) | | | | AMOX(µg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration of Zone inhibition** | **50** | **100** | **200** | **400** | **50** | **100** | **200** | **400** |
| *E.coli* | 12.1 | 11 | 15 | 18 | 12 | 11 | 16 | 19 |
| *S.aureus* | 15 | 13 | 17 | 19 | 0 | 8 | 11 | 13 |
| *B.subtilis* | 16 | 12 | 10 | 8 | 9 | 11 | 15 | 18 |
| *P.aeruginosa* | 13 | 12 | 11 | 9 | 8 | 12 | 16 | 17 |
| ***Fungal strains*** | | | | | | | | |
| *A.niger* | 11 | 10 | 9 | 13 | 12 | 11 | 16 | 19 |
| *C.albicans* | 12 | 16 | 10 | 8 | 0 | 8 | 11 | 13 |
| *C.tropicalis* | 11 | 10 | 9 | 5 | 11 | 8 | 13 | 15 |

**Table 4 : MIC and MBC**

| Strain | AE | | Amox | |
|---|---|---|---|---|
| **Bacteria** | **MIC (µg/ml)** | **MBC(µg/ml)** | **MIC (µg/ml)** | **MBC(µg/ml)** |
| *E.coli* | **50** | **100** | **50** | **22.5** |
| *S.aureus* | **28** | **50** | **6.25** | **25** |
| *B.subtilis* | **50** | **100** | **6.25** | **12.5** |
| *P.aeruginosa* | **50** | **100** | **5.25** | **12.5** |
| ***Fungal strains*** | | | | |
| *A.niger* | **40** | **50** | **5.5** | **11.5** |
| *C.albicans* | **30** | **100** | **6.5** | **20** |
| *C.tropicalis* | **35** | **50** | **5.0** | **10** |

## CONCLUSION

The results of present study conludes that MFE showed antibacterial as well as antifungal activity against tested organisms. We found that Gram positive bacteria are more susceptible than Gram negative bacteria. Flowers of *Musa paradisiaca* showed greater antifungal activity From the MIC values we can concluded that extract of *Musa paradisiaca*, required relatively lesser quantity for arresting the growth of tested

organisms and may be used in the food and beverage industries.Further molecular biology studies were warranted to understand the genes involved in the antimicrobial property.

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SP-46

# STUDY OF INDIAN PLANT TULASI & PEPPER MIXING MEDICINAL ACTIVITY

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## ABSTRACT

Indian long pepper is a plant. Botanically peppercorn belongs to the family of Piperaceae, in the genus of Piper and known scientifically as *Piper nigrum* Peppercorns are a good source of many anti-oxidant vitamins such as vitamin-C and vitamin-A. They are also rich in flavonoid polyphenolic anti-oxidants like carotenes, cryptoxanthin, zea - xanthin and lycopene. Ayurveda medicinal system has focused on healthy life style practices and regular consumption of adaptogenic herbs. Tulasi (*Ocimum tenuiflorum)* can be worshipped, ingested, made into tea and used for medicinal and spiritual purposes within daily life. Intermittent Tulasi leaf juice mixed with 2 g Maricha (Pepper) powder thrice a day is beneficial. Cough: Tulasi leaves juice with honey, twice or thrice a day is useful in cough and cold.

**KEYWORDS:** Pepper, vitamin-C and A. Adaptogen, Ayurveda, Tulsi antimicrobial activity,

## INDRODUCTION

Family: Piperaceae Genus: Piper : Species: nigrum : Black pepper is native to present-day Kerala in South India and pepper is at length cultivated there and elsewhere in tropical regions. Ground, dried and cooked peppercorns have been used since antiquity, both for flavor and as traditional medicine. Black pepper is the world's most traded spice and is one of the most common spices added to cuisines around the world. Its sharp taste is due to the chemical compound piperine, which is a different kind of spicy from the capsaicin characteristic of chili peppers.Family: Lamiaceae; Genus: Ocimum; Species: tenuiflorum *Ocimum tenuiflorum* commonly known as holy basil or Tulsi is an aromatic recurrent plant in the family Lamiaceae. It is native to the Indian subcontinent and rife as a cultivated plant throughout the Southeast Asian tropics. It is also often suggested to start your day by having two to three fresh Tulsi leaves on an empty stomach. It is a very great blood purifier and is known to flush out toxins and clean your internal systems. Along with this comes the advice to not chew Tulsi leaves but instead consume them[1,2].

## MATERIAL AND METHODS

Dry tulsi or extract was prepared by cold extraction methods. Dry pepper powder was prepared (0.5%, 1%, 2% ) concentration level. Honey 5ml. Tulsi + Pepper + honey mixed paste form, per person daily Owns two times take it. Cough is controlled, microbes also destroyed. So breathing was smooth.

## RESULT AND DISCUSSION

The biological role of *Piper nigrum* and *Ocimum tenuiflorum* Indian *Pepper nigrum* fruits are also used to produce white pepper and green pepper and are valued due to the presence of piperine including its different isomers (Figure 1). Black pepper can be used for different purposes such as human dietaries, as medicine, as preservatives, as biocontrol agents [5-7]. This plant and its active component piperine can stimulate the digestive enzymes of pancreas and intestines and also increases biliary bile acid secretion when orally administrated. Some reports have been demonstrated that black pepper consumption in humans increased orocecal transit time. Piperine prevents and minimizes diarrhea produced by various oil and chemicals and also reduce intestinal fluid assume-latin in mouse intestine (Figure 2)[8,9]. The active agents of *P. nigrum* activate the epithelial cells in rat jejunum to permeate the uptake of various amino

acids through the activation of membranes, enhance the production of proteins that are later used for the formation of cytoskeleton system due to surface adsorption property. This valuable species also has the power to minimizes different mutations like ethylcarbamte induced mutation in Drosophila. As compare to mutation, black pepper also reduces tumor formation in mice such as Ehrlich ascites tumour and Doltons lymphoma cells [10-12]. Other related activities included Anti-inflammatory activity, thermogenic action, growth stimulatory activity, anti-thyroid activity and chemopreventive. Secondary metabolites from P. nigrum play a defensive role against infections by microbes, insects and animals[13-14].

**Figure 1. *Pepper nigrum* fresh and dry fruits**

Pepper produces secondary metabolites that help in body metabolism and also used as system against various agents such as insect feeding plants and animals. Recently scientists from different biological fields screen plants for various secondary metabolites which can be used for the preparation of medicines and biocontrol agents and using different separation technique (Figure 3).[16]

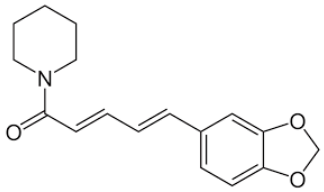


**Figure 2. Peperine structure**

**Antimutagenic and antitumor activity of *P. nigrum***

*P. nigrum* is not only used in the perfumery and food industry but also very effective against fatal diseases caused by mutations. El Hamss et al.[10] observed that when *Drosophila melanogaster* was exposed to mutation through promutagenethyl carbamate, in such an induced situation, the *P. nigrum* is effective to reduce mutational events. *P. nigrum* and its active derivatives especially peppercorn extract has been reported to inhibit tumors formation in experimental models. Such reduced antitumor activity by the oral administration was also reported [5]. The alcoholic extract of peppercorn and piperine is effective in immunomodulatory, antitumor activity and Dalton's lymphoma [14].


**Figure 3. Separation Technique**

**Ayurveda Tulsi**

Tulsi has been used for thousands of years in Ayurveda (Figure 4), a Hindu form of medical science, for its diverse healing properties. It is mentioned in the Charaka Samhita, an ancient Ayurvedic text. Tulsi is considered to be an adaptogen, balancing different processes in the body, and helpful for adapting to stress. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity.

If sprinkled over cooked food in stored water, Tulsi leaves prevent bacterial growth during the eclipses.

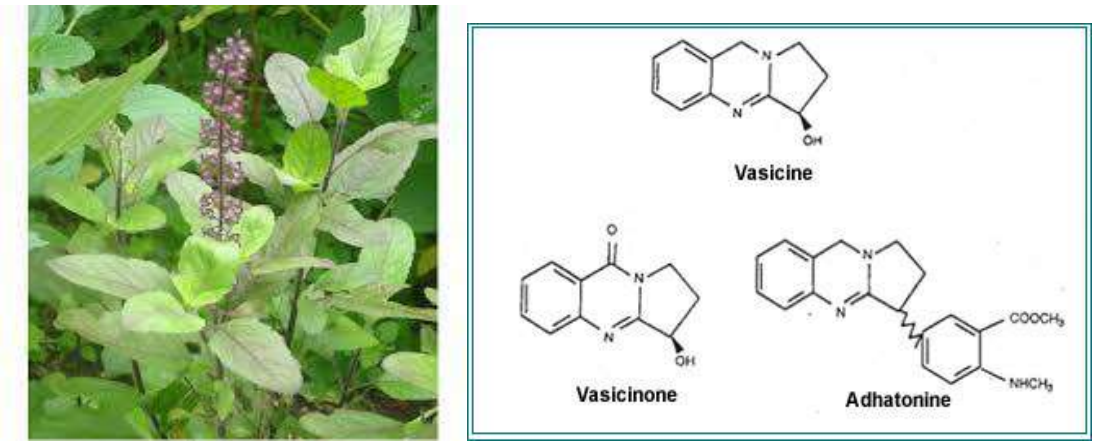


**Figure. 4. *Ocimum tenuiflorum* and different chemical compound structure**

**Pharmacognostic Study**

It is much-branched small herb and 30 to 75 cm in height. All parts of Tulasi are used in medicine, especially fresh and dried leaves. Leaves are oblong, acute with entire or serrate margin, pubescent on both sides and minutely gland-dotted. The leaves are green in color with aromatic flavour and slightly pungent taste. Flowers are purplish in the form of racemes. Nutlets are subglobose, slightly compressed, pale brown, or red. Seeds are reddish-black and subglobose.[17-20]

**Anti Microbial activity**

It inhibits the growth of microbes mixed paste is a remover of worms and parasites, when the freshly prepared juice and is taken with honey; the sweetness excites the parasites drawing them out of their hiding places. Digestive System Liver support generally contributes to healthy liver functions and counteracts liver diseases.

Liver Protective Improves the metabolic breakdown and elimination of dangerous chemicals in the blood is a purifier.

**Cardiovascular-Circulatory System**

(Heart, blood, circulation) Cardio tonic-prevents heart attack Lowers stress-related high blood pressure normalizes blood pressure Vascular protection-protects the heart and blood vessels, promotes even circulation Mild blood-thinning qualities thereby decreasing like the likelihood of strokes Lowers dangerous cholesterol protects against damage caused by foreign toxins in the blood(such as industrial chemicals) treatment of stress-related arterial hypertension (high blood pressure)[21,23].

**CONCLUSION**

Tulsi and Pepper is a popular home remedy for many ailments (per day 5 Tulasi & 3 Pepper mixing) such as a wound, bronchitis, liver diseases, catarrhal fever, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders1-2. Intermittent Tulasi leaf juice mixed with 2 g Maricha (Pepper) powder thrice a day is beneficial. Cough: Krishna Tulasi leaves juice with honey, twice or thrice a day is useful in cough and cold. Microbes are also destroyed. So, breathing was smooth.

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SP-47

# *AN IN VITRO* REGENERATION OF PROSPECTIVE CABBAGE *BRASSICA OLERACEA* L. (BROCCOLI)

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**Conduct number: 6381535084**

## ABSTRACTS

*Brassica oleracea* L. var. italic commonly called Broccoli is a horticulture plant and belongs to the family of Brassicaceae. *Invitro* rapid propagation was deliberated from shoot tip node and flower buds explants of *B. oleraceae*. The explants were cultured on (MSMurashige and Skoog 1962) medium supplemented with B5 vitamins. In various concentrations of Cytokines and Auxins ranging from 0.1mg to 3.0mg combinations of BAP and KN was good response from shoot tip, nodal and flower buds explants. The highest number of callus induced in the concentrations of 2.5mg BAP+2mgNAA.Multiple shoots was noticed in 2.5mg BAP and 1.5mg NAA and 2mg IBA. The present study enables the large scale production of *B. oleracea*using *invitro* conditions and disease-free plants.

**KEYWORDS:** *Brassica oleraceae,* Plant regeneration, Shoot regeneration, Sterilization, Growth regulators, (BAP, KN, NAA, IAA, and IBA), Medicinal uses.

## 1. INTRODUCTION

*Brassica oleracea* L. var. italic commonly called Broccoli and belongs to the family of Brassicaceae Broccoli is an edible green plant in the cabbage family whose large flower head is eaten vegetable, which include Kale, Cauliflower, Brussels sprouts, Bok Choy, collard greens and rutabaga[2]. This originated from Italy at 2000 years ago and is related to the cabbage and cauliflower, vegetable crop. It is well known for its high vitamins and calcium content. The plant has been reported containing Sulforaphane which functions as antioxidant[4,5,15]. As also contains multiple nutrients with potent anti-cancer properties, like Di-diindolylmethane (DIM) and selenium. DIM is a potent modulator of the innate immune response system with anti-viral, anti-bacterial and anti-cancer activity[2,17]. It also contains the compound glucoraphanin, an excellent source of indole-3-carbinol, a chemical DNA repair in cells and appears to block the growth of cancer cells. Broccoli it has been marketed as a health-promoting food because it naturally has a high content of bioactive phytochemicals such as glucosinolates, phenol compounds, vitamin C' and mineral nutrients[9,22]. Thus a diet rich in broccoli plays in role prevention of chronic diseases, cardiovascular disease, carcinogenic, breast and prostate cancer [6, 13]. The whole plant is highly medicinal important used in several preparations of ayurvedic and folk medicine. It has been reported that the phytochemical studies revealed the presence of secondary metabolites most highly present in orientin, isoorientin, D-pinitol, norepinephrine mucilage, tannins, non-protein amino acid, tannins, flavonoids, C-glycosides, steroids, terpenoids, fatty acids, saponins and coumarin, major and minor chemical constituents are also present[13]. The flower head has the treatment of inflammations, asthma and fatigue, and blood disease. The Unani system of medicine is resolving alternative treatment of blood impurities, bilious fevers, piles, jaundice. It is also used, amoebic, gynecological disorder to cure the traditional medicine healthcare system[8]. Other applications for extensively used as bronchitis, bleeding, amnesia, mental stress[7,15]. The present results showed potential plant has been applied for therapy to possess pharmacological actions human and treatment of anti-cancer[23,24].

## 2. Materials and methods

### 2.1 Plant collection and Sterilization of explants

*Brassica oleracea* L. var (Broccoli) was collected from Ooty, Kodaikanal (Dt) Tamilnadu, India, during the month January 2019. The explants of florets and stigma were collected from *invivo* plant flower heads. The explants were then prewashed 10% percent (W/V) of Bavistain methyl 1-3 Benzimidizole carbonate solution and washed thoroughly in running tap water. The explants were subsequently and disinfection surface sterilized with 0.12% $HgCl_2$ [mercuric chloride] solution for 3-5 minutes and washed 2-3 times in sterile distilled water. $HgCl_2$ was very penetrating that it destroyed the microorganism present in most tissues of the explants. The surface-sterilized explants were trimmed gently with the help of sterile surgical blade[1,7].

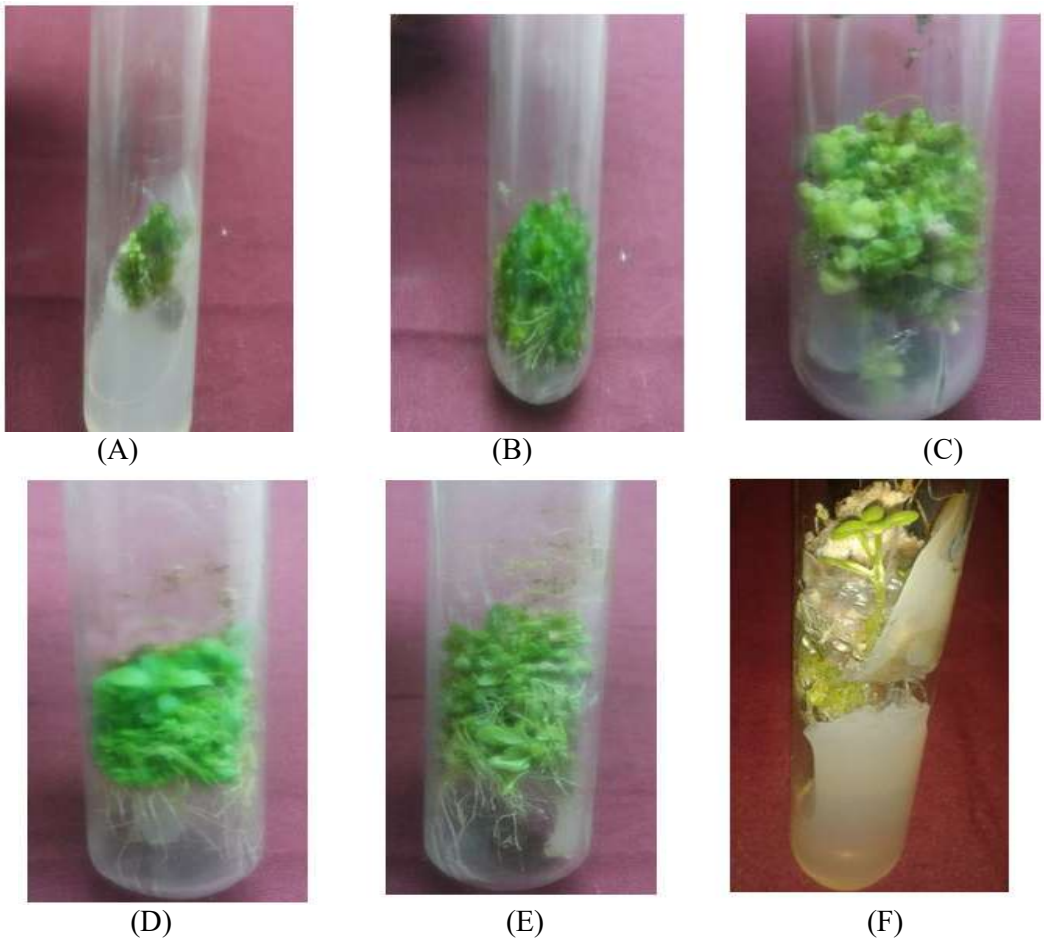
### 2.2 Culture medium

Nutritional support must be essential for optimal growth of tissue by *invitro*. The nutrient media consists of inorganic nutrients. The carbon source, vitamins, iron source, amino acids and natural supplements. All the stock solution was prepared and stored in Amber – sterilized colored bottles and preserved in a refrigerator at $4^0$C. In present study MS medium supplemented of different concentrations of growth regulators were added, like Benzyl amino purine (BAP) Kinetin (KIN) 2, 4-Dichlorophenl acidic acid (2,4-D), Naphtali acetic acid (NAA), Indole-3 butyric acid (IBA) were 0.5 to 3.5mg concentration was used. Multiple shoots were noticed BAP, NAA, IBA (0.1 to 3.5mg) were used. The required sucrose and other organic supplements nutrients were added. The final volume was made up of sterile distilled water. To the above-said media 0.8% (W/V) sugar and Hi-media (agar) were added. $P^H$ was adjusted to 5.8 – 5.9 with either 0.1 N Na OH or 0.1 N HCl using a $P^H$ meter[23, 21].

## 3. Results

The interactions of the main growth regulators can be used but at the simplest level auxins can cause cell enlargement and division. Cytokines cause cell division and shoot development.

**[A& B] Callus induction [C&D]Shoot initiation [E&F] root initiation and Shoot development**



(A) (B) (C)

(D) (E) (F)

The explantsflower buds for micro propagation were selected from the same. The result showed that shoot formation was generated from direct organogenesis for explants, were detected in all treatments. After 15 days growth of callus was observed [Fig-1]. The MS medium was supplemented with different concentration of hormones NAA, BAP, KN, IBA combination of it initiates basal callus and shoot and root proliferation [Table -1].

### 3.1 Callus induction

The requirement of various plant growth regulators for inducing callus and differentiation from flower bud explants. The explants began after 15 days of culturing and callus proliferation occurred [Fig-1]. The concentration of hormone BAP, KN, NAA, the large amount of callus induction from MS media at 4 weeks [Table 1].

**Table** 1. Effect of various concentrations of BAP, KN, and NAA in Callus induction on MS media from Flower bud explants after 4 weeks of culture.

| Growth regulators (mg/L) | | | Culture showing response (%) | Basal callus |
|---|---|---|---|---|
| BAP | KN | NAA | | |
| 0.5 | - | 0.5 | 55 | + |
| 1.0 | - | 1.0 | 65 | + |
| 1.5 | - | 1.5 | 72 | ++ |
| 2.0 | - | 2.0 | 85 | +++ |
| 2.5 | - | 2.5 | 95 | ++++ |
| - | 0.5 | 0.5 | 70 | + |
| - | 1.0 | 1.0 | 72 | +++ |
| - | 1.5 | 1.5 | 80 | +++ |
| - | 2.0 | 2.0 | 90 | +++ |
| - | 2.5 | 2.5 | 92 | +++ |
| 0.5 | 0.5 | 0.5 | 80 | ++ |
| 1.0 | 1.0 | 1.0 | 82 | ++ |
| 1.5 | 1.5 | 1.5 | 95 | +++ |
| 2.0 | 2.0 | 2.0 | 81 | +++ |
| 2.5 | 2.5 | 2.5 | 90 | +++ |

Callus induction: + poor ++ moderate +++ high response.

**Table 2**. Effect of various concentrations of BAP, IBA, KN in shoot regeneration on MS medium from flower bud explants after 4 weeks of culture

| MS Medium Growth regulators (mg/L) | | | Culture showing Response (%) | Mean shoot/ explants | Mean Shoot length (cm) | Basal callus |
|---|---|---|---|---|---|---|
| BAP | IBA | KN | | | | |
| 0.5 | 0.5 | - | 60 | 3 | 4.5 | +++ |
| 1.0 | 1.0 | - | 70 | 4 | 4.8 | +++ |
| 1.5 | 1.5 | - | 80 | 5 | 4.2 | ++ |
| 2.0 | 2.0 | - | 75 | 4 | 5.1 | +++ |
| 2.5 | 2.5 | - | 95 | 6 | 5.0 | +++ |
| - | 0.5 | 0.5 | 55 | 2 | 2.5 | ++ |
| - | 1.0 | 1.0 | 65 | 4 | 4.0 | +++ |
| - | 1.5 | 1.5 | 80 | 3 | 4.2 | +++ |
| - | 2.0 | 2.0 | 90 | 5 | 5.0 | +++ |
| - | 2.5 | 2.5 | 85 | 4 | 5.1 | +++ |
| 0.5 | 0.5 | 0.5 | 65 | 3 | 5.1 | +++ |
| 1.0 | 1.0 | 1.0 | 75 | 2 | 4.9 | +++ |
| 1.5 | 1.5 | 1.5 | 90 | 5 | 4.2 | +++ |
| 2.0 | 2.0 | 2.0 | 82 | 4 | 5.2 | ++ |
| 2.5 | 2.5 | 2.5 | 90 | 5 | 5.0 | ++ |

Shoot induction have been indifferent grades viz., No response + poor ++ moderate +++ high

**Table - 3 Effect of various concentrations of BAP, NAA, IBA in root formation on MS medium from flower bud explants after 4 weeks of culture.**

| MS Medium Growth regulators (mg/L) | | | Culture showing response (%) | Mean root/ explants | Mean root length | Basal callus |
|---|---|---|---|---|---|---|
| BAP | NAA | IBA | | | | |
| 0.5 | 0.5 | - | 60 | 10 | 09.95 | - |
| 1.0 | 1.0 | - | 65 | 12 | 11.05 | - |
| 1.5 | 1.5 | - | 72 | 12 | 11.05 | - |
| 2.0 | 2.0 | - | 85 | 14 | 13.45 | - |
| 2.5 | 2.5 | - | 92 | 14 | 13.45 | - |
| 0.5 | - | 0.5 | 70 | 13 | 12.75 | - |
| 1.0 | - | 1.0 | 75 | 12 | 11.05 | - |
| 1.5 | - | 1.5 | 82 | 10 | 09.95 | - |
| 2.0 | - | 2.0 | 90 | 15 | 13.75 | - |
| 2.5 | - | 2.5 | 95 | 14 | 13.80 | - |
| 0.5 | 0.5 | 0.5 | 80 | 13 | 12.75 | - |
| 1.0 | 1.0 | 1.0 | 85 | 12 | 13.45 | - |
| 1.5 | 1.5 | 1.5 | 75 | 10 | 09.95 | - |
| 2.0 | 2.0 | 2.0 | 90 | 15 | 13.75 | - |
| 2.5 | 2.5 | 2.5 | 95 | 14 | 13.80 | - |

Shoot induction have been indifferent grades viz., No response + poor ++ moderate +++ high

### 3.2 Shoot regeneration

The highest frequency of shoots from flower bud explants was observed in MS media containing 0.5 mg to 2.5 mg BAP, NAA, IBA, and KN was used.[Table-2]. The maximum shoot regeneration frequency i.e. 95% on MS basal medium supplemented with 2.5mg/L BAP, NAA 2.5mg/L and IBA 2.0mg/L respectively. The shoot regenerated shoots were obtained from both the explants. Shoot multiplication and elongation took place on the same medium.

### 3.3 Shoot multiplication

A large number of lateral shoots were recorded on the explants cultivated at media many authors emphasize the influence of proper cytokines and auxins combination on the formation of shoots in *in-vitro* conditions. Among the different concentrations of BAP, NAA, IBA 0.5 to 2.5mg/L used, IBA at 1.5mg/L has given 85% growth of shoot with a mean shoot length of 4.2cm [Table 3]. The maximum number of multiple shoots was observed at 2.5mg/L with 85% of responses with mean shoot length 4.0cm [Fig-3, 4]. The highest numbers of shoots from flower bud explants were observed by on 45 days it has given 15 shoots per explants [Table- 3].

### 3.4 Root formation

Among the different concentrations of BAP, NAA, IBA 0.5 to 2.5mg/L were used, IBA at 1.5mg/L has given 95% growth response of roots. A large number of lateral roots were recorded on the explants cultivated at MS media to proper cytokines and auxins combination on the formation of roots. [Table -3]. The maximum number of roots was observed at IBA 2.5mg/L [Fig-2, 3]. The highest number of roots from flower bud explants was observed by 45 days it has given 15 roots per explants [Table- 3].

## 4.DISCUSSION

The main edible parts of broccoli are sprouts and florets named the inflorescence[11, 23]. Broccoli is known as crown Jewel of Nutrition since possess the nutrients namely vitamins, minerals, secondary metabolites and fiber proclaiming its exceptional health benefits. The breakdown products of the sulfur-containing glucoseinolatesisothiocyanates are the active principles in exhibiting the anticancer property at stage[14, 24]. For the present study, the results revealed direct organogenesis of flower bud explants there was callus formation and the duration of shoot initiation on flower bud explants was 15-20 days this result is an

agreement with finding in Broccoli[7].For the present study, the results when subcultured on 35th day 15 shoots per explants were achieved [Table 3]. The shoots were aggregated. The mean shoot lengths were 13.80 measured. [7,9].KN, BAP, played a role in inducing shoot multiplication. It was suggested that the use of BAP as a cytokine and NAA as auxin in an appropriate ratio. They investigated that BAP induced more shoot produced. Many investigators examined that uses of Auxins and Cytokines KN, IBA, BAP was the optimum concentration for shoot and rootregeneration[3].This study has presented 95% regeneration at BAP 0.5 to 2.5mg KN 0.5mg to 2.5mg for initiation of shoot BAP, IBA, KN= 0.5 to 2.5 mg multiple shoot formation in explants[12, 10,24]. To know the effect of different media and cytokines on shoot regeneration of broccoli, shoot tip and nodal explants were cultured on three types of media supplemented with various concentrations of BAP and KIN. The degree of growth and differentiation varied considerably with the medium constituents[8,7]. Comparing the effect of cytokines type (BAP and KIN) on shoot production, the best response was achieved by BAP[19]. In general, and also found in the present study, higher concentrations of cytokines (above 2.5 mg/l) reduced the shot number as well as shoot length. This finding is also in line with the finding of who reported that higher concentrations of cytokinin reduced the number of micro propagated shoots. A similar response was also observed[16]. The regeneration of explants was cultured on the medium containing strong auxins and cytokines for the production moss of callus. These calli were transferred to medium supplemented with cytokinin and a weak auxin for shoot regeneration[13]. In the present study, the semi-friable calli obtained from shoot tip and nodal explants were transferred into MS-B5 medium augmented with a constant concentration of BAP (1.5 mg/l) with different concentrations of KIN (0.5-3.0 mg/l) in combinations for organogenesis[24]. The previous and earliest observation best rooting was achieved in the medium with reduced basal nutrients and IBA. This step is very important for the plant survival and critical step in the production of complete entire plantlets. In the present experiment from full to half strength in the basal medium was sufficient for the rooting of shoots. In many plant species, IBA is considered as an important and effective growth regulator for the induction of roots. In the present experiment 2 mg/l IBA produced the maximum number of healthy roots similar observation[11, 14]. In the present study, the highest frequency of shoots from leaf explants was observed in MS media containing 2.5 mg BAP +2.5 mg KIN this combination showed 82 % response. Root explants (*E. axillare*) were cultured on MS medium supplemented with (KIN, BAP) used alone or in combinations (2.22 m in combination with 4.64 m of KIN induced the maximum number of adventitious shoot buds (24.60 + 0.54) shoots per explants with the shoot length of 1.54 + 0.36). Explants from in vitro shoots in BAP supplemented medium was found with more response than those of wild plants[26,27]. The type of callus is determined by the explants used and organ of the plant, the hormones and their concentrations, the chemical constituents of the culture medium. The combinations of external growth regulators (Cytokinin with Auxin) are an essential requirement to stimulate shoots formation from callus. The difference in response depends on regeneration media could be due to the kind of endogenous hormones in cells that control many circumstances expressed by cells[10]. MS medium supplemented with different concentrations of BAP/KN resulted in the initiation of callus and shoots from shoot tip and nodal explants (Table 1). A maximum number of multiple shoots were induced in MS medium supplemented with 1.5 mg/l BAP (Fig. 2.a, b) when compared to other and higher concentrations used. Hence it is suggested that this optimum concentration of BAP promotes multiple shoot induction. Similar reports were also obtained with the cultures of *Phyllanthus amarus*[2] *Celastruspaniculatus*[3,4].

## 5. CONCLUSION

*Brassica oleracea* L. highly nutritional and medicinal plant. The *invitro* regeneration protocol is an efficient means of ex-situ conservation of plant diversity. We have demonstrated in this study the effects of growth regulators on the morphogenic response of broccoli cultivated *in-vitro*. Appropriate combinations and concentration of plant hormones result in a higher yield of plant biomass and mass propagation by tissue culture technique.

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SP-48

# MAJOR MINERAL CONTENT IN PRAWNS COLLECTED FROM DIFFERENT NICHES IN AND AROUND THANJAVUR

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## ABSTRACT

Minerals found in the human body It was comparatively studied between the marine, estuary and freshwater prawns. They are *penaeus monodon*, *penaeas indicus* and *Macrobracnhium rosenbergii*. They are collected along the thanjavur district Freshwater niche-I, Kaveri river at Thirukattupalli, Niche- II, Brackish water kollukadu estuary, and sethubava chattram marine niche-III. prawn muscle was determined by oven-dried at $60^0$C for 24 hours and used for the estimation of mineral content. Minerals content of estuarine shrimp *penaeus indicus* was found to be high (69.92±0.13$\mu$g/g) in potassium, (51.12±0.02 $\mu g/g$) low in *macrobranchium.* The highest Value of Sodiumcontent (69.74±0.13 $\mu g/g$) was estimated in *penaeus monodon* and the lowest value of sodium was noted (49.74±0.17 $\mu g/g$) in Freshwater prawn *Macrobranchium rosenbergii*. The minimum magnesium was recorded (12.95±0.08 $\mu g/g$) in Freshwater prawn. Maximum mineral content magnesium was recorded (19.52±0.13$\mu$g/g) marine shrimp *penaeus monodon*. The highest value of *calcium* content *penaeus indicus* (1.63±0.09 $\mu g/g$) was estimated and the lowest value of *calcium* noted (1.05±0.07 $\mu g/g$) in freshwater prawn *Macrobranchium regenbergii*. Values of various minerals obtained from both shrimp species show a significant difference and samples examined in this study contained appreciable concentrations of Na, K, Ca and Mg suggesting that these shrimp species are a good source of nutrient minerals. Comparatively high in maximum minerals were observed in the marine shrimp *penaeus monodon*.

**KEYWORDS:** *Macrobranchium rosenbergii*, *Penaeus indicus*, *penaeus monodon,* niches and major minerals.

## INTRODUCTION

Minerals elements are required for growth, repair and regulation of vital body functions. Quantitative mineral requirements of penaeid shrimp need investigations. Minerals found in the human body are a group in two categories such as major minerals and trace minerals. The meat of the shrimp contains minerals like calcium (Ca), magnesium (Mg) and phosphorus (P) which are essential for tooth and bone developments of, especially children. Iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are constitutive elements because they have a role in biological systems.[1] Marine foods are very rich sources of both macro and micro mineral components.[2] Perceiving this in mind, a study was planned to identify the significance of niche in the nutrition of prawn and shrimps, since prawn and shrimps are the major food in Thanjavur District.[3-10]

## MATERIALS METHOD

### Estimation of minerals

The samples were oven-dried at 60°C for 24 hours and used for the estimation of mineral content.[11]

### Sample preparation and derivatization

100 mg of dried sample was digested by microwave sample preparation system (Anton Paar Multiwave 3000) using an acid mixture containing nitric acid and perchloric acid (3:1V/V). The residues were dissolved in 2N hydrochloric acid and filtered through Whatman No. 1 filter paper and the volume was

made up to 25 ml with de-ionized water in a standard flask. The clear solution was used to measure the concentration of different minerals. Minerals such as sodium, potassium and calcium were analyzed using digital flame photometer (Model CL 22 D) pre-calibrated with respective standards. Magnesium, phosphorus, iron, zinc, copper, manganese, nickel and cobalt determinations were performed by Optical emission-spectrophotometer (Perkin Elmer Model Optima 2100 DV).

**Statistical Analysis**

The result obtained in the present investigation were subject to statistical analysis like mean and standard deviation by Zar (1984).[13]

$$\text{Mean } (\times) \frac{\Sigma x}{N}$$

The standard deviation (SD) was calculated by the following formula.

$$\text{standard deviation } (\sigma) = \sqrt{\frac{\Sigma (x-x)^2}{N}}$$

**RESULT**

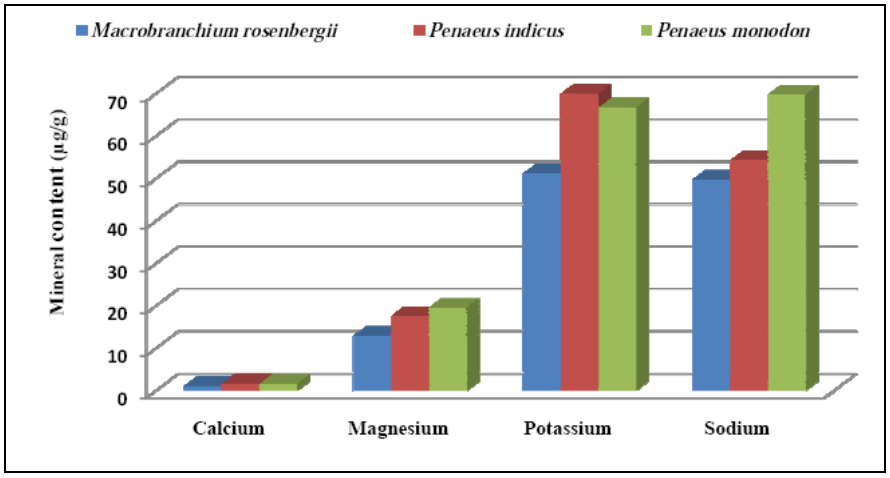
**Minerals content**

In the present study, four major mineral content were observed in the three prawns and shrimps. (Table-1). Minerals content of estuarine shrimp *penaeus indicus* was found to be high (69.92±0.13μg/g) in potassium, (51.12±0.02 $\mu g/g$) low in *macrobranchium.* The highest Value of Sodium content (69.74±0.13 $\mu g/g$) was estimated in *Penaeus monodon* and the lowest value of sodium was noted (49.74±0.17 $\mu g/g$) in Fresh water prawn *Macrobranchium rosenbergii*. The minimum magnesium were recorded (12.95±0.08 $\mu g/g$) in Feshwater prawn. Maximum mineral content magenesium was recorded (19.52±0.13μg/g) marine shrimp *penaus monodon*. The highest value of *calcium* content *penaeus indicus* (1.63±0.09 $\mu g/g$) was estimated and the lowest value of *calcium* noted (1.05±0.07 $\mu g/g$) in freshwater prawn *Macrobranchium regenbergii*.

**Table : 1. Major mineral contents ($\mu g/g$) of *Macrobranchium rosenbergii, Penaeus indicus* and *Penaeus Monodon* (Values are mean ±SD).**

| Sl.No | Minerals | *Macrobranchium rosenbergii* ($\mu g/g$) | *Penaeus indicus* ($\mu g/g$) | *Penaeus monodon* ($\mu g/g$) |
|---|---|---|---|---|
| 1 | Calcium | 1.05 ⊥0.07 | 1.63 ⊥0.09 | 1.61⊥0.05 |
| 2 | Magnesium | 12.95 ±0.08 | 17.54 ±0.14 | 19.52±0.13 |
| 3 | Pottassium | 51.12 ±0.02 | 69.92 ±0.14 | 66.72 ±0.11 |
| 4 | Sodium | 49.74 ±0.17 | 54.22 ±0.19 | 69.74±0.13 |

**Figure : 1. Major mineral contents ($\mu g/g$) of *Macrobranchium rosenbergii, Penaeus indicus* and *Penaeus Monodon* (Values are mean ±SD).**



## DISCUSSION

In the present study analyzing minerals in three niches prawn and shrimps were made. Mineral content was observed in the three prawn and shrimps. Minerals' content of estuarine shrimp *penaeus indicus* was found to be high (69.92 $\mu g/g$) in potassium (66.72 $\mu g/g$) in *penaeus monodon*. The highest value of sodium content (69.74 $\mu g/g$) was estimated in *penaeus monodon* and the lowest value was noted (49.74 $\mu g/g$) in freshwater prawn *Macrobranchium rosenbergii* was maximum mineral content magnesium recorded (19.52 $\mu g/g$) in marine. shrimp *penaeus monodon* and minimum mineral content magnesium recorded (12.95 $\mu g/g$) in freshwater prawn *macrobranchium rosenbergii* calcium was noted in estuarine shrimp *Penaeus indicus* (1.63 $\mu g/g$) and low amount of calcium was recorded in (1.05 $\mu g/g$) Fresh water prawn *macrobranchium rosenbergii.* Values of various minerals obtained from both shrimp species show a significant difference and samples examined in this study contained appreciable concentrations of Na, K, Ca, Mg, and P, suggesting that these shrimp species are a good source of nutrient minerals.[10] [3,4,5,6,7,8,9] The calcium content and this is in agreement with [13,14] The magnesium content in this study was similar to that recorded.[15,16] reported values for Ca, Mg, P, K, Na, Cu and Mn for *P. monodon* respectively which is also similar to the findings of this study.

## CONCLUSIONS

Comparatively high in maximum minerals were observed in the marine shrimp *penaeus monodon*. From this investigation, it is concluded that each niche group of prawn and shrimps has its biological values.

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# FT-IR SPECTROSCOPIC ANALYSIS OF LEAF EXTRACTS OF *NARINGI CRENULATA* (ROXB.) NICOLS.

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## ABSTRACT

Medicinal plants play a major role in the life of human beings throughout the globe. These plants are commercially important because of the presence of various chemical substances with the power of curing different diseases and healing several ailments affecting the normal activity of human beings. These economically important plants are found growing in remote forests, hills, and mountains where human invasion and intervention are minimum. Special knowledge about the location, usage and benefits. The Traditional Aboriginal Knowledge (TAK) is prevalent among the members of certain tribal communities inhabiting these geographical locations. The importance of medicinal plants was known to the outside world with the advent of communication technology and messages published in mass media. Increasing commercial pressure on these medicinal plants and competition among the drug manufacturers reflected on the purity and genuinely of the drugs. This ultimately resulted in several side effects in the physiological functions of the persons using these drugs. Hence pharmacognostical study of such wild plants is needed to safeguard the users. The native tribes Irulars and Malasars in the Velliangiri Hills of the Western Ghats use different parts of *Naringi crenulata* (*N.crenulata*) (Roxb.) Nicols (Plate 1) to cure different kinds of ailments. From the analysis of data of FTIR, strong absorption bands at alcohols, phenols, alkyls, amines, and some functional groups presented.

**KEYWORDS:** *Naringi crenulata* methanolic extract, FT-IR analysis, photo components.

## INTRODUCTION

The Traditional medicinal plant for several thousand years[1]. Medicinal plants are a large number of users as an alternative medicine for diseases of humans and animals since most of them are without side effects when compared with synthetic drugs. The chemical compounds' identification of the chemical nature of present in the medicinal plants provided some information on the different functional groups responsible for their medicinal properties. Studying the in-vitro efficacy of bioactive extracts of fifteen medicinal plants against multidrug resistant microbial[2]. The plants are used as remedies for human disease photochemical components of therapeutic value that produce physiological action on the human body[3]. The mainly used to various human diseases, treatment of ailment and health of affected organs from the period immemorial[4]. The world's population 80% traditional remedies for their healthcare. Today, about 70,000 to 80,000 plants are used for medicinal. This is because of some biological active and naturally occurring phytochemical present in the various parts of plants The plant '*Naringi crenulata*' (Roxb.) Nicolson belongs to the Rutaceae family, commonly known as 'kattunarakam', 'Malanarakkam', in Malayalam, 'Mahavilvam' in Tamil, a widespread species of the genus "Naringi". It has been used as folk medicine[5]. The root extract is used for vomiting, dysentery and colic disorders[6]. It is reported that its methanolic extract showed significant anthelmintic activity[7]. The photochemical compounds major groups as the most activity in plant extracts by infrared spectroscopy screened the bioactive group of chemicals in the dry leaf powder by FTIR analysis[8]. Numerous species of *Naringi crenulata* are known to possess a variety of biological activities including anti aging[9]. leaves are used for curing mental disorders[10] folk

medicine[11]. Fever[12] Intestinal worms[13]. Hence, an attempt is made in the present study to analyze the functional groups of phytochemical compounds present in the leaf extracts (in different solvents such as petroleum methanolic of *Naringi crenulata* by FTIR spectroscopic analysis. The present study was carried out the bioactive functional groups of phytochemical compounds present in the *Naringi crenulata* leaves in methanol extract with the aid of FT-IR techniques which may provide an insight in its use of traditional medicine.

## MATERIALS AND METHODS

### Collection of plant materials

The fresh and healthy leaves of the plant N.crenulata were collected from in and around Thanjavur, Tamil Nadu, India., during the 2017 and identified with Rapinat Herbarium by Principal Scientific Officer Dr.S.John Britto, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

### Extraction of plant material (Soxhlet method)

Soxhlet method used for the extraction process. Finally, crude extracts were collected and dried at room temperature,30˚C after which yield was weighed and taken for further analysis.

### Methanolic extract

Plant powder 25g was dissolved in 100ml hot water conical flask was kept on the shaker for 12hrs and allowed to run at 80rpm. Residues were collected for further process.

### Fourier Transform Infrared Spectrophotometer (FTIR)

**FTIR** is the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical as seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant material were used for FTIR analysis. The extract powder was encapsulated in 100 mg of KBr pellet, to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm 1 with a resolution of 4 cm respectively.

## RESULTS AND DISCUSSION

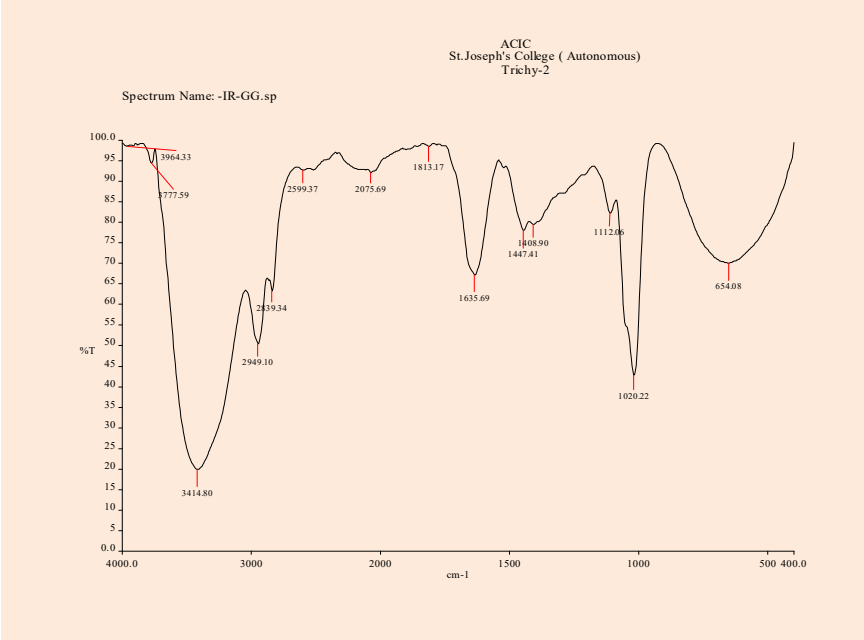
The phytochemical compounds of N. crenulata leaves have been evaluated using FT-IR. The chemical compositions of methanol extract leaves of N. crenulata were investigated using Fourier Transform Infrared Spectrophotometer. The FTIR spectrum of leaf extracts of N. crenulata is in data on the values and the probable functional groups (obtained by FTIR analysis) present in the leaf extracts are N. crenulata presented functional groups alcohols, phenols, amides alkanes and alkyls, alkyl nitrites, I socyanides, α, β-Unsaturated, aryl, acyclic, amines, ≡C-H bend fourteen functional groups recorded respectively (Table-1). The present study generated the FTIR spectrum profile for the medicinally important plants of Phyllanthus amarus, Senna auriculata, Phyllanthus maderaspatensis and Solanum torvum can be used in the aquaculture industry plants (14).The spectral analysis for saponins in the crude dry powder of 11 plants (15). Methanolic leaf extract of Cassia Alata. (16) The different kinds of biologically active compounds and their activities depending on their therapeutic uses.

**Table 1: Identification of the functional group of N. crenulata leaf by FTIR method**

| S.No | Group frequency $cm^{-1}$ of the samples | Functional group | Origin |
|---|---|---|---|
| 1 | 3964.33 | alcohols, phenols | O–H stretch free hydroxyl |
| 2 | 3777.59 | alcohols, phenols | O–H stretch free hydroxyl |
| 3 | 3414.80 | Amides | N-H stretch |
| 4 | 2949.10 | Alkanes and alkyls | C-H stretch |
| 5 | 2839.34 | Alkyl nitrites | C≡N Stretch |
| 6 | 2599.37 | Alkyl nitrites | C≡N Stretch |

| | | | |
|---|---|---|---|
| 7 | 2075.69 | Isocyanides | C≡N Stretch |
| 8 | 1813.17 | α,β-Unsaturated and aryl, acyclic | Anhydride stretching |
| 9 | 1635.69 | Amines | N-H bend |
| 10 | 1447.41 | Alkanes | C-H bend |
| 11 | 1408.90 | Phenol | O-H bend |
| 12 | 1112.06 | Alkyl halides | C-F stretch |
| 13 | 1020.22 | Alkyl halides | C-F stretch |
| 14 | 654.08 | ≡C-H bend | ≡C-H bend |

**Fig:1. Functional group of photochemical compounds of *Naringi crenulata* leaf by FTIR method**




## CONCLUSION

The presence of various bioactive phytochemical compounds justifies the use of the *Naringi crenulata* leaves for various ailments by traditional practitioners. However functional group of phytochemical compounds constituents and subjecting it to the biological activity will be analysed. It can be concluded that *Naringi crenulata* contains various bioactive compounds.

## ACKNOWLEDGMENT

The authors are thankful to Principal, A.V.V. M. Sri Pushpam College (Autonomous) Poondi, Thanjavur District, Tamil Nadu, India, and IBRI Research Laboratory for providing facility and encouragements during the study period.

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# PHYTOCHEMICAL CHARACTERIZATION OF *NARINGI CRENULATA* (ROXB) LEAF WITH METHANOLIC EXTRACT BY GC-MS METHOD.

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## ABSTRACT

The bioactive compounds of *Naringi crenulata (Roxb)* leaves have been evaluated using GC-MS. The active chemical compounds were identified by using Gas Chromatography-Mass Spectrometry. GC-MS analysis of leaves extracts *Naringi crenulata* in *the* characterization of photochemical sixteen components were identified. GC-MS results were Octane, 2,4,6-trimethyl, 1-Iodo-2-methylundecane, Hexadecane, Methoxy acetic acid, Pentadecanoic acid etc., the existence of other major components was also presented.

**KEYWORDS:** Methanol extract, *Naringi crenulata*, GC-MS analysis and photochemical.

## INTRODUCTION

Herbal medicine is one of the most remarkable uses of plant-based diversity. As many as 75 to 90% of the world's rural people rely on herbal medicine. The success of any health care system depends on the availability of suitable drugs on a sustainable basis. Natural medicine improves the strength of the body. The knowledge medicinal plant has been accumulated in many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plants species 100 species plant serves as regular sources of medicine during the last decades there had been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. According to the reports of the world health organization (WHO), as many as 80%of the world's people depend on traditional medicinal for their primary health care needs due to the considerable economic benefit in their development and All parts of this tree viz. root, stem, bark, leaf and fruit has been used as folk medicine[1]. It has been used as folk medicine[1]. The root extract of this plant is used for curing vomiting, dysentery, and colic disorders.[2,3] bioactive components of leaves and bark of *Naringi crenulata* using GC-MS[4]. Analysis of methanolic extract stem[5].Various parts of this plant have been employed in indigenous medicine and it is used as antiepileptic, purgative, sudoferic, colic trouble and cardialgia.[6] Leaves are used as a remedy for epilepsy. Bark is aromatic and cooling and is useful in vitiated conditions of Pitta[7]. Crenulatine along with twenty known indole alkaloids were isolated from the stem of the plant[8]. GC-MS is a combination of two different analytical techniques Gas chromatography (GC) and Mass Spectrometry (MS), used to analyze biochemical and organic samples. GC can separate semi-volatile and volatile compounds present in the sample with great resolution, but it cannot identify them. While the MS can provide detailed structural information so that they can be identified but cannot be quantified. Application of GCMS is to monitor and clean the environment, criminal forensics, law enforcement, security, food, beverage, and perfume analysis. It can also be used in astrochemistry and medical field[9].

## MATERIALS AND METHODS

### *Collection of plant materials*

The fresh and healthy leaves of the plant *N.crenulata* were collected from around Thanjavur, Tamil Nadu, India., during the 2017 and identified with Rapinat Herbarium by Principal Scientific Officer Dr.S.John Britto, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

***Preparation of Plant* extract**

Fresh stem parts were collected and air-dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered stem were extracted with methanol solvent by hot extraction using soxhlet apparatus collected and stored in a vial for further analysis.

***Gas chromatography-Mass spectrometry analysis*[10]**

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanolic leaf extracts was performed using a GC-MS (Model; QP 2010 series, Shimadzu,) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm dia., and 0.25mm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51ml/min. injector and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed from 70to 220°C at 10°C/min, held isothermal for 1min, and finally raised to 300°C at 10°C/min. 2ml of respective diluted samples were manually injected in the splitless mode, with a split ratio of 1:40 and with a mass scan of 50-600 amu.The total running time of GC-MS is 35min. The relative percentage of each extract constituents was expressed as a percentage with peak area normalization.

***Identification of components***

The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literature. NIST08s.LIB (10) ,WILEY8.LIB (11) library sources were also used for matching the identified components from the plant material.

## RESULTS AND DISCUSSION

The present investigation suggested that the principles in the *N.crenulata* leaves of Methanolic extract by GC-MS analysis clearly showed the presence of sixteen compounds. Most of the photochemical compounds which were reported from leaves were found to be rich in Hexadecanoic acid, Hexadecanoic acid, 15-methyl-, E-11-Hexadecenoic acid, Eicosanoic acid, ethyl ester, 7-Methyl-Z-tetradecane-1-ol acetate, 7-Hexadecenoic acid,(Z)-, 9,12-Octadecadienoic acid, Docosanoic acid, Pentadecanoic acid, 14-methyl-,3,7,11,15-Tetramethyl-2-hexadecane-1-ol, Octane,2,4,6-trimethyl,1-Iodo-2-methylundecane, Hexadecane, Methoxyacetic acid, 3-tridecyl ester, Hexadecanoic acid, 15-methyl-, 1-Iodo-2-methylundecane respectively.

**Table 1: Characterization of phytochemical compounds of *Naringi crenulata* leaves GCMS method**

| S. No | Retention Time | Compound name | Molecular Weight (g/mol) | Molecular formula |
|---|---|---|---|---|
| 1 | 14.982 | Octane, 2,4,6-trimethyl | 156 | $C_{11}H_{24}$ |
| 2 | 17.738 | 1-Iodo-2-methylundecane | 296 | $C_{12}H_{25}I$ |
| 3 | 21.680 | Hexadecane | 226 | $C_{16}H_{34}$ |
| 4 | 23.930 | Methoxyacetic acid, 3-tridecyl ester | 272 | $C_{16}H_{32}O_3$ |
| 5 | 25.117 | Hexadecanoic acid, 15-methyl-, methyl ester | 284 | $C_{18}H_{36}O_2$ |
| 6 | 26.800 | 1-Iodo-2-methylundecane | 296 | $C_{12}H_{25}I$ |
| 7 | 27.584 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 296 | $C_{20}H_{40}O$ |
| 8 | 28.793 | Pentadecanoic acid, 14-methyl-, methyl ester | 270 | $C_{17}H_{34}O_2$ |
| 9 | 29.581 | Pentadecanoic acid, 14-methyl-, methyl ester | 270 | $C_{17}H_{34}O_2$ |
| 10 | 30.983 | Docosanoic acid, ethyl ester | 368 | $C_{24}H_{48}O_2$ |
| 11 | 32.761 | 9,12-Octadecadienoic acid, methyl ester | 294 | $C_{19}H_{34}O_2$ |
| 12 | 32.891 | 7-Hexadecenoic acid, methyl ester, (Z)- | 268 | $C_{17}H_{32}O_2$ |
| 13 | 33.150 | 7-Methyl-Z-tetradecen-1-ol acetate | 268 | $C_{17}H_{32}O_2$ |
| 14 | 33.433 | Hexadecanoic acid, 15-methyl-, methyl ester | 284 | $C_{18}H_{36}O_2$ |
| 15 | 34.218 | E-11-Hexadecenoic acid, ethyl ester | 282 | $C_{18}H_{34}O_2$ |
| 16 | 34.638 | Eicosanoic acid, ethyl ester | 340 | $C_{22}H_{44}O_2$ |

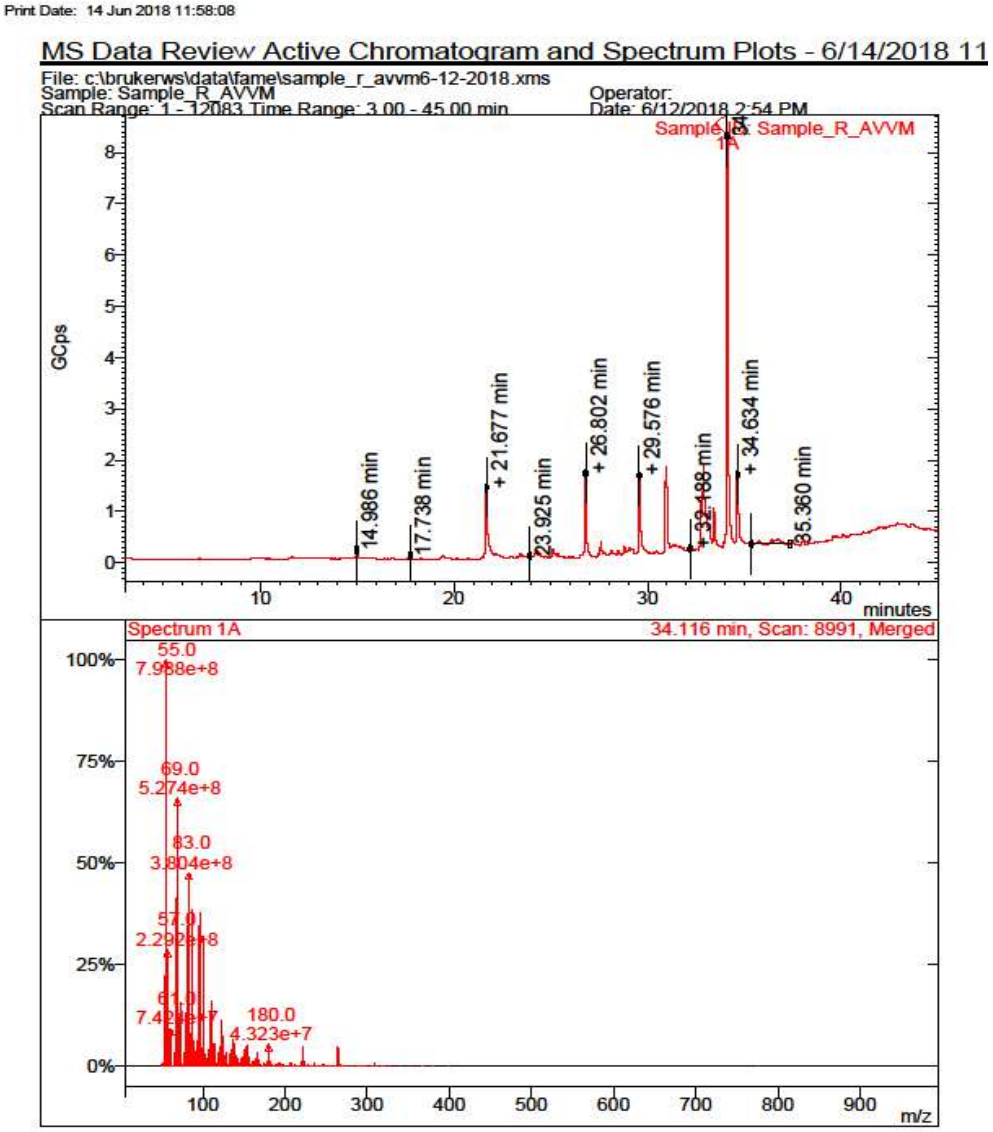


**Fig:1.**
**Characterization of phytochemical compounds of *N. crenulata* leaf by GCMS Method**

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The authors are thankful to Principal, A.V.V. M. Sri Pushpam College (Autonomous) Poondi, Thanjavur District, Tamil Nadu, India, and IBRI Research Laboratory for providing facility and encouragements during the study period.

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# IDENTIFICATION OF FECAL FATTY ACIDS IN SHEEP (*OVIS ARIES*) DURING DIFFERENT REPRODUCTIVE STAGES CONCERNING ESTRUS DETECTION

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## ABSTRACT

*Sheep* (Ramnad White) is an important indigenous breed in Tamil Nadu and has a decreasing trend in population, which is alarming as the breed is critically endangered. Artificial insemination is needed for frequent breeding and the conservation of these animals. Further, artificial insemination is also difficult in sheep because of few external estrus signs. Normally, reproduction depends upon the odors released from the female during the estrus phase. It is well known that mammals excrete chemical signals to the environment through urine, saliva, feces and specialized scent glands. Measurement of fecal fatty acids as a non- invasive technique is broadly used to monitor the reproductive status of free-ranging farm animals. Hence, the present study is planned to analyze the fecal fatty acids in sheep during different reproductive stages like pre-pubertal, estrus, pregnant and lactating animals. Samples of each stage were prepared by the FAMES method for GC analysis. The results showed that a total of 33 different fatty acids were identified in pre-pubertal stages, whereas, estrus animal shows 33 fatty acids, of which 15 belongs to saturated fatty acids, 8 belongs to monounsaturated fatty acids and 10 belongs to polyunsaturated fatty acids. Like the estrus animal, the pregnant animal also shows 30 compounds, of which 12 belonged to saturated fatty acids, six belong to monounsaturated fatty acids and 12 belong to polyunsaturated fatty acids. The lactation animal shows 29 fatty acids of which 13 belongs to saturated fatty acids, 6 belongs to monounsaturated fatty acids and 10 belongs to polyunsaturated fatty acids. Among all the stages, palmitic acid, stearic acid, elaidic acid, and oleic were identified in high concentrations in estrus when compared to all other phases. The saturated and monounsaturated fatty acids were also significantly higher in the estrus stage. The present study concluded that variation in the concentration of certain fatty acids during the reproductive stage especially in estrus helps us to monitor the reproductive status of animals.

**KEYWORDS: Ramnad white, GC, Fatty acids, reproduction, estrus**

## I. INTRODUCTION

Sheep play a vital role in the production of meat, fiber and skin, especially in ecologically difficult areas. India is one of the ecologically difficult countries, which has a rich repository of sheep resources, diversity and has the third-largest sheep population in the world. Tamil Nadu is gifted with eight sheep strains in which Ramnad White is an example for an indigenous breed, that has resorted to indiscriminate breeding over the past few decades, which results in dwelling population size[1]. Normally, the indigenous animal develops superior heat tolerance and disease resistance. They are relatively better than a crossbreed. This indigenous sheep contributes greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical and play an important role in the live- hood of a large proportion of small and marginal farmers and landless laborers. However, intermixing nearby exotic breeds and changes in the farming system have resulted a decrease in the pure breed population and dilution of genetic merits. No effort could be made until today for the conversation of sheep genetic resources[2]. Hence, it now appears that a very serious situation has arisen which may lead to complete loss of the available indigenous breeds.Reproduction in sheep is very similar to other cattle, but it is complicated by two main traits, silent heat and coupled with poor expression of estrus. A further alternative approach to providing information on

reproductive status might be the detection of pheromones. It is well established that chemical communication plays a pronounced role in governing mammalian social and reproductive behavior and it has been described that males attract towards the urine expelled from females during estrus, have been described in many animals. It is well known that the physiological and biochemical profiles of females, particularly lipids, vary according to reproductive status[3].Sexual readiness, physiological condition, and individual identity can be conveyed through chemical signals by the sender through the investigation of scent marks or excretory material. The essential tools need to study the reproductive status of sheep are fecal and urinary steroids and these techniques do not require chemical immobilization, such as used for blood sampling. Urine and feces are the two most primitive as well as a common source of mammalian pheromones [4,5,6]. Apart from urine and feces, several specialized cutaneous gland secretions has evoked to elicit pheromonal responses in mammals [7,8,9]. Estrus detection is a requirement for efficient reproductive management in farm animals [10]. Further, artificial inseminations performed in farm animals are successful only if it is done exactly during the estrus phase. Hence, this study focused on the information available on the fecal fatty acids profile of sheep during different reproductive stages which may useful for the detection of estrus stages.

## II. MATERIAL AND METHODS

### *2.1. Animals*

Six healthy sheep of the Ramnad White breed from four different stages like prepubertal, estrus, pregnant and lactating animals were used in the present study from the District Livestock Farm, Pudukkottai, Tamil Nadu, India. The animals were fed with standard diet in appropriate facilities that had floor and that was well ventilated.

### *2.2. Estrus determination*

The conventional estrus behavior in sheep is not easy to detect when compared to other cattle. The characteristic behaviors of estrus, such as restlessness, reddened and swollen vulva, are often difficult to detect because of the wool and small size of the vulva. Some of the secondary behaviors, such as rapid tail wagging and raised tail in the presence of ram are also considered as signs of estrus. The stages of the estrous cycle were carefully determined for two to three consecutive cycles with the help of a veterinarian. The sheep were considered to be in estrus if they accepted the mounting by another sheep. The period within three to five days before estrus was considered as pro-estrus and that within two to four days after estrus as post-estrus.

### *2.3. Sample collection*

The fecal samples were collected from sheep during various reproductive phases at every morning between 6.00 and 8.00 a.m. The samples were collected in the vial within 5 minutes after excretion. The vials were labeled appropriately and placed in a freezer at - $20^0$C for gas chromatography (GC) analysis.

### *2.4. Fatty acid profile*

The lipid was extracted from the collected feces using chloroform and methanol (1:1) [11]. One ml of extract was taken and mixed with a saponification reagent. The tubes were tightly closed and kept for 30 minutes at 60°C in a water bath. Two ml of methylation reagent was added to each tube and kept again in the water bath at 80°C for 20 minutes. Finally, a sufficient amount of extraction solvent (200 ml hexane + 200 ml of diethyl ether) was added to each tube and closed tightly and shaken thoroughly for 10 minutes. About 2/3 of the organic phase (upper layer) containing the fatty acid methyl esters (FAME) was transferred into screw cap glass vials. From each vial 1µl of the FAME was injected into the GC column[12].

### *2.5. Statistical Analysis*

The obtained data were computed by the ANOVA test followed by the post hoc Duncan's test. All the data analyses were significant at $P < 0.05$[13].

## III. RESULTS AND DISCUSSION

It is also interesting to note that fatty acid levels may vary according to the reproductive phases. Hence, the present work is aimed to detect the fatty acid profiles in sheep feces during various reproductive stages.

The fecal samples were subjected to GC analysis and the results showed the variation in qualitatively and quantitatively among the stages. The total fatty acids content in the sheep feces of various reproductive stages was quantitatively and qualitatively assistance by GC. In all the samples, various proportions and different kinds of fatty acids were identified and the fatty acids ranging from C8 to C22:6n3. GC analysis of pre-pubertal animals revealed a total thirty-three different types of fatty acids during all reproductive stages of females. Among these, 15 were saturated fatty acids; seven belong to monounsaturated and the remaining eleven belongs to polyunsaturated fatty acids. The saturated and monounsaturated fatty acids were significantly higher in early pre-pubertal, whereas polyunsaturated fatty acid level was significantly high in late pre-pubertal animals when compared to other stages. The level of laurel acid (0.65±0.02 mg/g) was found to be higher in late pre-pubertal feces followed by palmitic acid and behenic acids when compared to other saturated fatty acids. Oleic acid (0.64±0.02 mg/g) belongs to monounsaturated fatty was found to be higher in the early pre-pubertal stage when compared to other fatty acids. Among them, arachidonic acid (0.98±0.04 mg/g) was present in a high level at the late pre-pubertal stage. Further, arachidonic acid was found to be predominant when compared to all other fatty acids. Polyunsaturated fatty acids level was found to be higher in early pre-pubertal stages when compared to saturated fatty acids and monounsaturated fatty acids. The Estrus stage is considered as important in the present study, which shows thirty-three different fatty acids, of which fifteen belongs to saturated fatty acids, seven belongs to monounsaturated fatty acids and eleven belongs to polyunsaturated fatty acids. During the estrus period, the high level of saturated, monounsaturated and polyunsaturated fatty acids were present when compared to pro-estrus and post-estrus stages. Palmitic acid (8.13±0.53 mg/g), stearic acid (9.82±0.05mg/g), oleic acid (3.78±0.07mg/g), elaidic acid (2.42±0.13mg/g) and cis-4, 7, 10,13,16,19-docosahexaenoic (1.23 ± 0.21mg/g) were found to be predominantly higher in estrus phase when compared to other fatty acids. The analysis of fecal fatty acids during the estrous cycle showed 33 different types of fatty acids; some of them like palmitic acid, stearic acid, oleic acid and oleic acid were significantly higher in concentration at the estrus phase when compared to other phases. This finding is consistent with the report of Achiraman and Archunan [15] who suggested that the level of palmitic acid and oleic acid were found to be higher in mice urine during the estrus period. Further, Kannan and Archunan [8,9,15] reported the presence of palmitic acid in preputial and flank glands of house rat during the estrus phase. Selvaraj,[16] also suggested that the male rat (*Rattus rattus*) spent more time to oleic acid than the female rat. Mattina[17] suggested that palmitic acid was excreted in the reproductive phase of bobcat urine and involved in sexual attraction of conspecific. Yahia Khandoker[18] stated that the higher composition of palmitic and oleic acids in reproductive stages indicates that both may serve as a storage pool of metabolism precursor. The early, mid and late pregnant animals exhibited thirty-three different types of fatty acids. Among these fourteen belongs to saturated fatty acids, seven belong to monounsaturated fatty acids and twelve belongs to polyunsaturated fatty acids. However, the saturated and polyunsaturated fatty acids were present predominantly in early pregnant with higher concentration and monounsaturated fatty acids were found to be high in mid-pregnant periods. The fatty acids like palmitic acid (0.93±0.28 mg/g), arachidic acid (0.91±0.11 mg/g), cis-8,11,14-Eicosatrienoic acid (0.91±0.34 mg/g) and Cis-4,7,10,13,16,19-Docosahexaenoic acid (0.81±0.01mg/g) were found exclusively in early pregnant animals. GC analysis of lactating animals also revealed thirty-three types of fatty acids, of which fourteen saturated fatty acids, seven monosaturated fatty acids and twelve were polyunsaturated fatty acids. During the lactation period, polyunsaturated fatty acids were higher when compared to monounsaturated acids and saturated fatty acids. The linoleic acid (0.98±0.01 mg/g) belongs to PUFAs was found to be high in early lactation when compared to other fatty acids followed by stearic and arachidonic acids (Table - 1). In the present study, thirty-five different types of fatty acids were detected in sheep feces. Among them, four fatty acids namely palmitic acid, stearic acid, oleic acid and cis-4,7,10,13,16,19-Docosahexaenoic acid were present in all twelve stages, but the concentration of these fatty acids varied considerably across all the reproductive phases. Further, palmitic acid, stearic acid, oleic acid and cis-4, 7, 10,13,16, 19-Docosahexaenoic acid were found to higher in estrus when compared to other phases. Cis-4, 7, 10, 13, 16, 19 – Docosahexaenoic acid was present in proestrus and estrus but it was absent during the post estrus stage. The level of saturated and monounsaturated fatty acids was found to be significant in the estrus phase, whereas polyunsaturated fatty acids were found to be significant in the late lactation period (Table - 2).

A total of thirty-five fatty acids were detected among them lauric acid, myristic acid, palmitic acid, oleic acid, stearic acid and cis-5,8,11,14,17-eicosapentaenoic were present in all stages, but the concentration of all these fatty acids varied considerably across all stages. The amount of certain fatty acids during the

estrus phase is present in higher concentration when compared to that of other reproductive stages and it suggests that it may act as a chemical attraction. Further study is needed to collect more information regarding the functional role of these fatty acids through the behavioral analysis. In this study, there are evidence for specific fatty acid during estrus phases but it is remarkably noted that certain fatty acids concentration seems to be higher during the estrus phase. It is also further noted that urinary fatty acids showed attraction in mice [19], bovine [20,21] and tiger [22,23]. Besides, the difference in the level of fatty acid present samples may give a basis for individual identity and it may act as a scent marker.The feces of prepubertal animals contain nearly 29 fatty acids; among these, arachidonic acid is present in higher concentrations as compared to other fatty acids. The reason for the higher concentration of arachidonic acid in the prepubertal stage, because it is a precursor for prostaglandin biosynthesis, which has been implicated in ovarian function [24,25,26]. GC analysis of pregnant animal feces shows 30 fatty acids of which gamma-linoleic acid is the higher concentration when compared to other fatty acids. The feces of lactation animals shows 28 different types of fatty acids and the concentration of linoleic acid was found to be higher when compared to other fatty acids. These variations in the fatty acids during pregnancy and lactation depend on the physiology state of females and could be due to the modification in the reproductive status of animals [20].The level of saturated fatty acids was found to be significantly higher when compared to monounsaturated fatty acids and polyunsaturated fatty acids. Many reports are indicated that the increased level of saturated fatty acids is common during spawning seasons, while PUFA values significantly decreased during the same period [27]. Similarly, in Sparidae species, fatty acid compositions studied about the reproductive cycles especially increased in saturated fatty acids and decreased in PUFA [28], but in bobcat urine predominantly contains unsaturated fatty acids [17]. The level of polyunsaturated fatty acids was found to be significantly higher in late lactation when compared to other stages.These results supported by Pudelkewiez, [29] that females deprived of PUFAs abort or pups die soon after birth and males are sterile and refuse to mate and they reported that low-fat diet showed impairment in reproductive success. The present interpretations indicate that lipids are varied in the feces during different reproductive phases of sheep in addition to this, the quantitative and qualitative estimation of fatty acids also varied [20,21,29].

**Table 1: Fatty acids profile (mg/g) of sheep (*Ovis aries*) feces during different reproductive stages**

| Car.chain | Fatty acids | Early Prepubertal | Mid Prepubertal | Late Prepubertal | Proestrus | Estrus | Postestrus | Early Pregnant | Mid pregnant | Late pregnant | Early Lactation | Mid Lactation | Late Lactation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C8:0 | Caprylic acid | 0.04 ± 0.01 | 0.34 ± 0.03 | - | - | 0.30 ± 0.02 | 0.01± 0.01 | - | 0.46 ± 0.05 | - | 0.35 ± 0.01 | | 0.15 ± 0.01 |
| C10:0 | Capric acid | - | 0.37 ± 0.01 | - | 0.03 ± 0.13 | 0.37 ± 0.01 | 0.02 ± 0.01 | 0.36 ± 0.52 | 0.70 ± 0.12 | 0.48 ± 0.13 | 0.37 ± 0.03 | 0.28 ± 0.07 | 0.50 ± 0.07 |
| C11:0 | Undecanoic acid | 0.50 ± 0.01 | 0.30 ± 0.02 | 0.60 ± 0.02 | 0.05 ± 0.12 | 0.15 ± 0.20 | 0.05 ± 0.01 | - | - | - | - | - | - |
| C12:0 | Lauric acid | 0.40 ± 0.01 | 0.51 ± 0.02 | 0.65 ± 0.02 | 0.07 ± 0.01 | 0.80 ± 0.02 | 0.18± 0.01 | 0.67 ± 0.15 | 0.12 ± 0.02 | 0.09 ± 0.18 | 0.38 ± 0.07 | 0.49± 0.06 | 0.81 ± 0.03 |
| C13:0 | Tridecanoic acid | - | - | - | 0.01 ± 0.05 | 0.03 ± 0.01 | 0.02 ± 0.01 | - | 0.02 ± 0.01 | - | 0.23 ± 0.01 | - | - |
| C14:0 | Myristic acid | 0.21 ± 0.01 | 0.16 ± 0.03 | 0.11 ± 0.01 | 0.11 ± 0.35 | 0.94 ± 0.23 | 0.37 ± 0.02 | 0.28 ± 0.14 | 0.37 ± 0.12 | 0.37 ± 0.25 | 0.4 ± 0.08 | 0.3 ± 0.12 | 0.32 ± 0.12 |
| C15:0 | Pentadecanoic acid | 0.31 ± 0.01 | 0.21 ± 0.01 | 0.16 ± 0.01 | 0.05 ± 0.06 | 0.54 ± 0.02 | 0.17 ± 0.21 | - | 0.33 ± 0.25 | 0.67 ± 0.16 | 0.03 ± 0.15 | - | - |
| C16:0 | Palmitic acid | 0.61 ± 0.02 | 0.32 ± 0.02 | 0.04 ± 0.08 | 1.69 ± 0.19 | 8.13 ± 0.53 | 2.58 ± 0.41 | 0.93 ± 0.28 | 0.18 ± 0.05 | 0.11 ± 0.02 | 0.6 ± 0.07 | 0.39 ± 0.15 | 0.54 ± 0.15 |
| C17:0 | Heptadecanoic acid | 0.03 ± 0.01 | 0.33 ±0.01 | - | 0.03 ± 0.08 | 0.38 ± 0.02 | 0.11± 0.07 | - | 0.16 ± 0.05 | 0.19 ± 0.12 | 0.24 ± 0.02 | - | - |
| C18:0 | Stearic acid | 0.03 ± 0.01 | 0.26 ± 0.03 | 0.96 ± 0.02 | 0.60 ± 0.26 | 9.82 ± 0.05 | 2.55 ± 0.01 | 0.22 ± 0.19 | 0.15 ± 0.11 | 0.92 ± 0.15 | 0.58 ± 0.07 | 0.44 ± 0.75 | 0.90 ± 0.35 |
| C20:0 | Arachidic acid | 0.20 ± 0.02 | 0.11 ± 0.02 | 0.16 ± 0.01 | 0.25 ± 0.67 | 0.11 ± 0.24 | 0.04 ± 0.03 | 0.91 ± 0.11 | - | - | - | 0.18 ± 0.03 | 0.22 ± 0.12 |
| C21:0 | Henicosanoic acid | - | 0.11 ± 0.02 | - | 0.03 ± 0.42 | 0.08 ± 0.21 | 0.02 ± 0.13 | - | - | - | - | - | - |
| C22:0 | Behenic acid | 0.61 ± 0.01 | - | 0.30 ± 0.23 | 0.02 ± 0.18 | 0.11 ± | 0.04 ± 0.21 | 0.43 ± 0.25 | - | 0.17 ± 0.01 | - | - | - |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 0.02 | | | | | | | |
| C23:0 | Tricosanoic acid | 0.09 ± 0.04 | - | - | 0.08 ± 0.67 | 0.11 ± 0.02 | 0.08 ± 0.15 | - | - | - | - | 0.23 ± 0.07 | 0.16 ± 0.03 |
| C24:0 | Lignoceric acid | 0.55 ± 0.02 | - | - | 0.25 ± 0.22 | 0.11 ± 0.02 | 0.03 ± 0.10 | 0.18 ± 0.28 | - | 0.34 ± 0.12 | - | 0.08 ± 0.08 | - |
| **£SFAs** | **3.54 ± 0.06** | **3.07 ± 0.04** | **2.70 ± 0.06** | **3.29 ± 0.07** | **21.89 ± 0.07** | **6.32 ± 0.05** | **3.65 ± 0.09** | **2.50 ± 0.04** | **3.35 ± 0.02** | **3.18 ± 0.09** | **2.39 ± 0.07** | **3.61 ± 0.03** | |
| C14:1 | Myristoleic acid | 0.16 ± 0.01 | - | 0.02 ± 0.15 | 0.02 ± 0.15 | 0.03 ± 0.03 | 0.01 ± 0.01 | 0.23 ± 0.13 | 0.42 ± 0.15 | - | 0.02 ± 0.60 | 0.23 ± 0.04 | 0.23 ± 0.67 |
| C15:1 | cis-10-Pentadecenoic acid | 0.43 ± 0.02 | 0.17 ± 0.01 | 0.03 ± 0.45 | 0.03 ± 0.45 | 0.32 ± 0.22 | 0.05 ± 0.08 | - | - | - | - | - | - |
| C16:1 | Palmitoleic acid | 0.16 ± 0.01 | 0.17 ± 0.01 | 0.04 ± 0.29 | 0.04 ± 0.29 | 0.19 ± 0.05 | 0.10 ± 0.67 | 0.34 ± 0.22 | 0.23 ± 0.21 | - | 0.39 ± 0.09 | - | 0.18 ± 0.15 |
| C17:1 | cis-10-Heptadecenoic acid | - | - | - | - | 0.02 ± 0.02 | 0.02 ± 0.57 | - | 0.15 ± 0. 15 | - | 0.18 ± 0.15 | - | - |
| C18:1 n9t | Elaidic acid | - | 0.02 ±0.02 | - | 0.04 ± 0.01 | 2.42 ± 0.13 | 0.27 ± 0.01 | 0.13 ± 0. 11 | 0.18 ± 0.03 | 0.24 ± 0. 23 | 0.06 ± 0.14 | 0.13 ± 0.01 | 0.17 ± 0.16 |
| C18:1 n9c | Oleic acid | 0.64 ± 0.02 | 0.54 ± 0.02 | 0.04 ± 0.01 | 0.02 ± 0.03 | 3.78 ± 0.07 | 0.05 ± 0.01 | 0.03 ± 0.24 | 0.17 ± 0.01 | 0.61 ± 0.15 | 0.22 ± 0.03 | 0.15 ± 0.13 | 0.15 ± 0.05 |
| C20:1n 9 | cis-11,Eicosenoic acid | 0.19 ± 0.01 | 0.18 ± 0.02 | 0.02 ± 0.03 | 0.01 ± 0.23 | 0.03 ± 0.45 | - | - | - | - | - | - | - |
| C24:1n 9 | Nervonoic acid | 0.50 ± 0.01 | - | 0.04 ± 0.02 | - | 0.50 ± 0.01 | 0.07 ± 0.07 | 0.54 ± 0.31 | 0.72 ± 0.24 | - | - | 0.58 ± 0.07 | 0.07 ± 0.09 |
| **ΣMUFAs** | **1.58 ± 0.02** | **1.09 ± 0.02** | **2.06 ± 0.02** | **0.18 ± 0.02** | **7.29 ± 0.02** | **0.52 ± 0.02** | **1.28 ± 0.02** | **1.87 ± 0.02** | **0.86 ± 0.02** | **0.88 ± 0.02** | **1.09 ± 0.02** | **0.81 ± 0.01** | |
| C18:2 n6t | Linolelaidic acid | 0.64 ± 0.01 | 0.91 ± 0.01 | 0.74 ± 0.03 | 0.01 ± 0.01 | 0.25 ± 0.01 | 0.03 ± 0.05 | 0.15 ± 0.01 | - | 0.27 ± 0.02 | 0.16 ± 0.03 | 0.23 ± 0.15 | 0.16 ± 0.33 |
| C18:2 n6c | Linoleic acid | - | 0.06 ±0.01 | - | - | 0.16 ± 0.02 | 0.02 ± 0.01 | - | 0.54 ± 0.22 | 0.72 ± 0. 11 | 0.98 ± 0.01 | 0.48 ± 0.03 | 0.65 ± 0.12 |
| C18:3n 3 | alpha-Linolenic acid | | | | 0.02 ± 0.04 | 016 ± 0.01 | 0.03 ± 0.04 | - | 0.18 ± 0. 13 | - | - | - | - |
| C18:3n 6 | gamma-Linolenic acid | 0.05 ± 0.02 | - | 0.02 ± 0.01 | - | 0.42 ± 0.01 | 0.02 ± 0.01 | 0.28 ± 0.32 | 0.94 ± 0. 14 | 0.17 ± 0.01 | 0.01 ± 0.17 | 0.73 ± 0.15 | 0.17 ± 0.01 |
| C20:2 | cis-11,14-Eicosadienoic acid | 0.84 ± 0.01 | 0.63 ± 0.02 | 0.12 ± 0.05 | 0.01 ± 0.01 | 0.03 ± 0.08 | - | 0.02 ± 0.03 | - | 0.33 ± 0.14 | 0.62 ± 0.07 | 0.36 ± 0.02 | 0.24 ± 0.52 |
| C20:3n 6 | cis-8,11,14-Eicosatrienoic acid | 0.19 ± 0.01 | 0.18 ± 0.02 | 0.17 ± 0.01 | - | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.91 ± 0.34 | 0.13 ± 0. 11 | 0.11 ± 0.13 | 0.22 ± 0.07 | 0.99 ± 0.14 | 0.74 ± 0.22 |
| C20:4n 6 | Arachidonic acid | 0.91 ± 0.01 | 0.84 ± 0.01 | 0.98 ± 0.04 | 0.01 ± 0.02 | 0.34 ± 0.02 | 0.01 ± 0.01 | 0.15 ± 0.01 | - | 0.27 ± 0.02 | 0.85 ± 0.03 | - | - |
| C20:3n 3 | cis-11,14,17-Eicosatrienoic acid | - | - | - | - | - | - | 0.48 ± 0.23 | 0.39 ± 0.02 | 0.92 ± 0. 21 | - | - | 0.2 ± 0.12 |
| C20:1n 9 | cis -11-Eicosenoic acid | - | - | - | - | - | - | 0.36 ± 0.04 | - | - | | | |
| C20:5n 3 | cis-5,8,11,14,17-Eicosapentaenoic | 0.77 ± 0.04 | 0.71 ± 0.01 | 0.79 ± 0.02 | 0.07 ± 0.01 | 0.40 ± 0.01 | 0.02 ± 0.03 | 0.61 ± 0. 13 | 0.13 ± 0. 11 | 0.14 ± 0.03 | 0.19 ± 0.07 | 0.79 ± 0.02 | 0.65 ± 0.12 |
| C22:2 | cis-13,16-Docosadienoic acid | - | - | - | - | 0.63 ± 0.02 | - | 0.47 ± 0.05 | 0.42 ± 0.01 | - | 0.36 ± 0.12 | - | 0.25 ± 0.24 |
| C22:6 n3 | cis-4,7,10,13,16,19-Docosahexaenoic | - | 0.40 ± 0 .02 | - | 0.05 ± 0.03 | 1.23 ± 0.21 | - | 0.81 ± 0. 01 | 0.05 ± 0.23 | 0.14 ± 0. 13 | - | 0.34 ± 0.19 | 0.46 ± 0.32 |
| **ΣUFAs** | **3.91 ± 0.01** | **3.73 ± 0.03** | **2.76 ± 0.01** | **0.17 ± 0.03** | **4.17 ± 0.01** | **0.62 ±0.0 3** | **4.47 ± 0.02** | **2.78 ± 0.02** | **2.80 ± 0.01** | **3.39 ± 0.01** | **3.92 ± 0.03** | **4.33 ± 0.02** | |

***Values are expressed in Mean ± SEM***

**Table 2: Level of different fatty acids in sheep (*Ovis aries*) feces during various reproductive stages**

| Stages | Fatty acids mg/g of lipid | | |
|---|---|---|---|
| | SAFs | MUFAs | PUFAs |
| Early - Prepubertal | $3.54 \pm 0.06^{c}$ | $1.58 \pm 0.02^{d}$ | $3.73 \pm 0.01^{e}$ |
| Mid - Prepubertal | $3.07 \pm 0.04^{e}$ | $1.09 \pm 0.02^{f}$ | $3.91 \pm 0.01^{d}$ |
| Late - Prepubertal | $2.67 \pm 0.06^{f}$ | $2.06 \pm 0.02^{b}$ | $2.76 \pm 0.01^{g}$ |
| Proestrus | $3.29 \pm 0.07^{d}$ | $0.18 \pm 0.02^{i}$ | $0.17 \pm 0.03^{i}$ |
| Estrus | $21.89 \pm 0.07^{a}$ | $6.79 \pm 0.02^{a}$ | $4.17 \pm 0.01^{c}$ |
| Post estrus | $6.32 \pm 0.05^{f}$ | $0.52 \pm 0.02^{h}$ | $0.62 \pm 0.03^{h}$ |
| Early - Pregnant | $3.65 \pm 0.09^{c}$ | $1.28 \pm 0.02^{e}$ | $4.47 \pm 0.02^{a}$ |
| Mid - Pregnant | $2.50 \pm 0.04^{g}$ | $1.87 \pm 0.02^{c}$ | $2.78 \pm 0.02^{g}$ |
| Late - Pregnant | $3.35 \pm 0.02^{d}$ | $0.86 \pm 0.02^{g}$ | $2.80 \pm 0.01^{g}$ |
| Early - Lactation | $3.18 \pm 0.09^{de}$ | $0.88 \pm 0.02^{g}$ | $3.39 \pm 0.01^{f}$ |
| Mid - Lactation | $2.39 \pm 0.07^{g}$ | $1.09 \pm 0.02^{f}$ | $3.92 \pm 0.03^{d}$ |
| Late - Lactation | $3.61 \pm 0.03^{c}$ | $0.81 \pm 0.01^{g}$ | $4.33 \pm 0.02^{b}$ |

***Values are expressed in Mean ± SEM and the values with different alphabets in vertical rows are significantly different at P < 0.05 % level***
***SFA saturatedfatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids***

## IV. CONCLUSION

The presence of one or two fatty acids in the estrus phase along with other components may involve in the estrus indicating compounds. The amount of certain fatty acids during the estrus phase is present in higher concentrations when compared to that of other reproductive phases and suggested that they may be involved in chemo-attraction. Even though the present study did not reveal any estrus-specific urinary fatty acids, it is interesting to note that the concentration of certain fatty acids seems to be higher during the estrus phase. Moreover, experiments are now in progress in determining the effect of these fatty acids during the estrus period. Further, if we conduct behavioral analysis using these fatty acids it will give the exact role of them.

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# ANALYSIS OF FAT SOLUBLE VITAMIN CONTENTS IN THREE DIFFERENT HABITAT FISHES

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## ABSTRACT

Fish contains the most important nutritional constituents and serves as a source of energy, the best source of ω-3 fatty acids, high grade of protein, vitamins and minerals loaded food for young as well as old age humans. The poor man's diet fish is the most wanted foodstuff for the popular societies in the world. The flesh of fish encompasses relatively large quantities of vitamins, particularly A and D. Three different habitats of fishes were selected to analyze the vitamin content. They were freshwater fish *Oreochromis mossambicus*, estuarine fish *Mugil cephalus* and marine fish *Lates calcarifer*. Tissue samples were taken from each fish was homogenized. Three fat-soluble vitamins such as vitamin A, vitamin D and vitamin E were estimated from the fishes utilized.

**KEYWORDS**: Fishes, *Oreochromis mossambicus, Mugil cephalus, Lates calcarifer*, vitamins A, D and vitamin E.

## INTRODUCTION

Fish contains the most important nutritional constituents and serves as a source of energy, the best source of ω-3 fatty acids, high grade of protein, vitamins and minerals loaded food for young as well as old age humans. Based on their solubility, vitamins are differentiated as fat-soluble - A, D, E and K and water-soluble vitamins B and C. Hepatic reserves of vitamin A in aquatic animals are much more when compared to mammals and birds. Fish is a good source of vitamin A and D[1]. The deficiency of Thiamine shows poor growth, high mortality, and stress susceptible[2]. Fish account for 60% of the animal protein consumed by the population and also provide essential vitamins, minerals and fatty acids[3]. The proximate composition is habitually used as a marker of the dietetic value of food materials[4]. The proximate composition has been reported that the key components of fish are moisture, protein, fat, minute quantities of carbohydrates, vitamins and minerals[5]. Fish is also a home town of vitamin A, which is needed for healthy skin and eyes and vitamin D, which is needed to help the body absorb calcium to strengthen teeth and bones. Fish is one of the main sources of vitamins[6]. In fish such as the fat-soluble vitamins A, D, E and K also have therapeutic effects on the prevention of particular diseases[7]. More and more attention is being given to fish products as a source of vitamins and minerals. This is particularly true for small-sized species consumed whole, with heads and bones.Hence the study was carried out to determine fat-soluble vitamin composition of the three different habitat fish species *Oreochromis mossambicus, Mugil cephalus* and *Lates calcarifer*.

## MATERIALS AND METHODS

### Collection of samples

Fresh fishes such as *Oreochromis mossambicus, Mugil cephalus,* and *Lates calcarifer* were collected from nearby areas and brought to the laboratory for vitamin analysis.

### Estimation of fat-soluble vitamins in the study fishes

Fat soluble vitamins such as A, D and E in the fish tissue were determined by HPLC with UV - Visible diode assay detector Agilent 1100 series[8, 9].

### Analysis of Results

The results obtained were subject to statistical analysis such as mean and standard deviation.

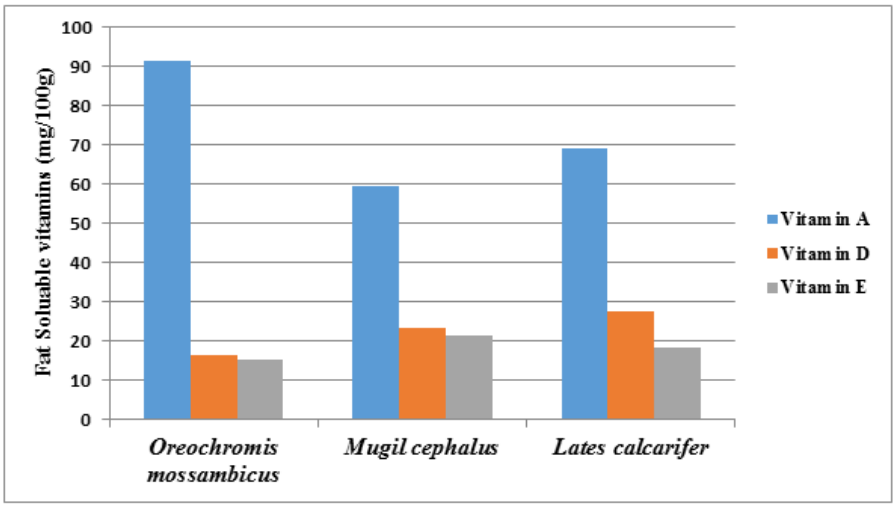
## RESULTS

Three different fishes chosen for the study were *Oreochromis mossambicus, Mugil cephalus* and *Lates calcarifer*. The fat-soluble vitamins were estimated and the results were presented in (Table 1). Three fat-soluble vitamins were found out from study fishes. The maximum amount of vitamin A (91.53±0.18mg/100g) was noted in freshwater fish *Oreochromis mossambicus* and minimum vitamin A was recorded in estuarine fish *Mugil cephalus*. The highest amount of vitamin D (27.46±0.78) was noted in *Lates calcarifer* and the lowest amount of vitamin D (16.35±0.67) was noted in*Oreochromis mossambicus.* The maximum amount of vitamin E (21.45±1.16) was noted in *Mugil cephalus* and the minimum amount of vitamin E (15.41±0.67) was noted in *Oreochromis mossambicus* (Table 1 and Fig 1).

**TABLE-1. Fat and water-soluble vitamins of (mg/100g) of *Oreochromis mossambicus*, *Mugil cephalus* and *Lates calcarifer* (Values are mean ± SD).**

| Solubility | Vitamins (mg/100g) | Fishes | | |
|---|---|---|---|---|
| | | *Oreochromis mossambicus* | *Mugil cephalus* | *Lates calcarifer* |
| Fat-soluble Vitamins | Vitamin A | 91.52 ± 0.18 | 59.47 ± 0.96 | 69.02 ± 0.26 |
| | Vitamin D | 16.35 ± 0.53 | 23.57 ± 1.04 | 27.46 ± 0.78 |
| | Vitamin E | 15.41 ± 0.67 | 21.45 ± 1.16 | 18.58 ± 1.04 |

**Fig 1. Fat-soluble vitamins (mg/100g) of *Oreochromis mossambicus*, *Mugil cephalus* and *Lates calcarifer*.**




## DISCUSSION

In this study, fat-soluble vitamin content of the three fishes was observed. Three fat-soluble vitamins were found out from study fishes. The maximum amount of vitamin A (91.53±0.18mg/100g) was noted in freshwater fish *Oreochromis mossambicus* and minimum vitamin A was recorded in estuarine fish *Mugil cephalus*. The highest amount of vitamin D (27.46±0.78) was noted in *Lates calcarifer* and the lowest amount of vitamin D (16.35±0.67) was noted in *Oreochromis mossambicus.* The maximum amount of vitamin E (21.45±1.16) was noted in *Mugil cephalus* and the minimum amount of vitamin E (15.41±0.67) was noted in *Oreochromis mossambicus.* Catfishes have more vitamin content than *Oreochromisnilotieus* (vitamin A 0.00025%, 0.00046%, and 0.00084, vitamin C 0.0023mg/g, 0.0040mg/g and 0.0023mg/g) in raw, skin- dried and electric- dried fish sample[10]. The total vitamin contents of vitamin A (145.6 mg/g), vitamin C (45.65mg/g) are the highest concentration in the head and bone region of *Oreochromis mossambicus*[11]. Seven vitamins were found in catfish samples such as vitamins A, C, D, E, B6, B1 and vitamin B12. Vitamin E (4.28mg, 7.52mg) (3.28mg, 5.45mg) is the predominant vitamin in the head and body of A.maculatus, P.lineatus[12]. The nutritional parameters are attributed to the diet which they consume

and their ecological conditions. This study reveals that all fishes have an essential source of fat-soluble vitamins.

## CONCLUSION

Fish occupy a complete food for man and so it is commonly considered as a superfood of nature. The maximum amount of vitamin A (91.53±0.18mg/100g) was observed in freshwater fish *Oreochromis mossambicus.* The maximum amount of vitamin E (21.45±1.16) was noted in *Mugil cephalus.* The maximum amount of vitamin D (27.46±0.78) was recorded in *Lates calcarifer. Oreochromis mossambicus* is a freshwater fish, *Mugil cephalus* is an estuarine fish, *Lates calcarifer* is a marine fish. Each habitat group of fishes has its nutritional parameters with their different habit and food preferences.

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# DETOXIFICATION STUDIES OF *O*-CRESOL CONTAMINATED WATER SAMPLE BY IMMOBILIZED CELLS IN A PACKED BED COLUMN REACTOR

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## ABSTRACT

Increasing population and industrialization have polluted our environment immensely. Biodegradation is the process of complete removal of toxic compounds from our environment to protect and safeguard our earth from pollution. A large number of microorganisms are being utilized for this purpose as they have proven nontoxic, eco-friendly and cost-effective and have gained more public acceptance than the conventional chemical methods. Although they have limited disadvantages the control and optimization of the biodegradation process is a complex system and have to consider many factors like the availability of a microbial population capable of degrading the pollutants, their growth conditions, the availability of nutrient factors including the pollutant of our concern and the environmental factors like temperature pH, presence of oxygen, etc. In the present research, an attempt has been made to study the various scenario of detoxification of *o*-cresol (methylated phenol) by a bacteria isolated from petroleum-contaminated soil. The isolated novel bacteria *Pseudomonas monteilii* SHY immobilized in an appropriate matrix showed increasing the removal efficiency of *o*-cresol from samples. When agar entrapped *P. monteilii* SHY cells were packed in the bed column and used, the removal efficiency of *o*-cresol was increased many folds.. The process was applied to treat 2L of water from a river source and it could remove 700mg/L of *o*-cresol from the sample in 8 days and showed a removal efficiency of 99.42%.

**KEYWORDS:** *Pseudomonas monteilii* SHY, immobilized beads, water treatment, Detoxification

## INTRODUCTION

The anthropogenic activities such as rapid industrialization and urbanization have spread their deadly tentacles in the form of air, water and land pollution to engulf our environment. Environmental preservation for sustainable development is one of the major concerns for a rapidly developing country like India. The chief xenobiotic compounds include phenolic derivatives, halogenated benzenes, benzoates, polychlorinated biphenyls, chlorinated pesticides, etc. The cresols are organic aromatic methylated phenolic compounds and are also known as hydroxytoluene, methyl phenol, methylhydroxybenzene, tricresol, etc. Cresolhas 3 isomers which are ortho-Cresol(1,2-cresol), meta-cresol(1,3-cresol) and para-cresol(1,4-cresol) depending upon the position of the methyl group present. *O*-cresols are used in the production of herbicides, pesticides, dye intermediates, antiseptic, and antioxidants and directly as a valuable solvent. (Handbook of Commercial Catalysts: Heterogeneous Catalysts)[1]. *O*-cresols after reacting with formaldehyde are widely used as Coatings, electronic insulation materials, adhesives and also for automotive applications. Even though they are used for many purposes their toxicity is of high concern because of their disastrous effect to flora and fauna [2] and it is in the EPA list since 1979.The *o*-cresol isomer which is more toxic than the other two isomers was selected for the present study.

## MATERIALS AND METHODS

### Microorganism used

The microorganism used for the study was a novel strain of *Pseudomonas monteilii* isolated from petroleum-contaminated soil. The organism was named *Pseudomonas monteilii* SHY and the sequence of the novel isolate was deposited in GeneBank (NCBI, USA) with Accession number: MF278026. The cells proved highly efficient in removing o-cresol in soil and water. Entrapping these cells in the agar matrix increased their removal efficiency many folds. This agar entrapped *P. monteilii* SHY cells were used in the present study.[3,4]

### Removal of *o*-cresol by the immobilized cells through a packed bed column reactor

A packed bed column reactor (PBCR) using agar - entrapped cells of *P. monteilii* SHY was designed for bioremediation of simulated *o*-cresol containing effluents. An experiment was performed in a glass column of height 50 cm, with a diameter of 3.2 cm was packed with immobilized cells up to 30 cm height under sterilized conditions. It was operated under optimal growth and immobilization conditions. The column was partially filled by the immobilized beads and the efficient bed heights from 5cm to 30cm were checked and selected for the study. The concentration of the substrate was 500mg/L. The flow rate of 5ml/hour was maintained. At the end of each batch circle, the sample collected from the column was estimated for residual *o*-cresol [5]

### Reusability of agar immobilized P. monteilii SHY cells by repeated batch cultivation

The reusability of the agar immobilized cells was checked by repeated batch degradation experiments. The reactions were carried out in $30^0$C with 500mg/L of o-cresol. After each cycle of incubation (48 h/cycle), the spent solution in the packed bed column was decanted and the agar beads were washed with sterile water and a fresh solution of o-cresol was added. The process was repeated under identical conditions and the spent solution was analyzed for the residual of o-cresol by 4 AAP method [5].

### Detoxification of o-cresol from a water sample

To check the efficiency of *P. monteilii* SHY in treating *o*-cresol contaminated water source, the water body called Kallai river which is used as a soaking yard for wood by many timber industries in and around the Mancave region of Kozhikode was selected. The water in the river is highly polluted with wood preservatives as the wood after adding preservatives containing o-cresol is dumped in the river body for prolonged storage before use.

The *o*-cresol removal efficiency was calculated using the formula:

$$\%\ \text{removal efficiency} = \frac{\text{Initial concentration of } o\text{-cresol (mg/L)} - \text{Final concentration of } o\text{-cresol (mg/L)}}{\text{Initial concentration of } o\text{-cresol (mg/L)}} \times 100$$

The water for analysis (2 L) was collected in sterile bottles and a sample was transferred to the packed bed column set up having immobilized beads of *P. monteilii* SHY cells (50 gm cells having 0.78 mg protein / g of wet beads) and was operated in the optimized conditions formulated earlier. The eluted samples were centrifuged at 15000 rpm for 10 min at $4^0$c.Cell-free supernatants were used to estimate o-cresol (4-Aminoantipyrene method) and COD every 24 hours.

### Determination of COD

COD determines the number of organic pollutants found in surface water and in wastewater. The Chemical Oxygen Demand of the *o*-cresol solution before and after treatment with *P. monteilii* SHY determined to

check the ability of the organism to decrease the pollution load according to IS 3025 (part 58), 2006.[6] The samples were centrifuged at 15000rpm for 10 min at $4^0$c.Cell-free supernatants were used to estimate *o*-cresol and COD. The GC-MS analysis of the sample was also done to check the removal efficiency of the organism.The COD was calculated using the formula:

The COD was calculated using the formula:

$$COD\ (mg\ O_2 / L) = (A - B) \times M \times 8000 / \text{vol of sample}$$

Where, A= volume of FAS used for blank(ml)

B = ml FAS used for sample

M = molarity of FAS

8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

## RESULT AND DISCUSSION

### Removal of *o*-cresol by the immobilized cells through PBCR

The packed bed column with agar immobilized beads of *P. monteilii* SHY cells proved to be highly efficient in removing *o*-cresol from the medium provided with 700 mg/L of *o*-cresol. The removal efficiency is directly proportional to the bed height of the column.[7] (Fig:2), has claimed that the removal efficiency of methanol and toluene was directly proportional to the column heights and has reported the same results. The packed bed column set up used for the treatment process is shown in Figure1. The immobilized agar beads of *P. monteilii* SHY cells in the packed bed reactor were able to remove 99% of *o*-cresol (700mg/L to 7mg/l ) by 7days. Figure 3 shows the residual *o*-cresol concentration at different time duration.

**Fig:1 The packed bed column set up**

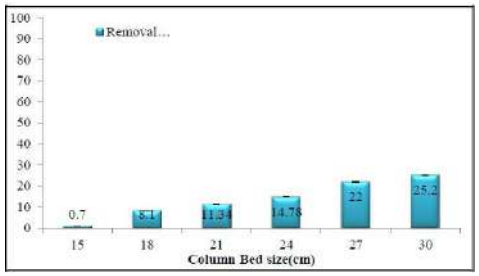


**Fig:2 Removal efficiency of various column bed size**

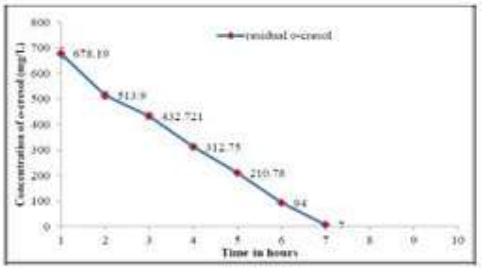



**Fig:3 Residual *o*-cresol in the PBCR set up**

**Table 1. Removal efficiency of *P. monteilii* SHY in PBCR set up**

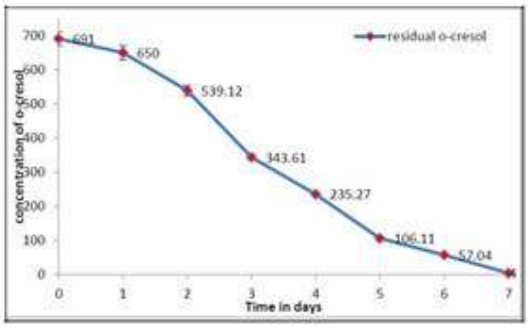
| Number of Days | Residual *o*-cresol | Removal efficiency (%) |
|---|---|---|
| 1 | 678.19 | 3.11 |
| 2 | 513.9 | 26.58 |
| 3 | 432.72 | 38.18 |
| 4 | 312.75 | 55.32 |
| 5 | 210.78 | 69.88 |
| 6 | 94 | 86.57 |
| 7 | 7 | 99 |

Virender Kumar,[8] have reported the increase in biodegradation efficiency of cyanide by *Serratia marcescens* RL2b immobilized in alginate beads in packed bed column. Phenol Degradation in a Packed Bed Reactor by immobilized cells of *P. aeroginosa* MTCC has been reported[9] . The degrading efficiency of the cells is increased in the immobilized stage when used in a packed column set up. The degradation efficiency of the cells was increased far better than in the shake flask. (Table 1).A progressive reduction in removal efficiency may be due to severe mass transfer limitations. Niladevi and Prema,[10] have also employed the use of a packed column for phenol removing and found efficiency increase and the immobilized system maintained 50% of its efficiency after eight successive runs. The decrease in the degradation efficiency might be due to the substrate diffusion limitations in the reactor.[11] This situation may also lead to cell death. The deformation and detachment of cells may be the other reasons for the reduction in the degradation rate.

## Detoxification of o-cresol from a water sample

The packed bed column set up used for river water analysis is shown in Figure 4 The treatment process could remove 99.4 % of the *o*-cresol present in the river water within 7day. The effluent was highly contaminated and the initial concentration of *o*-cresol in the sample was 691.4 mg/L of *o*-cresol and it was reduced to 4 mg/L. (Figure 5.)

**Fig 4. PBCR set up for *o*-cresol removal from river water**




**The residual *o*-cresol in the eluted river water samples**

Not much work has been carried out on the *o*-cresol degradation in a packed bed reactor. The only work reported so far is by Yasin Kaymaz in a continuous packed bed with *Pseudomonas putida* DSM 548 (pJP4). He compared the efficiency between Ca-alginate and pumice immobilized beads and Ca-alginate was found to be more efficient. The degradation rate was found to be reduced after second use and the degradation time required by the cells was 18 hrs. and 22hrs respectively for Ca-alginate beads and pumice beads (Table2)

**Table 2. The residual o-cresol and the removal efficiency of river water in PBCR**

| Days | Residual *o*-cresol | Removal efficiency (%) |
|---|---|---|
| 0 | 691.0 | 1.28 |
| 1 | 650 | 7.14 |
| 2 | 539.12 | 22.9 |
| 3 | 343.61 | 50.91 |
| 4 | 235.27 | 66.39 |
| 5 | 106.11 | 84.8 |
| 6 | 57.04 | 91.85 |
| 7 | 4 | 99.4 |

**Determination of COD**

The organism could reduce the chemical oxygen demand of the solution containing *o*-cresol to the acceptable limit. The treatment time for reducing the COD was directly proportional to the concentration of *o*-cresol. The initial COD was estimated before adding to the PBCR and a 94% reduction in the COD of the sample was found after treatment in the column. Fig: 5. The chemical Oxygen Demand of the water sample was reduced from 1873 mg/L to 107 mg/L which is under permissible limits. GC/MS analysis confirmed the removal of *o*-cresol from the water. (Figure 6)

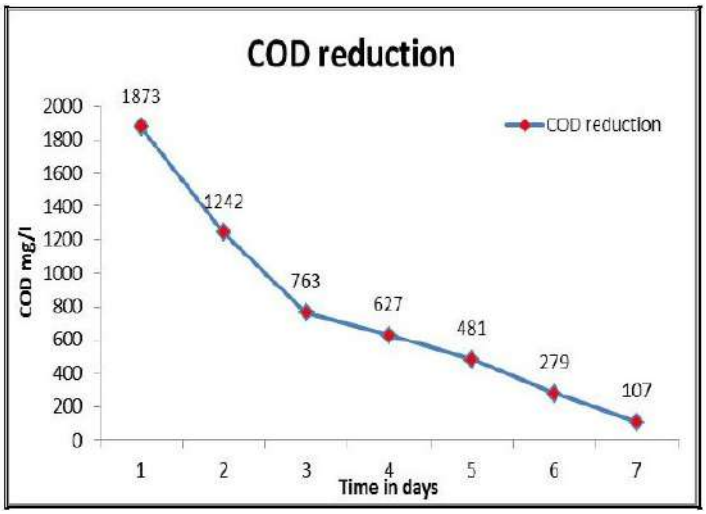



**Fig:5 COD reduction in river water**

## GC-MS ANALYSIS

The continuous reduction in the o-cresol concentration can be observed from the chromatogram. This result provides the concrete evidence that the immobilized beads in the column bearing the *P. monteilii* SHY is highly capable of reducing the substrate concentration from the water sample. The compound *o*-cresol at 15.884 retention time shows the highest peak. GC – MS served as an important tool in confirming the absence of *o*-cresol in the degraded sample. No secondary metabolites were observed in GC analysis which is the same as observed by many.[12,13] (Figure 6). Thus, based on the available information, we could report that cresols degrade rapidly in soils,possibly becoming incorporated into soil microorganisms, without leaving any secondary metabolites.

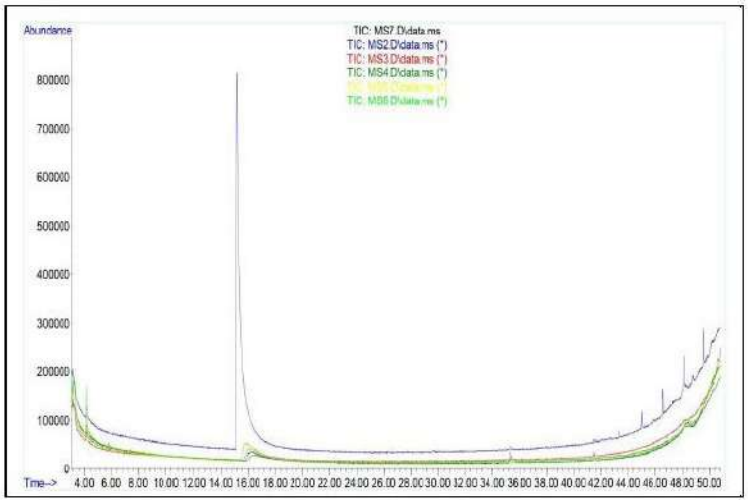



**Figure 7: GC-MS analysis of water samples collected at different time interval of river water treatment**

## CONCLUSION

The novel strain of *P. monteilii* SHY is a highly efficient organism which could remove *o*-cresol from the contaminated water bodies

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SP-54

# STUDIES ON ETHANOL EXTRACT OF RHIZOMES OF*Alpinia galanga* AGAINST HUMAN BREASTCANCER CELL LINE

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## ABSTRACT

Breast cancer starts when cells the breast begin to grow out of content. These cells usually form a tumor that on often be seen on an x-ray or felt as a lump. The tumor is cancer if the cells can grow into invade .surrounding tissues or spread metastatic to distant to the area of the body. Breast cancer occurs almost entirely in women, but men can get breast cancer too. The symptoms of breast cancer, breast pain or a lump, may be caused by normal breast changes or a begin (not cancer) breast cancer in. A change in size or shape, a lump or area that feels thicker than the rest of the breast, Redness or rash on the skin and/ or around the nipple, swelling in your armpit or around your collarbone. Medical treatments for breast cancer are surgery, radiation oncology, and medical oncology. Breast cancer is the second leading cause of cancer deaths among women. Unfortunately, the development of resistance to chemotherapeutic agents is a common obstacle in the treatment of different types of cancers including breast cancer Therefore, in the present study the quercetin, a flavonoid compound was isolated, purified and characterized by the rhizomes of *Alpinia galanga* by column chromatography, TLC, HPTLC, UV, FTIR, $^{1}$H NMR, $^{13}$C NMR, HPLC, FT-IR, preparative TLC, HPTLC. The cytotoxicity studied for MCF-7 human breast cancer cell lines dose-depended inhibition using an in-vitro cytotoxicity effect. The antioxidant activity of the compound quercetin was also examined by using a free radical 1,1-diphenyl-2-picryl hydroxyl (DPPH) scavenging method. From this study, the different concentrations of the extract have potent radical scavenging activity using DPPH as a substrate.The isolated compounds exhibited significant of MTT assay, MCF-7 cell line was treated with different concentration of 20, 40, 60, 80,100µg/ml and incubation for 24hr showed the percentage of cell viability of the cells in a dose depended on manner the inhibition concentration $IC_{50}$value of 58.46 µg/ml, inhibitory activities. The ethanolic extracts of *Alpinia galanga* were showed growth inhibitory activity against *Escherichia coli* (11 mm), *Enterococcus aerogenes* and *Staphylococcus aureus* (10 mm) at concentration 100 mg/ml. The highest activity was demonstrated against *Candida albicans* (11 mm zone of inhibition) at 100 mg/ml, followed by the highest activity against *Candida tropicalis* (0 mm zone of inhibition) at 100 µg/ml). Therefore, it is suggested that the compounds quercetin isolated from the rhizomes of *Alpinia galanga* is a potential source for natural cytotoxicity and antioxidant compounds and could have potential use in the management of anticancer activities.

**KEYWORDS:** Breast cancer, *Alpinia galanga,* FTIR, HPLC, Cytotoxicity

## INTRODUCTION

Breast cancer is the second leading cause of cancer death among women in the US. An estimated 39,620 breast cancer death and 232,340 new cases are expected among women in 2013. Approximately 184,000 women are diagnosed with breast cancer each year in the United States, and 90% of these women will live at least 5 years[1]. The existing cytotoxic agents used for breast cancer treatment are found to be expensive and inefficient because they induce service side effects due to their toxicity in nano cancerous tissue. Now a day's silver nanoparticles are emerging as promising agents for cancer therapy. The biologically synthesized silver nanoparticles have activity against human breast cancer cells[2]. Breast cancer starts when cells the breast begin to grow out of content. These cells usually form a tumor that on often be seen on an x-ray or felt as a lump. The tumor is cancer if the cells can grow into invade .surrounding tissues or spread metastatic

to distant to an area of the body. Breast cancer occurs almost entirely in women, but a man can get breast cancer too. India is a subcontinent with wide ethnic culture religious and economic diversity and variation in the health care infrastructure. Breast cancer is the commonest cancer in urban Indian females, the second commonest. Breast cancer awareness programs are more concentrated in the cities and have not reached the remote and rural parts of the country[3]. Familial and genetic breast cancer in Indian women has to 5%are believed to be hereditary with the BRCR1 and BRCA2 gene mutations having been identified as the major genetic causes in an Indian study on226 breast cancer patients are have a positive family history[4]. Then the TNM staging of breast cancer patients is having a distant metastatic disease at presentation with a higher incidence of skeletal metastases the compounded are vitamin D and calcium intake in Indian women[5.] Systemic and adjuvant chemotherapy regimens are used for the anthracycline-based combinations of first-line chemotherapy for the most patients, doxorubicin cyclophosphamide results in 51.2% and CMP in 40% of patients.[6]. India is a subcontinent with wide ethnic culture religious and economic diversity and variation in the health care infrastructure. Breast cancer is the commonest cancer in urban Indian females, the second commonest, cancer in rural Indian women. Breast cancer awareness programs are more concentrated in the cities and have not reached the remote and rural parts of the country.*A. galangl* is useful against lumbago, rheumatic pains, sore throat, pain in the chest, diabetes, tubercular glands, and disease of the kidney, bronchitis, and catarrhal affections[7]. *Alpinia galanga* in commonly has known as Greater galangal. Its rootstocks are tuberous and slightly aromatic. Leaves are oblong-lanceolate, acute, glabrous, and green above, paler beneath with slightly callus white margins. Sheaths are long and glabrous and ligules are short and rounded. Flowers are greenish-white, in densely flowered, 15-30 cm panicles, bisexual, irregular and bracts ovate-lanceolate. The leaves, flowers, barks and stem of *Alpinia galanga* root are used to treat hypertension, tumor, pain, gastritis, bleeding piles, dysentery, scorpion poison, skin diseases and malaria This medicinal plant was used to treat diseases such as microbial infections, rheumatic pains, chest pain, fever, dyspepsia, kidney diseases, tumors, diabetics and even HIV. And also the plant has a potent role in the treatment of diseases such as eczema, bronchitis, coryza, mobile, pityriasis Versicolor, otitis internal, gastritis, ulcers, and cholera. The seed of this plant is also helpful for use for emaciation and to clean mouth It enhances the digestive power, appetite and also acts as purgative.

## MATERIALS AND METHODS

### COLLECTION OF PLANT MATERIAL

The rhizomes of *Alpinia galangal* were collected in December from the Kolli Hills, Tiruchirappalli, Tamilnadu, India. The plant was identified and confirmed by Dr. S. John Britto, Director, Rapinat Herbarium, St. Joseph College, Tiruchirappalli, Tamilnadu. The voucher specimen number PP001 dated 11.01.2018.

### PREPARATION OF ETHANOL EXTRACTS

The rhizomes of *Alpinia galanga* were washed in running water, cut into small pieces and then shade dried for a week at 35-40˚C, after which it was ground to a uniform powder of 40 mesh size. The methanol extracts were prepared by soaking 100 g of the dried powder plant materials in 1 L of ethanol using a soxhlet extractor continuously for 10 hr. The extracts were filtered through Whatman filter paper No. 42 (125mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure.[8] The final dried samples were stored in labeled sterile bottles and kept at -20˚C. The filtrate obtained was used as a sample solution for further isolation.

### PHYTOCHEMICAL SCREENING TEST OF ETHANALIC EXTRACT OF RHIZOMES OF *ALPINIA GALANGA*: QUALITATIVE ANALYSIS

Phytochemical screenings were performed using standard procedures (Yadav M *et al*., 2014)[9].

### CHARACTERIZATION TECHNIQUES

### ISOLATION OF QUERCETIN BY COLUMN CHROMATOGRAPHY

The condensed ethanol extract of the rhizomes (100 g) of the sample was subjected to column chromatography over TLC grade silica gel. Elution of the column first with n-hexane, and finally with methanol yielded several fractions. The preparation of solvent systems used to obtain quercetin (56 mg/100

g) was ethyl acetate-methanol (80:20) from fraction 5. The compound was detected on TLC plates by spraying with Libermann Burchard reagent and heated at 100°C for 10 minutes. [10]

## PURIFICATION OF ISOLATED COMPOUNDS

### Preparative Thin-layer chromatography (TLC)

The isolated pure compound was dissolved in appropriate solvents. 5 µl of isolated compounds (red fraction) was applied to silica gel plates, Merck (Germany) 20×20 cm, 0.25 mm in thickness. Plates were developed using the solvent system n-Hexane: Ethyl acetate (80:20v\v) for quercetin. The separated zones were visualized with freshly prepared LibermannBurchard reagent and heated at 100°C for 10 minutes. Chromatograms were then examined under daylight within 10Minutes[11].

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### High-performance liquid chromatography (HPLC)

The analytical HPLC system (Shimadzu) was equipped with a diode array detector, a 20µl loop, 200 x 4.6 mm C18 column, methanol (HPLC grade, 0.2mm filtered) used as a mobile phase. The isolated quercetin compound was separated using a mobile phase of methanol: water (75:25 v/v) at a flow rate of 1.0 ml/min, column temperature 30 °C. Injection volume was 40 µl and detection was carried out at 346 nm[12].

### Structural elucidation study of the isolated compound

Different spectroscopic methods including UV, FTIR, $^{1}H$ NMR, $^{13}C$ NMR and GC-MS were used to elucidate the structure of isolated compounds. The UV–visible spectrum of the isolated compounds in methanol was recorded using a Shimadzu 160A UV–visible spectrophotometer. The Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm-1 and a wavenumber range from 400 to 4000 cm-1 using the KBr pellet technique. 1H and 13C NMR spectra were acquired on Bruker WP 200 SY and AM 200 SY instruments (1H, 200.13 MHz; 13C, 50.32 MHz) using TMS as internal standard and CDCL3 as solvent[13].

## ANTIOXIDANT ACTIVITY (DPPH FREE RADICAL SCAVENGING ACTIVITY) DETERMINATION

The antioxidant activity of the ethanolic extract was examined based on the scavenging effect on the stable DPPH free radical activity[14]. Ethanolic solution of DPPH (0.05mM) (300µl) was added to 40µl of synthesized AgNPs aqueous extract with different concentrations (20 - 100µg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Synthesized AgNPs aqueous (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as a percentage of inhibition were calculated according to the following equation[15]. Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100Where B and A are the absorbance values of the test and the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of the sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

## *IN VITRO* ANTICANCER STUDIES OF ISOLATED COMPOUND QUERCETIN

### Cell culture[16]

MCF-7 (human breast carcinoma cells) cell line was cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 ug/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% $CO_2$ at 37°C.

### MTT assay

The isolated compound quercetin was tested for *in-vitro cytotoxicity,* using MCF-7 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured MCF-7 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of $1\times10^{5}$ cells/ml cells/well (200 µ L) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the herbal extract in a serum-free DMEM medium. Each sample was replicated three times

and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were visible under an inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

**Collection of test organisms**

To examine the antimicrobial activity of rhizomes of *Alpinia galanga*, three strains [*Escherichia coli* (MTCC 25922),*Enterococcus aerogenes* (MTCC 29212). *Staphylococcus aureus* (MTCC 27853),] were prepared as test organisms. The clinical fungal test organisms used for the study are *Candida albicans*(MTCC 282), *Candida tropicalis* (MTCC No.184). All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

**Antibacterial activity of ethanolic extract of rhizomes of *Alpinia galanga*(disc diffusion method)**

Antibacterial activity of crude ethanolic extract rhizomes of *Alpinia galanga* was determined using the disc diffusion method. The Petri dishes (diameter 60 mm) were prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six-millimeter widths was impregnated with 10 µl of crude ethanolic extract at various concentrations of 20-100 µg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minutes at room temperature for compound diffusion. Negative control was prepared using the respective solvent. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice[17] (Karumaran *et al*., 2016).

**Determination of the antifungal activity of ethanolic extract of rhizomes of *Alpinia galanga***

Antifungal activity of ethanolic extracts of rhizomes of *Alpinia galanga* was determined using the disc diffusion method The Petri dishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six-millimeter width were impregnated with 10 µl of crude extract at various concentrations of 20-100 µg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minutes at room temperature for compound diffusion. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimetres[18] (Vivek *et al*., 2013).

**RESULTS AND DISCUSSIONS**

The presence of different phytochemical compounds viz, flavonoids, tannins phlorotannins, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, leucoanthocyanin, anthocyanins, anthoquines, proteins, coumarins, glycosides, phenols, alkaloids, xanthoproteins, Modine and carbohydrate were analyzed inethanolic extract of rhizomes of *Alpinia galanga.* The ethanolic extract of rhizomes of *Alpinia galanga* indicated the presence of anthocyanins, saponins, flavonoids, tannin, steroids, terpenoids, cardiac glycosides, leucoanthocyanin, coumarin, glycosides, Modine, phenol, alkaloids, and absence of anthraquinone, carbohydrates, phlorotannins, and protein. (Table-1) Quantitative analysis of important phytochemicals in the ethanolic extract of rhizomes of *Alpinia galanga* contains these phytochemicals in varying amounts in the leaf. The phytochemical with the highest quantity was alkaloids followed by saponin, flavonoids, phenol, tannin and terpenoids respectively. The highest concentration of alkaloids (0.163mg/g), saponin (0.007mg/g), flavonoids(0.015mg/g),phenol(0.046mg/g), tannin(0.055mg/g) and terpenoids (0.004mg/g) .The preparation of solvent systems used to obtain quercetin (46 mg/100 g) was ethyl acetate-methanol (80:20) from fraction 8. The compounds were detected on TLC plates by spraying with Libermann Burchard reagent and heated at 100°C for 10 minutes. Column fractions from 110 to 119 with ethyl acetate: ethanol (80:20) in the TLC mobile phase solvent ratio of chloroform: methanol (1:1) showed Rf value of 0.46 equal to that of standard quercetin. The fractions were then combined and crystallized and the final yield approximately 100 mg. This process was repeated several times by using a bulk quantity of samples until the desired amount of quercetin has been obtained[19] (BharathiSambandam*et al.*, 2016).

**Table:1- qualitative analysis of an ethanolic extract of rhizomes of *Alpinia galangal***

| S.No | Phytochemical Constituents | *Alpinia Galanga* |
|---|---|---|
| 1 | Tannin | +++ |
| 2 | Phlobatannin | - |
| 3 | Saponin | +++ |
| 4 | Flavonoids | ++ |
| 5 | Steroids | +++ |
| 6 | Terpenoids | +++ |
| 7 | Cardiac glycosides | +++ |
| 8 | Leuco anthocyanin | ++ |
| 9 | Anthocyanine | ++ |
| 10 | Anthoquione | - |
| 11 | Protein | +++ |
| 12 | Coumarin | ++ |
| 13 | Glycosidase | ++ |
| 14 | Phenol | +++ |
| 15 | Alkaloids | +++ |
| 16 | Xanthoprotein | +++ |
| 17 | Emodin | +++ |
| 18 | Carbohydrate | - |

***(+ =slightly present, ++ = moderately present , +++ = strongly present)***

A previous study indicates an in vitro study that shows the maximum amount of total flavonoid in *Cyperusrotundus L.* (Cyperaceae). Quercetin (Rf-0.78; UV – fluorescent yellow; Ammonia – deep yellow; FeCl3 – Bluish grey; mp – 309-310°C). The characteristic IR spectral peaks were found to be superimposable with those of their respective standard reference compound of quercetin. *C.rotundus* roots has the highest amount of quercetin[20](Samariya Krishna and SarinRenu, 2013). The previous study shows the absorption peaks positioned at 1612 cm-1, 1516 cm-1 and 1429 cm-1 are assigned to the C---C, C=O and C=C aromatic stretching vibrations respectively. OH, bending vibrations of phenols were observed at 1359 cm-1. The absorption peak at 1315 cm-1 and the peaks at the lower frequencies between 950 cm-1 and 600 cm-1 were assigned to the C-H bending vibrations of aromatic hydrocarbons. C-O stretching vibrations of aryl ether and phenols were observed at 1240 cm-1 and 1210 cm-1 respectively. C-CO-C stretching and bending vibrations of ketones were observed at 1163 cm-1, which confirms that the isolated compound is flavonoid quercetin. This result is in good agreement with the previous literature for the molecular structure of quercetin[19](BharathiSambandam *et al.*, 2016). The previous study shows 1H – NMR spectrum (400 MHz, DMSO – d6, TMS) of the glycoside. The C4' hydroxyl group resonates at d 9.55 ppm. The C3' and C5' protons occur at d 7.01 ppm and the C2' & C6'protons show up at d 7.9 ppm. The protons of C6 & C8 resonate respectively at d 6.16 ppms. The 1H" of glucose resonates at d 5.45 ppm. The 1H" of rhamnose resonates at d 5.45 ppm. The remaining sugar protons appear in the region of d 3.25 ppm to d 3.51 ppm. The rhamnosyl CH3 appears as a doublet at d 0.88ppm (J=7Hz). The various signals noticed the 13C – NMR spectrum (100 MHz, DMSO – d6, TMS) of the glycoside can be assigned to different carbon (Fig-1).

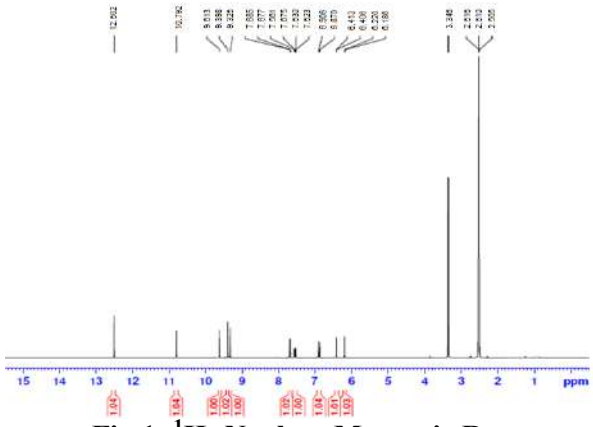


**Fig.1 -$^{1}$H- Nuclear Magnetic Resonance**

The previous study confirms the 13C-NMR spectrum showed carbonyl group at 176.2 ppm and aromatic carbon group from 93.8-164.3 ppm. The peaks present in the NMR spectrum showed resemblance with the pure quercetin. Thus, it can be confirmed that the isolated compound is found to be quercetin[19] BharathiSambandam*et al.*, 2016). The isolated compounds quercetins contain the best antioxidant activity at high concentrations when compared with ascorbic acid. The plant showed 82.25% activity at 100 µl/ml at the same time, ascorbic acid gave 85.03 at the same concentration.The anti-cancer activity of isolated compounds quercetin from *Alpinia galanga* plant extract was assessed by the MTT method. MCF-7 cell line was treated with different concentration of 20,40,60,80,100µg/ml and incubation for 24hr showed percentage of cell viability (graph) of the cells in a dose depended manner(figure:1) the inhibition concentration $IC_{50}$ value of 58.46µg/ml. Who reported the anticancer effects of *D.inoxia* leaf extract on human breast cancer cell line MCF-7. Thus the cytotoxicity study reveals the morphological changes are a consequence of characteristic, molecular and biochemical events occurring in an apoptotic cell. Another hand the cytotoxicity studies showed that the synthesized AgNPs exhibited dose-depended toxicity on human breast cancer (MCF-7) cell lines the $IC_{50}$ value for MCF-7 cells is found to be 5µg/ml of AgNPs, where a 50% noticed cell death is observed[21].(Liu, Q *et al.,*2017).The crude extracts showed growth inhibitory activity against *Escherichia coli* (11 mm), *Enterococcus aerogenes,* and *Staphylococcus aureus* (10 mm) at concentration 100 mg/ml. At concentration 80 mg/ml, the crude extracts exhibited the antibacterial activity all three bacteria but were more susceptible against *Escherichia coli* (10 mm), *Staphylococcus aureus* (10mm), *Enterococcus aerogenes* (9mm). The methanolic extracts were the most effective and the highest activity was demonstrated against *Candida albicans* (11 mm zone of inhibition) at 100 mg/ml, followed by the highest activity against *Candida tropicalis* (0 mm zone of inhibition) at 100 µg/ml).

## CONCLUSION

Thus the present work showed the ability of quercetin which is isolated and characterized by the stem of *Alpinia galanga* shows anticancer activity under *in vitro* conditions and it may reduce the side effect of chemical drugs. More comprehensive studies related to this compound will enhance pharmaceutical exploration in the field of cancer activities.

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SP-55

# NATURAL PREPARATION OF EFFECTIVE MICROORGANISMS FROM FRUITS AND THEIR ANTIMICROBIAL ACTIVITY AGAINST URINARY TRACT PATHOGENS

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## ABSTRACT

The environmentally friendly Effective Microorganisms (EM) technology claims an enormous amount of benefits EM appears to be a safe growth promoter without any associated risk. EM is a fermented mixed culture of naturally occurring species of co-existing microorganisms in acidic medium ($P^H$ below 3.5), normally photosynthesis bacteria (*Rhodopseumonas palustris*, and *Rhodabacter sphacrodes*), Lactobacilli (*L. Plantarum*, *L. casei*, and *Streptomyces lactis*), Yeast (*Saccharomyces cerevisiae*). EM technology has not only helped agricultural but it also assisted in the field of live stocks, the environment, construction, industry and health. Mostly bacteria and fungus were isolated and identified by standard methods. EM solution is standardized into 25, 50, 75 and 100µl of extract. The aim of the project work was planned for natural preparation of effective microorganisms using fruits against urinary tract pathogens.

**KEYWORDS**: IndexTerms - Effective microorganisms, Antimicrobial activity, Urinary tract pathogens.

## 1. INTRODUCTION

Effective Microorganisms strands for (EM). Effective Microorganisms comes in the liquid form and consist of naturally occurring beneficial microorganisms. Effective Microorganism was developed by the University of the Ryukyus, Japan. Approximately 80 different microorganisms are capable to positively influencing decomposing organic substances such that is reverts into a "Life is promoting" process1. EM release antibiotic is called bacteriocins that selectively reduce the growth of other pathogens, including yeast and probiotics. EM can also quickly harmful bacteria or fungal growth and work directly eradicate time. EM produce antimicrobial biochemical that manage damage or kill pathogenic microorganisms2. Urinary tract infection is one of the most common frequently occurring nosocomial infections. Normally UTI is caused by a variety of gram-negative and gram-positive bacteria. The gram-positive bacteria include *Staphylococcus* sp, *Streptococcus* sp. The gram-negative includes a large number of aerobic bacilli such as *E.coli, K. pneumonia, P.aeruginosa*, *Proteus* sp. Among the 80-90% of UTI is caused by *E.coli* and ambulatory patients and of nosocomial infection[3].

## 2. MATERIALS AND METHODS

### *2.1 Fruit samples collection*

The fruit samples were purchased from the Uzhavarsanthai Mannargudi market, Thiruvarur District, Tamil Nadu, India. Fresh fruits were selected. The fruits were covered with polythene bags. These samples are stored in 20˚c until further use.

### *2.2 Preparation of Effective Microorganisms*

Purchase of papaya (1kg), pumpkin (1kg), grapes (1kg) as the equal volume. The ingredients process in to chopped and mixed well and this content mixed with banana (2), cane molasses (1/2 kg), jaggery (100g) egg(1), and neem powder (1/2 kg). 5 liter of distilled water mixed well and tightly sealed. This ingredient is mixed well in twice a day rotating the container. The process is continuing for 45 days. After 45 days the

white layer was formed on the surface. The fermented EM solution was collected by filtered and transferred to a sterile bottle[4].The bottle was stored in the coolest environment available (preferably about 50 days) in a closed container away from the light of the three-element that awakens the dormant microbes, light is the most influential, then heat, then air. To avoid contamination do not use the cap as a serving spoon. Consumed within one month after opening. The product is sterilized and does not contain any live microorganisms.

### *2.3 Extension (EMe) and activation (EMa) of EM stock solution[5].*

Isolation of microorganisms from EM solution. The EM liquid culture containing was isolated as per the standard method.EM stock culture containing a mixture of Lactic acid bacteria- *Lactobcillus casei* ($10^5$), photosynthetic bacteria *Rhodopseudomonas palustris* ($10^1$), and yeast-*saccharomyces cerevisiae* ($10^2$).

### *2.4 Urine sample collection*

The urine sample was collected from a urinary tract infected (UTI) Patient. It is collected in sterile, dry-necked, leak-proof container. About 20 ml of the sample should be collected clean-catch method is used to collected midstream urine. First voided urine is not collected because it is a container with microbes from the lower portion of the urethra. If immediate delivery to be laboratory is not possible, the urine should be refrigerated at $4^0$C. Specimens containing boric acid needed not to be refrigerated[6].

### *2.5 Isolation of microorganisms from the urine sample*

### *Serial dilution[7]*

Serial dilution was performed by using the collected urine sample to isolate the bacteria.1 ml of urine sample was diluted in the tube containing 9 ml of sterile distilled water and mixed thoroughly to make 1:10 dilution ($10^{-2}$).1 ml of diluted sample was transferred to the next tube and serially diluted into the series of the test tubes having 9 ml of sterile pipettes up to$10^{-7}$ dilution. For the isolation of bacteria, the nutrient agar medium was sterilized at $121^0$ C for 15 minutes $P^H$ (6.5±0.2). Petri plates were sterilized and labeled properly .1 ml of samples from $10^{-3}$, $10^{-5}$ and $10^{-7}$ dilution was transferred into respective plates. Finally, the cooling medium was poured into the sample containing. The plates were incubated under the anaerobic condition at $30^0$c for 46-72 hours and the colonies were counted. Different colonies were observed and transferred to other specific media for identification. In the same way was applied the isolation of fungi using rose Bengal agar, potato dextrose agar and sabouraud dextrose agar.

### 2.*6 Identification of bacteria of was done by standard biochemical tests*

Gram 's staining[8]

Motility test[9]

### *2.7 Biochemical test[10]*

### *2.8 Identification of fungi was done by Lactophenol cotton blue technique[11]*

### *2.9 Well diffusion method[12]*

Using the bacterial and fungal culture plates for the well diffusion method. The agar plates were prepared and spread on the specific medium coated agar plates. One well of 5 mm size made in the agar plates with the help cork borer under an aseptic condition in the laminar airflow chamber. The wells were loaded with 100µl of EM extracts. Incubated the plates at 28˚c for 48 hours. Observe the plates after 48 hours for a clear zone around the well. The zones of inhibition were collected by measuring the diameter of the inhibition zone around the well, measure the zone of diameter.

### *2.10 Statistical analysis*

All experiments were carried out in the mean values and standard deviation was calculated. Those data were presented as mean for each sample by using formula[13]

$$\text{Mean} = \bar{X} = \frac{\Sigma X}{N} \qquad \boxed{\text{Mean} = X = \Sigma X}$$

Where, N = Number of observations, Σ = Sum of all the values of variables.

## 3. RESULT

The management of UTI is very important because of the prevalence of the pathogenesis and development of drug resistance caused by the uropathogenic. Due to that our research work is planned to naturally preparation of EM solution and their control measures on UTI. The fruit samples were collected from the Uzhavarsanthai Mannargudi market, Thiruvarur District, Tamil Nadu, India. Fresh fruits were selected. The fruits were covered with polythene bags. These samples are stored in 20˚c until further use.

***Preparation of Effective Microorganisms***

EM solution was prepared by the standard protocol. The process is continuing for 45 days. After 45 days the white layer was formed on the surface. The fermented EM solution was collected by filtered and transferred to a sterile bottle. EM solution was a yellowish liquid with a pleasant odor and sweet-sour taste with a $p^H$ of 3. EM stock solution was stored for 12 months in a cool place without refrigeration.

***Isolation and Identification of Microorganisms from EM Solution***

*Lactobacillus*, photosynthetic organisms, yeast are isolated and identified from the prepared EM solution and their CFU/ml are ($1x10^8$), ($1x10^3$) and ($1x10^6$) (Table1).

***Isolation of Microorganisms from the Urine Sample***

Urine samples were collected from the urinary tract infected patients and pathogens are isolated by using agar medium (bacteria) and potato dextrose agar medium (fungi). The bacteria were isolated in suitable dilution are $10^{-5}$, $10^{-6}$,$10^{-7}$ and the colonies as 25,22,and 15 CFU/ml recorded and fungal colonies as 16,13 and 17 CFU/ml in the dilution of $10^{-3}$,$10^{-4,}$ and $10^{-5}$ from the dilution factors recorded tabulated ( Table 2).

***Identification of Bacteria from Urine Sample***

Five bacteria were isolated and identified based on microscopic evaluation and biochemical characterization. *E.coli* is positive in, motility indole, methyl red, catalase, Triple sugar iron test, and negative in gram stain Voges proskaeur, citrate utilization test, oxidase and urease. *Pseudomonas aeruginosa* showed positive reaction in catalase, oxidase and negative result in gram stain, indole, methyl red, voges proskaeur, citrate utilization test, urease, triple sugar iron test. Whereas *Klebsiella pneumonia* was recorded positive reaction in Voges proskaeur, citrate utilization, catalase, oxidase, urease, triple sugar and negative result in motility, gram stain, indole, methyl red. Likewise *Staphylococcus aureus* showed positive in gram stain, methyl red and negative result in indole, Voges proskaeur, citrate utilization test, catalase, oxidase, urease. *Bacillus* spare positive in gram stain Voges proskaeur, citrate utilization, catalase, oxidase and other negative results recorded respectively (Table 3).

***Morphological and Colonial Characteristic of Fungi***

- Colony morphology of *A.niger*- blackish-brown and morphologically observed as hyphae septate with conidiospore.
- *A.flavus* - conidial head yellow to green and hypaeseptate with conidiospore.
- *A.terrus* - ferruginous to morocco red and hyphae septate with conidiospore.
- *A.fumigatus* - black or green and hyphae septate with conidiospores.
- *Candida albicans*– pink-colored colony and hyphae septate with conidiospores.
- *Saccharomyces cerevisiae*-cream in color and pseudohyphae.

***Antibacterial Activity***

Four different concentration of EM solution was used for antibacterial activity of Urinary tract infected pathogen such as *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus* sp were tested. In 25µl of EM solution was showed maximum zone of inhibition on *E.coli* (7.00±2.31) followed by *P.aeruginosa* (2.67±0.89), *K.pneumoniae* (1.34±0.45), *S. aureus* (2.00±o.67), and *Bacillus* (3.00±0.01) were recorded respectively. The next level of 50µl of EM was showed maximum zone of inhibition on *E.coli* (5.00±1.16) followed by *P.aeruginosa* (2.26±1.0), *k.pneumoniae* (3.34±1.34), *S. aureus* (3.67±1.34) and *Bacillus* (0.00±0.00) were recorded respectively. In 75 µl of EM solution was showed maximum zone of inhibition on *E.coli* (6.00±2.00) followed by *K. pneumoniae* (5.00±1.87), *P. aeruginosa* (4.00±1.34), *Bacillus* (3.67±1.23) and *S.aureus* (2.67±0.87) were recorded respectively. In 100µl of EM solution was showed maximum zone of inhibition on *E. coli* (7.67±2.56), followed by *k. pneumoniae* (6.00±3.00), *P.aeruginosa* (5.00±1.67), *Bacillus* (5.00±1.68) and *S. aureus* (4.00±1.35). Among the dilution, 100µl of EM was effectively control the pathogen *E.coli*> *K.pneumomiae*> *P.aeruginosa*>

*S.aureus*>*Bacillus.* (Table 5).

***Antifungal activity***

A similar observation was revealed by the good diffusion of fungi. In *A. niger* was showed maximum zone of inhibition on 25µl (5.34±1.78), followed by *A. flavus* (1.67±0.56), *A. terras* (2.00±0.67), *A. fumigatus* (0.00±0.00) and *Candida albicans* (4.00±1.34). The next level of 50µl of EM was showed maximum zone of inhibition *A. niger* (6.00±2.00), followed by *A. flavus* (2.00±0.89), *A. terrus* (2.00±0.89), and *Candida albicans* (3.67±1.23) were recorded respectively. In 75 µl of EM solution was showed maximum zone of inhibition on *A.niger* (7.67±2.56) followed by *A. flavus* (2.00±0.62), *A.terrus* (4.00±1.34) and *Candida albicans* (5.00±1.68) were recorded respectively.Among 100µl of EM solution was effectively control the *A. niger*(8.00±2.67)>*A. fumigatus* (5.00±1.67) >*Candida albicans* (4.00±0.62) >*A. flavus* (3.00±1.00) >*A. terras* (1.17±0.56) (Table 6). As in furthermore, EM must suppress the growth of UTI pathogens respectively. This is the first primarily as trail work for the control of UTI pathogens in EM. Probably EM was effectively controlling all the bacterial pathogens than fungi.

**Table-1 Isolation of Microorganisms from EM Solution (CFU/ML)**

| S.NO | EM SOLUTION | NAME OF THE MICROORGANISMS | CFU/ML |
|---|---|---|---|
| 1 | Natural preparation of EM | *Lactobacillus casei* | $1x10^8$ |
| 2 | | *Rhodopseudomonas palustris* | $1x10^3$ |
| 3 | | Saccharomyces *cerevisiae* | $1x10^6$ |

**Table 2: Isolation of Bacteria and Fungi from Urine Sample**

| Name of the sample | Total no. of colonies (CFU/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Bacteria | | | Fungi | | |
| | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| Sample (Urine) | 25 | 22 | 15 | 16 | 13 | 7 |

**Table 3:** Biochemical characterization of isolated bacteria

***Note: (+) positive; (-) Negative***

| S.NO | Test | *E.coli* | *B.subtilis* | *S.aureus* | *P.aeruginosa* | *K.pneumoniae* | *L. casei* |
|---|---|---|---|---|---|---|---|
| 1 | Colony morphology | rod | bacilli | Cocci | Rod | Rod | Rod |
| 2 | Gram staining | G (-) | G (+) | G (+) | G (-) | G (-) | G (-) |
| 3 | Motility test | Motile | Motile | Non-Motile | Motile | Non- motile | Non- motile |
| 4 | Indole test | + | - | - | - | - | - |
| 5 | MR test | + | - | + | - | - | + |
| 6 | VP test | - | + | - | - | + | - |
| 7 | Citrate utilization test | - | + | - | - | + | - |
| 8 | Catalase test | + | + | - | + | + | - |
| 9 | Oxidase test | - | + | - | + | + | - |
| 10 | Urease test | - | - | - | - | + | - |
| 11 | Triple sugar iron test | + | - | - | - | + | - |

**Table- 4** Morphological and Colonial Characteristics Of Fungi

| S.No | Fungal species | Colony morphology | Microscopic observation |
|---|---|---|---|
| 1 | *A. niger* | Blackish brown | Hyphae septate with conidiospore |
| 2 | *A. flavus* | Conidial head yellow to green | Hyphae septate with conidiospore |
| 3 | *A. terrus* | Femuginous to marocco red | Hyphae septate with conidiospore |

| | | | |
|---|---|---|---|
| 4 | *A. fumigatus* | Black or green | Hyphae septate with conidiospore |
| 5 | *Candida albicans* | Pink colored colony | Hyphae septate with conidiospore |
| 6 | *Saccharomyces Cerevisiae* | Cream in color | Pseudohyphae |

**Table-5 Antibacterial Activity of EM Solution**

| Name of the Bacteria | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | 25µl | 50µl | 75µl | 100µl |
| *Escherichia coli* | 7.00±2.31 | 5.00±1.67 | 6.00±2.00 | 7.67±2.56 |
| *Pseudomonas aeruginosa* | 2.67±0.89 | 3.00±1.00 | 4.00±1.34 | 5.00±1.67 |
| *Klebsiella pneumonia* | 1.34±0.45 | 3.34±1.12 | 5.00±1.87 | 6.00±3.00 |
| *Staphylococcus aureus* | 2.00±0.67 | 3.36±1.34 | 2.67±0.87 | 4.00±1.35 |
| *Bacillus* sp. | 3.00±0.01 | 0.00±0.0 | 3.67±1.23 | 5.00±1.68 |

***Values are represented as Mean ± Standard Deviation***

**Table 6: Antifungal Activity of EM Solution**

| Name of the fungi | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | 25µl | 50µl | 75µl | 100µl |
| *Aspergillus niger* | 5.34±1.78 | 6.00±2.00 | 7.67±2.56 | 8.00±2.26 |
| *Aspergillus flavus* | 1.67±0.56 | 2.00±0.67 | 2.00±0.62 | 3.00±1.00 |
| *Aspergillus terreus* | 1.67±0.55 | 2.00±0.89 | 3.34±1.12 | 1.17±0.56 |
| *Aspergillus fumigatus* | 0.00±00 | 2.00±0.87 | 4.00±1.34 | 5.00±1.67 |
| *Candida albicans* | 4.00±1.34 | 3.67±1.23 | 5.00±1.68 | 4.00±0.62 |

Mean values are represented as Mean ± Standard deviation

## 4. DISCUSSION

In the present investigation suggested that the natural preparation of Effective microorganisms as an antimicrobial agent for UTI. In this study were reported that agreed to[14] antibacterial activity of EM health drink was analyzed against intestinal pathogens such as *E. coli* and *B. subtilis*. Aqueous extract of health drink and medicinal plant against *E. coli* and *B. subtilis* showed minimum activity (3.1mm±4.21) at 25% concentration and maximum activity at a 100% level. Maximum antibacterial activity was observed in aqueous extract of health drink against all tested bacteria namely *E. coli* and *B. subtilis*. In our study, 100µl EM solution effectively controls urinary tract pathogen such as *E. coli*, and *B. subtilis*. In *E. coli* showed some of the inhibition (7.00±2.31), at 25µl concentration and *B. subtilis* showed minimum activity (3.00±0.01) at 25µl concentration and maximum suppressed lead of inhibition at (5.00±1.68) at 100µl.In our results were correlated to the finding stated that [15] *E. coli* was the most predominant species were isolated from cases suffering from urinary tract infection patients. The isolated *E. coli* was identified by microscopic examination and biochemical reactions. The most frequent isolated species from UTIs patients was *E. coli* (43.9% positive in urine samples). In our study, *E. coli*, *K. pneumoniae*, *S. aureus*, and *P. aeruginosa* was the most predominant species that were isolated from UTI patients. These organisms were identified in the microscopic examination and biochemical reactions. The UTI pathogen was 50% positive result in urine samples.Scientists reported that effect of EM a health of lay hens commercial layer hens were since feed control 1,2,3 % of EM for 1-2 weeks. It did not influence body weight but increase egg production in birds given feed contains 1 at 2% of EM. Reports revealed that the effect of EM (Bokashi R) multi microbial probiotic preparation in the nonspecific immune response in pigs. It increases phagocytic activity in the experimental group.

## SUMMARY AND CONCLUSION

The aim of the study is related to the natural preparation of Effective Microorganisms using different kinds of fruits like banana, papaya, pumpkin, grapes, egg, cane molasses and neem powder, jaggary were collected

from uzhavarsanthai mannargudi market, Thiruvarur district. The duration of EM fermentation was 45 days. After than extension and activation of EM also done. The urine samples were collected from UTI patients. The identified bacteria such as *E. coli*, *K. pneuminiae*, *P. pseudomonas* and *S. aureus* and fungi like *A. niger*, *A. fumigatus*, *A .terrus*, *A. flavus* and *Candida albicans* recorded respectively. EM solution was prepared in 25µ, 50µl, 75µl and 100µl well diffusion was performed for the identified bacterial and fungal pathogens. Moreover, the antimicrobial of Urinary Tract Pathogen *E. coli* and *A.niger* was effectively controlled by the 100µl of EM solution. Therefore the purpose of the study is to be a plan that EM is a safe product, this technology is to be applied of the invitro controlling of pathogens that is clinically important. EM is the most popular microbial technique now used in the worldwide. EM consists of mixed culture of beneficial and naturally occurring microorganisms that promote in a way as excellent multi-dimensional probiotic. EM is safe to use become they are harmless are they 100% organic, not chemically synthesized. Also EM not ate a drug. EM consists of ray different kinds of effects and disease suppressing microorganisms. Future studies may also be needed to study the approval of the government quality standards parameter as biochemical digestion and oral administrative of EM against pathogens under clinical trial and compared with an antibiotic sensitivity pattern also.

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SP-56

# EFFICACY OF LIGNIN DEGRADED COMPOSTS ON GROWTH AND YIELD OF *Abelmoschos esculentus* (L).

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## ABSTRACT

The most efficient lignin degraders are Basidiomycetous, white and brown rot fungi which are taxonomically so close to each other that both types may sometimes appear in the same genus. In this present study, the compost materials such as cow dung degraded lignin compost, vermicompost degraded lignin compost, mushroom spent waste degraded lignin compost were collected from Biominin Laboratory, a unit supported by S.T.E.T Biofloral product Research and Development Centre, S.T.E.T Women's College, Mannargudi. The physicochemical parameter such as pH, Carbon, Potassium, Phosphorous, Temperature, biomass, Nitrogen, Electrical conductivity was tested before and after treatment. The seedling of *Abelmoschus esculentus* was transplanted in trial pots of equal size, which was noted as (T1), (T2), (T3). The uninoculated pot was denoted as control (T4). The morphological parameter such as Height of the plant, No.of leaves, No.of roots, Branches, Shoot length, Root length, leaves width, No. of flowers No. of fruits and biochemical constituents such as chlorophyll, Carbohydrates, Protein, Carotenoids content were analyzed at different intervals ($15^{th}$, $30^{th}$, and $45^{th}$ days) respectively, Among the overall treatment cow dung showed better performance than other treatments. In this study, to prevent environmental pollution from extensive application of chemical fertilizers. Lignin degraded compost could be recommended to farmers to ensure public health.

**KEYWORDS**: Lignin degradation, Cow dung, Mushroom spent waste, Vermicompost.

## INTRODUCTION

Lignin is the most common aromatic organic compound found in the lignocelluloses component of the plant cell wall. It's the characteristic ability to absorb UV (ultraviolet) radiation makes it susceptible to degradation, on being exposed to sunlight[1]. The source of lignin in the soil can be of plant origin or lignocellulosic waste from the food processing industry. It's represented a significant part of plant litter input (approximately 20%) into the soil. Lignin is anamorphic three – dimensional polymer composed of phenylpropanoid subunits. It acts as a binding material and is involved in cross-linking of cellulose that provides extra length, rigidity, and stiffness to the cell wall. Lignin protects plant cells from enzymatic hydrolysis and various other environmental stress conditions. The complex structure of lignin makes it recalcitrant to most degradation methods and continues to pose a critical challenge[2]. The most efficient lignin degraders are basidiomycetous, White and Brown rot fungi which are taxonomically so close to each other that both types may sometimes appear in the same genus[3]. However less than 10% of wood degrading basidiomycetous fungal species are brown rot fungi[4] wood-rotting Ascomycota and Deutromycotina, i.e., microfungi are considered to be soft rot fungi, while Basidiomycota are either white rot or brown rot fungi, [5&6] Cow manure can be used more directly to fertilize individual plants. It consists of three basic elements critical to plants to produce the protein needed to build living tissue for green stems, strong roots and a lot of leaves. Vermicompost is earthworm excrement, called castings, which can improve the biological, chemical,and physical properties of the soil. The Mushroom spent waste contain sufficient nutrients and generally nontoxic materials to the plant growth and made it feasible to be used as biofertilizer. The main components of MSW to use it as fertilizer are calcium, nitrogen, ash, and protein[7]. Lignin directly or indirectly influences soil microbial community structure, which in turn control soil, retaining soil nutrients, promoting soil aggregate formation, which reduces soil, erosion, bioremediation and detoxification of natural and man-made organic pollutants[8]. Therefore the present study was planned to improve the growth and yields *Abelmoschous esculentus* (L) using lignin degraded compost materials.

## 2.MATERIALS AND METHOODS

### *2.1 Collection of soil and composts*

The soil samples were collected from the S.T.E.T Herbal Garden,Sundarakkottai village,Mannargudi taluk,Thiruvarur district, Tamilnadu India.The lignin degraded compost material such as cow dung, vermicompost,and mushroom spent waste was obtained from the biominin laboratory of the S.T.E.T Biofloral products Development and Research Centre, S.T.E.T Women's college, Sundarakkottai, Mannargudi Taluk,Thiruvarur District.

### *2.2 Pot culture method*

The designed treatment pots were filled with the following materials namely, lignin degraded compost of cow dung, vermicompost, mushroom spent waste, sterilized garden soil. They are mixed with 1:1 ratio the pots were provided with water facilities, control was maintained without fertilizer.

### *2.3. Pot method*

The pot culture study was conducted to find out the growth parameters of the Okra (*Abelmoschus esculentus* (L).) plants and the effect of various substrates. The seeds were soaked in water overnight and the 8 seeds were sown in each treatment. The garden soil was sterilized in an autoclave at 15 lbs. Pressure for half an hour. The pH of the soil was adjusted to 7. And the pot was filled sterilized soil with lignin degraded compost of cow dung, vermicompost, and mushroom spent waste. When the plant grew randomly 3 seedlings from each treatment were uprooted without disturbing the root system and different parameters such as plant height, shoot and root length, several leaves and branches, leaf breadth, no of flowers, no of fruits, were measured 15$^{th}$, 30$^{th}$, and 45$^{th}$ day to observe the plant growth.

### *Treatments*

The pots were maintained in the open shade at the temperature of 27°C - 30°C. The ladies' finger plant" (*Abelmoschus esculentus" (L))* was treated with fertilizers.

T1 : Control without any treatment
T2 - Lignin degraded compost using Cow dung
T3 - Lignin degraded compost using Vermicompost
T4 - Lignin degraded compost using Mushroom spent waste.

### *2.4 Physicochemical analysis*

The analysis of soil samples for various Physicochemical parameters were studied using standard methods. The pH, temperature, moisture, electrical conductivity, nitrogen, phosphorous, potassium, carbon, content were assessed by electrometric and turbidity methods respectively.

### *2.5 Estimation of micronutrients*[9]

**Preparation of extractant solution**

Weighed 1.865 gm of DTPA (Diethylenetriamine Penda acidic acid) 0.005M
Plus 14.9 gm (13.1 ml) of triethanolamine – 0.01 M
Plus 14.7 gm (14.7 gm) of calcium chloride – 0.01 M, in a 1000ml volumetric flask and makeup to 1 liter with double distilled water.
Adjust the pH of the solution to 73 by using distilled HCL.
Preparation of distilled HCL
1 litre of double distilled water was taken 650 ml of conc. HCL (AR) in a round bottom flask. Then boil it, collect the distillate completely.

**Procedure**

10gm of soil sample was weighed in a 125 ml polythene conical flask.
20 ml of DTPA extractant was added to that soil sample and shake it for 2 hours.
The soil sample was filtered through Whatman 40 (or ) 41 filter paper.
The readings were taken in atomic absorption spectrophotometer (AAS) after following the usual procedure for operating the instrument.

Estimation of Fe, Mn, Zn, and Cu calculated by the following formula,

$$\text{ppm in soil} = \text{ppm solution} \times \frac{\text{Vol.of extract}}{\text{wt .of soil taken}}$$

### *2.6 Isolation of microorganism from the pot culture*

**Serial dilution**[10]

Microbiological analysis of the soil was also carried out to analyze the soil was carried out to analyze the soil quality. Serial dilution was performed by using the collected sample to isolate the bacteria. 1gram of soil sample was diluted in the tube containing 9 ml of sterile distilled water and mixed thoroughly to make 1:10 ($10^{-2}$). 1ml of diluted sample was transferred to the next test tube and serially diluted into the series of test tube having 9 ml of sterile pipettes up to $10^{-7}$ dilutions.For the isolation of bacteria, the nutrient agar medium was sterilized at 121°C for 15 minutes, pH (6.5± 0.2). Petri plates were sterilized and labeled properly. 1 ml of sample from $10^{-5}$, $10^{-6}$ dilutions was transferred into the respective plates. Finally, the cooling medium was poured into the sample containing plates. The plates were incubated under the anaerobic condition at 30°C for 48-72 hours and the colonies were counted. Different colonies were observed and transferred to other specific media for identification. In the same way was applied the isolation of fungi, the sample from the dilution $10^{-3}$ and $10^{-4}$ using sabouraud dextrose agar, potato dextrose agar.

**Identification of bacteria**

The identification of bacteria was done by the standard biochemical methods[11]

**Identification of fungi**

The identification of fungi was done by the standard methods[12]

### *2.7 Seed treatment*[13]

The (L.) "*Abelmoschus esculentus*" (Ladies finger) seeds were soaked in water for overnight.

### *2.8 Seedling treatment*[14]

The seeds were sown in the pots, at the depth of 2cm in each pot. After 5days, the seeds were germinated. The weeds were removed regularly and watering was done at regular intervals. After 15 days once, the plant growth parameters were analyzed and observed.

### *2.9 Seeds germination*[15]

Seedling growth was recorded on the 7 days after planting by counting the seedlings the percentage of seedling growth was determined of 7, 14,21. After planting, the number of seedling growth was counted and calculated by using the formula,

$$\text{Germination (\%)} = \frac{\text{number of seed germinated} \times 100}{\text{Number of seed sown}}$$

### *2.10 Morphological parameter*

After 15th, 30th,45th days of growth, the plants per pot were removed from all samples and studied for the following morphological parameters. They were,

- **Height of the plant (in cm)**
- **Number of leaves (per plant)**
- **Number of roots (per plant)**
- **Branches**
- **Shoot length (in cm)**
- **Root length ( in cm)**
- **Branches**
- **Leaves width (in dm)**
- **Number of flowers**
- **Number of fruits**

### *2.11 Microbial load*

A set of serial dilutions is made a sample of each is placed into a liquefied agar medium, and the medium poured into a Petri dish. The agar solidifies, with the bacteria and fungi cells locked inside the medium. Colonies grow with the agar, as well as a top of the agar and below the agar (between the agar and the lower dish). The procedure described above produces a set of pour plates from many dilutions, but spread plates (sample spread on top of the solidified agar) can be used also. The agar plate allows accurate counting of the microorganisms.

$$\textbf{Colony-forming unit} = \frac{\text{Number of colony} \times \text{Dilution factor}}{\text{Volume of sample}}$$

### *2.12 BIOCHEMICAL ANALYSIS*

Biochemical content such as chlorophylls, carotenoids, protein, carbohydrates were analyzed from the plants.The biochemical contents were analyzed by using the standard methods[16]

### *2.13 Statistical analysis*[17]

The decomposed materials of fertilizer are utilized by plants, and the growth and development and its parameters are measured by statistical analysis. The growth parameter such as root length, shoot length. Height of plants, no, of branches, leaves colour . All the experiments were reported as triplicates. The result obtained in the present investigation were subjected to statistical analysis like mean X and standard deviation (SD).

Mean (X) = sum of value of the variable
N = Number of observation

Where, together with X and $\bar{X}$. Divide the total by the number of observations. The standard deviation (SD) was calculated by a formula. The formula for calculating

$$\text{Standard deviation (SD)} = \sqrt{\Sigma(X - \bar{X}/N}$$

Where, $\bar{X}$ = Arithmetic mean
X = No of all values
N = total number of obser

## 3.RESULTS

Lignin biodegradation is responsible for much of the natural destruction of wood in use and creates substantial economic losses. Furthermore, lignin degradation may cause a role in plant pathogenesis. The efficacy of lignin degraded substrate materials was used for the treatment of *"Abelmoschous esculentus"* (L) ladies finger under pot culture treatment, microbial populations, physicochemical properties of the soil before and after treatment and a biochemical constituent of the plants were also analysed. 4 treatments are designed T1 ( Lignin degraded compost using cow dung ), T2 (Lignin degraded compost using vermicompost), T3 (Lignin degraded compost Mushroom spent waste), T4 (control).

### *3.1 Analysis of physicochemical parameters*

The physicochemical parameters such as pH, Temperature, Moisture, Electrical conductivity, Nitrogen, Phosphorus, Potassium and Carbon were tested before and after inoculation and the above said parameters were increased after fertilizer inoculation treatment. (Table – 1)

### *3.2 Biochemical characteristics of isolation and identification of microorganism from the soil.*

**Serial dilution**

A serial dilution technique was used to isolate the Bacteria and Fungi. Gram staining, Motility test and Biochemical tests were Indole, MR – VP, Citrate utilization test, Oxidase test, Catalase test. Lactophenol cotton blue was used to identify the fungi. The bacterial species are identified as their Morphological characters, Biochemical and Bergey's manual of determinative bacteriology. The bacterial species are *E.Coli, Pseudomonas sp., Bacillus sp., Azotobacter sp., Streptomyces sp., Staphylococcus sp., Klebsiella sp.,*

**Isolation of fungi**

Five different species were observed from the soil treatment. The colonies showed a characteristic color of green, yellow, grey, brown, white and they were confirmed by identifying their morphological characters and by using manual such as manual of soil fungi (Gillman, 1957), Dematiaceous Hypomycetes (Ellis,1971), Hyphomycetes (subramaniam, 1971).

***Identification of microorganism***

**Identification of bacteria**

Totally 8 bacterial species were isolated namely, *E.Coli, Pseudomonas sp., Bacillus sp., Azotobacter sp., Streptomyces sp., Staphylococcus sp., Klebsiella sp., Enterobacter sp.,*

G $(+)^{ve}$, Rod-shaped, Non-motile organism showed positive results for MR-VP and Catalase tests and negative results for indole, citrate and urease was identified as *Bacillus sp.,*

G$(-)^{ve}$ Rod-shaped, motile organism showed a positive result for MR-VP and catalase tests and a negative result for indole, MR-VP and urease was identified as *Pseudomonas sp.*.

G$(-)^{ve}$ rod-shaped, motile organism showed a positive result for catalase, citrate and VP tests and a negative result for indole, MR-VP and urease tests was identified as *Enterobacter sp*., G$(+)^{ve}$ , rod-shaped, motile organism showed positive results for Catalase, Citrate, Urease and Indole tests and negative results for MR-VP tests was identified as *Azotobacter sp.,*

G$(+)^{ve}$ cocci shaped, nonmotile organism showed a positive result for catalase, citrate, urease and VP tests and negative result for indole, MR and VP test was identified as *Streptomyces sp.,*

G$(-)^{ve}$ rod shaped, nonmotile organism showed a positive result for catalase, citrate, urease and VP tests and negative results for Indole and MR tests was identified as *Klebsiella sp.,*

**Identification of fungi**

Totally 5 fungal species were isolated namely *Aspergillus sp., Rhizopus sp., Pleurotus sp., Trichoderma sp., p.chrysogenum.*Septate hyaline hyphae, conidiospore, conidia globose and light green and yellowish colonies were identified as *Trichoderma sp.,* Hyphae meet they are joined by cramp connection, haploid basidiospore, conidia globose, white or more pale often lilac-grey colonies were identified as *Pleurotus sp.,* Septate branching, sporanjospore, large sporanjium and wooly white to yellow ten turns to a darker brown to black colour colonies was identified as *Aspergillus sp.,*Aseptate , conidiospore, conidio globose and white and fluffy in appearance sporangia turns blackish, brown was identified as *Rhizopus sp.,*Vegetative hyphae, conidiospore, club-shaped smoothy spores and white colour cylindrical to ellipsoidal shape was identified as *P.Chrysoporium.*

***3.3 Analysis of morphological parameters***

**Height of the plants**

In 45$^{th}$ day, cow dung(T1) increased height of the plant (58.6±9.8 cm) followed by vermicompost(T2) and control.(Table -2).

**Number of leaves**

In 45$^{th}$ day, the same result was obtained in cow dung (T1) applied pot was showed a better response in the number of leaves of plants (14.6±0.5). vermicompost (T2) applied plant was exhibited better response in the number of leaves of plants(13.3±0.4) followed by(T3) mushroom spent waste degraded lignin compost and (T4) control.(Table -2).

**Number of roots**

In the 45$^{th}$ day, the same result was also obtained in mushroom spent waste degraded lignin substrate (T3) was showed a better response in the number of roots of plants ( 60±16.6). cow dung degraded lignin substrate(T1) plant was exhibited better response in the number of roots of plants ( 56.6±6.2)followed by other treatments and control. (Table-2).

**Number of branches**

In the 45$^{th}$ day, among the overall treatments cow dung degraded lignin compost (T1) was showed a maximum response in branches in plants (14±0.77). mushroom spent waste lignin degraded compost (T3) was showed a better response in branches of the plants.(13.3±0.46) followed by other T2 and T4. (Tables - 2).

**Shoot length**
In the 45th day, among the overall treatments cow dung degraded lignin compost (T1) showed a maximum response in shoot length of plants(52.6±2.04 cm). In vermicompost degraded lignin compost (T2) was exhibited better response in shoot length of plants (48±8.03 cm).(Tables – 2).

**Root length**
In 45th day, among the overall treatments, mushroom spent waste degraded lignin compost (T3) was showed a maximum in root length of plants (17±1.4 cm). cow dung degraded lignin compost (T1) was shown a better response in the root length of the plants.(17±0,77 cm) followed by other treatments and control.(Table-2)

**Leaves width**
In the 45th day, cow dung degraded lignin compost (T1) was shown a better response in leaves width of plants (22.5±0.77 dm). Mushroom spent waste degraded lignin compost (T3) was showed a better response in leaves width of plants (19.3±1.7 dm) followed by other treatments and control.(Table- 2)

**Number of flowers**
Overall treatments in the 45th day, mushroom spent waste degraded lignin compost (T3) was showed a maximum response in the number of flowers in plants (2.3±1.2). cow dung degraded lignin compost (T1) was showed a better response in the number of flowers of plants(2±0.77) followed by (T2) Vermicompost degraded lignin compost and (T4) control.(Table- 2)

**Number of fruits**
In the 45th day, cow dung degraded lignin compost (T1) was showed a maximum response in the number of fruits in plants (7±0.77). mushroom spent waste degraded lignin compost (T3) was shown a better response in the number of fruits of plants(6.6±0.4) followed by (T2) Vermicompost degraded lignin compost and (T4) control. (Table-2)

***3.4 Biochemical analysis***
**Chlorophyll content**
In the 45th day, among the overall treatments, cow dung degrades lignin compost (T1) was showed higher content of chlorophyll a (0.65±0.65 mg/g), chlorophyll b (0.06±0.076mg/g) and total chlorophyll (0.05±21 mg/g) of plants. Next, mushroom spent waste lignin degraded compost (T3) was exhibited better response in chlorophyll a (0.54±0.63 mg/g), chlorophyll b (0.04±0.02 mg/g) and total chlorophyll content (0.026±0.16 mg/g) followed by other treatments and control. (Table-3)

**Carbohydrate content**
In the 45th day, among the overall treatments, cow dung degraded lignin compost (T1) was showed better response in the carbohydrate level of plants (7.75±0.67 mg). Mushroom spent waste degraded lignin compost treatment (T3) was shown a better response in carbohydrate of plants (7.75±0.67 mg) followed by other treatments and control. (Table-3)

**Protein content**
Cow dung degraded lignin compost (T3) was showed higher activity in the protein content of plants (5.64±0.06 µg). Mushroom spent waste degraded lignin compost (T3) was showed higher activity in protein content of plants (4.43±0.42µg) followed by other treatments and control. (Table -3)

**Carotenoids content**
In the 45th day, among the overall treatments, cow degraded lignin compost (T1) was shown a better response in carotenoids of plants (0.59±0.76 mg). Vermicompost degraded lignin compost (T2) was shown a better response in carotenoids of plants (4.43±0.042 mg) followed by other treatments and control.(Table-3)

***3.5 Microbial population***
Before the treatment of soils contains, bacterial populations are ($1.7 \times 10^{-3}$), ($1.3 \times 10^{-3}$) , ($1.6 \times 10^{-3}$), ($1.4 \times 10^{-3}$) CFU/g and, fungal population are ($6.9 \times 10^{-4}$), ($4.8 \times 10^{-4}$), ($5.7 \times 10^{-4}$), ($4.0 \times 10^{-4}$) CFU/g .After the treatment of soils, bacterial populations are ($2.8\times 10^{-3}$), ($2.4 \times 10^{-3}$), ($2.6 \times 10^{-3}$) ($1.2 \times 10^{-3}$) CFU/g and

fungal populations are ($8.0 \times 10^{-4}$), ($5.2\times10^{-4}$), ($6.2\times10^{-4}$), ($5.0\times10^{-4}$) CFU/g. Hence our study was highlighted that cow dung degraded lignin compost, mushroom spent waste degraded lignin compost and vermicompost degraded lignin compost could enhance the morphological parameters such as the height of the plant, number of leaves, number of roots, branches, shoot length, root length, leaves width, the number of flowers number of fruits and biochemical constituents such as chlorophyll, carbohydrate, protein, carotenoidswere increased. ,The comparatively maximum level of increase in the above said parameters. T1-(Cow dung degraded compost), showed better efficiency than others.

***Table 1: Physicochemical parameters of soil***

| S.no | Physicochemical parameters | TREATMENTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BEFORE TREATMENT | | | | AFTER TREATMENT | | | |
| | | T1 | T2 | T3 | T4 | T1 | T2 | T3 | T4 |
| 1. | pH | 6.4 | 5.9 | 6.0 | 5.4 | 7.2 | 6.3 | 6.9 | 5.4 |
| 2. | Temperature(°C) | 8.0 | 7.5 | 7.9 | 6.0 | 8.5 | 8.0 | 8.0 | 6.0 |
| 3. | Electrical conductivity | 1.2-1.8 | 0.7-1.2 | 1.1-1.5 | 1.0-1.1 | 1.4-2.3 | 0.9-1.5 | 1.4-1.9 | 1.0-1.1 |
| 4. | Moisture | 58 | 54 | 55 | 50 | 64 | 54 | 62 | 50 |
| 5. | Nitrogen(ppm) | 88.7 | 86.6 | 87.5 | 80.5 | 89.7 | 87.4 | 88.6 | 80.5 |
| 6. | Potassium(ppm) | 37 | 30 | 35 | 29 | 40 | 35 | 39 | 40 |
| 7. | Phosphorous(ppm) | 76 | 70 | 74 | 69 | 80 | 66 | 84 | 69 |
| 8. | Carbon | 0.75 | 0.50 | 0.74 | 0.49 | 0.96 | 0.65 | 0.89 | 0.50 |

***Table2-***
***Effect of lignin degraded substrate on morphological parameters ofAbelmoschous esculentus(L)***
**($45^{th}$ Day)**

| Treatment | Height of the plant(cm) | No.of leaves (per) | No.of roots (per) | Branch | Shoot length (cm) | Root length (cm) | Leaves width (dm) | No.of flowers | No.of fruits |
|---|---|---|---|---|---|---|---|---|---|
| **T1** | 58.6±9.8 | 14.6±0.5 | 56.6±6 | 14± 0.77 | 52.6±2.04 | 17±0.77 | 22.5±0.7 | 4±0.77 | 7±0.77 |
| **T2** | 41.3±11.6 | 11±0.77 | 50.6±2 | 11±0.77 | 48±8.03 | 16±0.77 | 12.6±1.2 | 3.3±0.8 | 6±0.77 |
| **T3** | 46±3.0 | 13.3±0.4 | 60±16.6 | 13.3±0.4 | 46±7.9 | 17±1.4 | 19.3±1.7 | 5±0.77 | 6.6±0.4 |
| **T4** | 45±3.25 | 7.6±1.7 | 46±8.3 | 10±0.77 | 40±1.8 | 12.6±1.7 | 9.6±1.24 | 3.3±0.8 | 5±0.77 |

***Values are triplicates, mean ± standard deviation***

***Table 3- Biochemical constituents in Abelmoschus esculentus(L)***
*($45^{th}$ day)*

| Treatment | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Total chlorophyll (mg/g) | Carbohydrate (mg) | Carotenoids (mg) | Protein (µg/g) |
|---|---|---|---|---|---|---|
| **T1** | 0.65±0.65 | 0.06±0.076 | 0.05±0.21 | 7.75±0.67 | 0.59±0.76 | 5.64±0.06 |
| **T2** | 0.43±0.49 | 0.03±0.89 | 0.038±0.18 | 4.54±0.039 | 0.39±0.75 | 3.49±0.032 |
| **T3** | 0.54±0.63 | 0.04±0.072 | 0.026±0.16 | 5.68±0.042 | 0.47±0.93 | 4.43±0.042 |
| **T4** | 0.12±0.19 | 0.01±0.035 | 0.019±0.04 | 0.18±0.54 | 0.13±0.43 | 2.65±0.021 |

## SUMMARY AND CONCLUSION

In this present study, the fertilizers such as cow dung degraded lignin compost, vermicompost degraded lignin compost, mushroom spent waste degraded lignin compost were collected from Biominin Laboratory, a unit supported by S.T.E.T Biofloral product Research and Development Centre, S.T.E.T Women's College, Mannargudi. The physicochemical parameter such as pH, Carbon, Potassium, Phosphorous, Temperature, biomass, Nitrogen, Electrical conductivity was tested before and after treatment. The seedling

of *Abelmoschous esculentus* were transplanted in trial pots of equal size, which was noted as (T1) – Cow dung degraded lignin compost, (T2) – Vermicompost degraded lignin compost, (T3) – Mushroom spent waste degraded compost, (T4) – Control. The uninoculated pot was denoted as control. The morphological parameter such as Height of the plant, No.of leaves, No.of roots, Branches, Shoot length, Root length, leaves width, No.of flowers No.of fruits and biochemical constituents such as chlorophyll, Carbohydrates, Protein, Carotenoids content were analyzed at different intervals ($15^{th}$,$30^{th}$, and $45^{th}$ days) respectively. The result of this study clearly showed that cow dung degraded lignin compost was more effective than treatment. Besides, Mushroom spent waste degraded compost to increase the rate of organic matter mineralization to results in an increase in plants available nutrients. Composting is an alternative technology for sustainable solid waste management. cow dung degraded lignin compost can be used to promote soil fertility and soil quality, enhance crop yield and quality accelerates the production of quality fertilizer by promoting the decomposition of waste and inorganic matter used in agriculture and lower the hazards of continued cropping in an open and greenhouse environment. To prevent the environmental pollution from extensive application of chemical fertilizer, the Cow dung degraded lignin compost and mushroom spent waste degraded lignin compost could be recommended to farmers to ensure the public health. Further study is to need in terms of field trials.

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SP-57

# BIOSYNTHESIS OF ZINCOXIDE NANOPARTICLES USING *TRICHODERMA* SP. AND ITS ANTIFUNGAL ACTIVITY

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## ABSTRACT

Synthesis of nanomaterials by advanced Green Nanotechnology method with well-defined dimensions and high monodispersity is to use of microorganisms. The present study reports the synthesis of zinc oxide nanoparticles using fungus *Trichoderma* sp. The production of zinc oxide nanoparticles was confirmed by the formation of white aggregates of zinc oxide nanoparticles. The optical properties of nanoparticle was analyzed by UV Vis Spectroscopy. Fourier transform infrared spectroscopy is used to characteristic functional groups of nanomaterial. Particle size and morphology were analyzed using Scanning Electron Microscopy. The antifungal activity of zinc oxide nanoparticles against *Sarocladium oryzae* was studied using a well diffusion method.

**KEYWORDS**: Nanoparticle, *Trichoderma* sp., Zinc oxide, *Sarocladium oryzae*, UV-FTIR, SEM

## INTRODUCTION

Fungal growth and pathogenicity are the main cause of considerable economic loss in the paddy field. Today several synthetic fungicides have been found to cause adverse effects to humans and the environment and for this reason, many of them have been banned. It is difficult to control fungal growth because fungi have developed resistance to many conventional fungicides such as benzimidazoles and dicarboximides[2]. To overcome resistance, it is important to explore novel antifungal agents, which may replace current control strategies. In recent years, nanoparticle (NP) materials have received increasing attention due to their unique physical and chemical properties, which differs significantly from their conventional counterparts[18]. Synthesis of nanoparticles employing microorganisms has attracted much due to their usual optical, chemical, photoelectron chemical and electronic properties and many biological organisms, such as bacteria, fungus, yeasts and plants either intra or extracellular[1]. In agriculture, zinc compounds are mainly used as fungicides[20]. The 50% lethal dose (LD50) of oral toxicity for ZnO is relatively high reaching 240 mg/kg for rats[17]. It is believed that smaller sizes of ZnO, correlated with its stronger antimicrobial activity [21]. However, to the best of our knowledge, the effect and mode of action of ZnO NPs on the growth of fungi *Trichoderma harzianum* have not been studied. Therefore, in the current study the antifungal activity of ZnO NPs against plant pathogenic fungi, *Sarocladium oryzae* was investigated.

## MATERIALS AND METHODS

### Isolation and identification of the fungal isolate

*Sarocladium oryzae* and *Trichoderma harzianum* were isolated from different paddy field soil samples of Thanjavur district. The pure culture was maintained on PDA media (Hi media Laboratories Ltd. Bombay, India) at 25±1°C. Identification of the fungal isolates was carried out by morphological and microscopic examination using the identification keys of (3, 19 and 4)

### Synthesis of nanoparticle

Nanoparticles can be synthesized chemically or biologically. The development of these eco-friendly methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology especially Zinc nanoparticles, which have many applications.

**Metal tolerance profiles of *T. harizianum***

A maximum tolerable concentration assay was performed to determine the zinc metal tolerance ability of *T.harzianum*. The experimental plates were prepared by supplementing the PDA medium with varying amounts of zinc acetate to obtain final conc. of $Zn^{+2}$ ions range of 100, 200, 300, 400 500µg $ml^{-1}$. Plates without $Zn^{+2}$ ions were used as control. Inoculums of test fungi ($10^6$ CFU $ml^{-1}$) were spotted on the media surface of each plate. After inoculation, the plates were incubated at 28•C for 4 days under dark conditions and examined the fungal growth. The experiment was done in triplicate. The maximum concentration of $Zn^{+2}$ ions in the medium which allowed the growth of a fungus[7].

**Extracellular synthesis of zinc oxide nanoparticles**

*Trichoderma harzianum* was selected for the reduction of zinc oxide nanoparticles. The *T.harzianum* was inoculated in liquid media containing (g/l) $KH_2PO4$, 7.0., $K_2HPO4$, 2.0., $MgSO_4.7H_2O$, 0.1., $(NH_4)2SO_4$, 1.0., Yeast extract, 0.6., glucose, 10.0. The flask was incubated at 25•C for 3 days in a rotary orbital shaker at a speed of 150rpm. The biomass was harvested after 72h of growth by sieving through a plastic sieve. The biomass was washed with sterilized distilled water to remove any medium component. 20g of biomass (fresh weight) was minced with 200ml of deionized water in a 500ml Erlenmeyer flask and agitated in the same condition for 72h at 25•C. After incubation, the cell filtrate was obtained by passing it through Whatman filter paper no.1. The filtrate was collected and used for further studies[12]. Aqueous Zincoxide conc. 1.0mM was added to flasks containing 100ml of fungal cell-free filtrate and incubated for 72h under the same conditions as described above. Controls containing fungal cell-free filtrate (without zinc oxide; positive control) and pure zinc oxide solution (without fungal cell-free filtrate; negative control) were also run simultaneously along with experimental flasks in three replicates. The viability of the fungal cells after incubation in Mili-Q deionized water for 72 h was also checked.

**Characterization of synthesized zinc nanoparticles**

Three different spectroscopical techniques were used to characterize the zinc oxide nanoparticle. They include absorption UV-Visible light spectroscopy, FTIR and SEM analysis.

**UV-Visible and FTIR spectroscopic analysis**

The reduction of zinc ions was confirmed by qualitative testing of supernatant by UV-Visible spectrophotometer between 400-600nm and FTIR spectra were recorded in the range 4000-450$cm^{-1}$, to identify the possible biomolecules responsible for the reduction of the metal ions by the cell filtrate. 1ml of sample supernatant was withdrawn after 24h and absorbance was measured.

**Scanning Electron Microscope (SEM)**

The ZnO NP sample and freeze-dried sample was sonicated with distilled water; a small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of $10^{-5}$ torr. The accelerating voltage of the microscope was kept in the range 10-20KV and was used to characterize the mean particle size, the morphology of nanoparticles.

**Antifungal activity of Zinc oxide nanoparticles**

The antifungal activity of nanoparticle compounds was tested against *Sarocladium oryzae* by using a good diffusion method. An aliquot (0.02ml) of inoculums was introduced to molten PDA and poured into a Petri dish by pour plate technique. After solidification, the appropriate wells were made on the agar plate by using a sterile cork borer. 0.05ml of each nanoparticle synthesizing compounds were added into separate well 28•C for 48h. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the nanoparticle synthesis compounds.

**RESULTS**

Cultural characteristics comprising growth rate, colour and colony appearance were examined. On PDA, *T. harzianum* formed 1-2 concentric rings with green conidial production. The conidia production was denser in the center than towards the margins. Colonies growing rapidly, moist to slimy, pink or orange; reverse remaining colourless or turning pink to orange. Conidiophores simple, occasionally branched. Phialides slender, arising from submerged or slightly fasciculate aerial hyphae for

*Sarocladium oryzae*. *T.harzianum* has 90% metal tolerance ability was selected for further studies on the extracellular synthesis of ZnO nanoparticles. The appearance of yellow colour, indicate the formation of zinc oxide nanoparticle in the reaction mixture during the period of incubation. The control showed no colour change of the mixture when incubated in the same conditions. In the UV-Visible spectrum, a strong peak was observed at 220nm-300nm which indicated the presence of Zinc Oxide nanoparticle which was also supported by FTIR (Fig.1). The ZnO particles produced by the biological method were observed by SEM which revealed that the particles were more or less spherical and the size of the particles ranged from 60-75nm, which was absent in the case of control. ZnO nanoparticles were exhibited in a maximum inhibition zone (19.8±1.25mm) against *Sarocladium oryzae* and control. Since, the antifungal activity of the ZnO nanoparticles was concentration-dependent, due to the rupture of the fungal cell membrane resulting in the possible decrease in fungal enzymatic activity (Table.1).

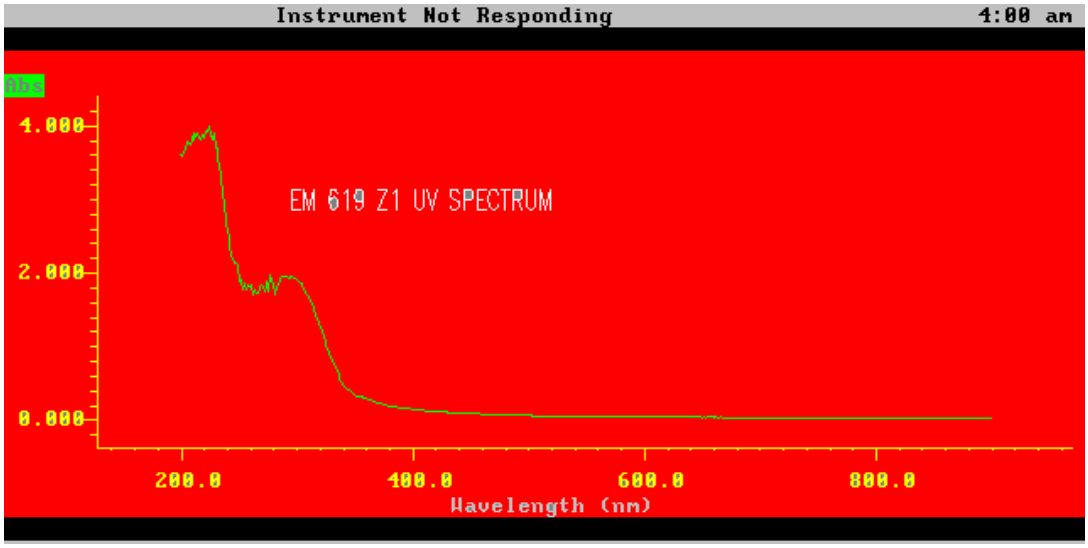


**Fig:1 UV-λmax of the nanoparticle zinc oxide synthesised by *T.harzianum*.**

**Table 1: Antifungal activity of Nanoparticles against *Sarocladium oryzae***

| S.No. | Sample | Zone of inhibition (mm) |
|---|---|---|
| 1. | Control (AgNo3 without culture) | 5.1±1.75 |
| 2. | Control (ZnO without culture) | 5.1±1.75 |
| 3. | Standard (*T.harzianum* culture filtrate) | 9.8±1.75 |
| 4. | Nano ZnO | 19.8±1.25 |

## DISCUSSION

In the UV-Visible spectrum, a strong peak was observed at 220-300nm was indicated the presence of zinc oxide nanoparticles, can be attributed to a band-to-band emission of ZnO which represents a direct bandgap of 3.3 e.V(Prasad and Hha, 2009). The presence of a broad absorption peak in FTIR spectra indicated the presence of ZnO nanoparticles of varying sizes. The main goal of FTIR is to determine the chemical functional groups from the sample. Hence, the present study revealed the antifungal compounds from *Trichoderma* sp. Similarly, several workers have been reported [14,11,15 and 6] Zinc is an essential micronutrient for plants. Plants generally absorb Zn as a divalent cation ($Zn^{2+}$), which acts either as the metal component of enzymes or a regulatory cofactor of a large number of enzymes. Based on an analysis of 298 soil samples collected from different countries in the world, Zn deficiency is the most widespread micronutrient deficiency[16]. In India, Zn is now considered the fourth most important yield-limiting nutrient after nitrogen, phosphorus, and potassium.Particle size may affect the agronomic effectiveness of Zn fertilizers. Decreased particle size results in an increased number of particles per unit weight of applied Zn (10). Since granules of 1.5mm weigh less than granules of 2 or 2.5mm, smaller granules were used for the same weight, resulting in a better distribution of Zn, and the higher surface area of contact of Zn fertilizer resulted in better Zn uptake[9,13]. ZnO nanoparticles were found to maximum inhibition zone (6.1±1.04) on fungal pathogen

*S.oryzae* cause sheath rot disease in paddy was compared to the control. Closely, related work was reported by[5] have investigated the antifungal activities of ZnO nanoparticles against two important postharvest fruit diseases fungi namely *B.cinerea* and *P.expansum*. The mode of action of ZnO nanoparticles on the growth of fungal hyphae was studied by SEM and Raman spectroscopy. ZnO nanoparticles at a concentration greater than 3mmol-1 can significantly inhibit the development of *B.cinerea* and *P.expansum*. The nanoparticles studied were more effective against fungi[8].

## CONCLUSION

In the present study concluded that ZnO NPs synthesizing compound was effectively controlling the growth of *S.oryzae* and also increase the yield of the rice plant.

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SP-58

# ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI FROM MARINE ASSOCIATED PLANT (*AVICENNIA MARINA*)

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## ABSTRACT

Endophytic fungi were isolated from mangrove plants of *Avicennia marina* in Sethubavasathiram southeast coast of India. The new mode of actions creating a need for the discovery of novel products for human health and welfare. Researchers have shown a new untapped area where fungal endophytes have come at a glance due to the broad range of hosts in biodiversity. About 16 different fungal species were isolated by using the PDA medium and identified by using Standard Manual. The present review explores various sources and prospects of endophytic fungi.

**KEYWORDS:** *Avicennia marina*, endophytic fungi, biodiversity sustainability, PDA

## INTRODUCTION

*Avicennia marina*, commonly known as grey mangrove or white mangrove, is a species of mangrove tree classified in the plant family Acanthaceae. (Formerly in the Verbenaceae or Avicenniaceae). It is more highly valued for timber, firewood, charcoal, dye and tannin production. Research has shown that several medically active components are present in the plant including iridoid glucosides, flavonoids and naphthoquinone derivatives. Some of these have shown strong antiproliferative and moderate cytotoxic activities, as well as antibacterial effects and yet others, produced compounds that can be utilized industrially, such as enzymes and solvents[1]. Microbes that spend at least part of their life cycle inside the healthy plant tissues without causing disease symptoms are called 'endophytes'[2]. Association of Endophytic fungi with mangrove plants confers protection from adverse environmental disasters. India is considered Karnataka, Tamil Nadu, Bihar and Uttar Pradesh have great biodiversity for endophytic fungi[4]. Hence, our study was aimed to isolate endophytic fungi from *Avicennia marina* of leaves, roots and stem. The present review article is about sources of Endophytic fungi and their prospects.

### Classification of Endophytic fungi

There are two major groups of endophytic fungi have been reported based on evolutionary relatedness, taxonomy, plant hosts, and ecological functions (Table: I). The *Clavicipitaceous* endophytes (C-endophytes), which infect some grasses; and the *nonclavicipitaceous* endophytes (NC-endophytes), which can be recovered from asymptomatic tissues of nonvascular plants such as ferns and allies, conifers, and angiosperms[4,5&6].

Apart from these two main groups, there are four classes of endophytic fungi
I) Class I Endophytic fungi
II) Class II endophytic fungi
III) Class III endophytic fungi
IV) Class IV endophytic fungi

### These four classes are explained as follows.

The Class I Endophytic fungi are Clavicipitaceous endophytes of grasses which were first reported by European investigators in the late 19th century from seeds of *Lolium temulentum, Lolium arvense, Lolium linoleum,* and *Lolium remotum*[5]. From their earliest discovery, investigators hypothesized a link to toxic syndromes experienced by animals that consume infected tissues. However, these hypotheses were largely untested until[6]. linked the endophyte *Neotyphodium coenophialum* to the widespread occurrence of summer

syndrome toxicosis in cattle grazing tall fescue pastures (*Festuca arundinaceae*). As this hypothesis became widely known, investigations on endophyte natural history, evolution, ecology, and physiology followed author[1].Class II endophytic fungi consists a diversity of species, prominently related to the Dikaryomycota (Ascomycota or Basidiomycota) although, mostly belonging to Ascomycota and Basidiomycota. The Basidiomycota includes Agaricomycotina and Pucciniomycotina. A *Phoma* sp. in *Calluna vulgaris* was first reported by Rayner in 1915 as an II class of endophytes. These species are always colonized in all the parts of the plant including the seed coat. According to a recent analysis, *Phoma* sp. are common root endophytes that confer fitness benefits to plants.During the 20th century, brown alga *Ascophyllum nodosum* was reported which requires the fungus *Mycophycia.* The Class III endophytic fungi are differentiated based on their occurrence i.e. above-ground tissues; the formation of highly localized infections; horizontal transmission; the potential to confer benefits on hosts that are not necessarily habitat-specific; and extremely high in plant biodiversity. This class includes the hyperdiverse endophytic fungi associated with leaves of tropical trees[7&8] as well as above-ground tissues of nonvascular plants, seedless vascular plants, conifers, and woody and herbaceous angiosperms in biomes ranging from tropical forests to boreal and Arctic/Antarctic communities[9]. These Class of endophytes is found in flowers and fruits, in asymptomatic wood and inner bark[10]. The Endolichenic fungi were also found under class III endophytes. Scientist[11] recovered more than 80 endophyte species from *Juniperus communis* in Switzerland Author[12] isolated 78 species from leaves and twigs of *Quercus petraea* in Austria. Like, Class II endophytes, the majority of the Class III endophytes are members of the Dikaryomycota (Ascomycota or Basidiomycota), with inference to Ascomycota. Members of the Basidiomycota belonging to the Agaricomycotina, Pucciniomycotina and Ustilaginomycotina[13].The Class IV endophytes were revealed during the study of ectomycorrhizal fungi when[14] observed a brown to blackish, pigmented fungus associated with terrestrial plant roots called MRA. These species were often found with mycorrhizal fungi and referred to as *Pseudomycorrhizal* fungi[14]. Presently, these fungi are referred to as dark septate endophytes (DSE) and are grouped as Class IV endophytes. These Class IV endophytes are distinguished upon a functional group, based on the presence of darkly melanized septa, and their restriction to plant roots. However, almost a century after their discovery, little is still known about the role of ismysterious and rather elusive fungal symbionts[5].

## MATERIALS AND METHODS

### Collection of plant material

Endophytic fungi were isolated from the mangrove plant of *Avicennia marina* collected from Sethupavasathiram – Pattukottai, Tamil Nadu, India. Three parts of the plant such as leaf, stem, and root (each a wedge of 0.5 $cm^2$ of the epidermal tissue along with about 0.3–0.5 cm of internal tissue and mucilage) were cut off with an ethanol-disinfected cutter. Each part was placed separately in sterile polythene bags to avoid moisture loss. The materials were transported to the laboratory within 12 h and stored at 4°C until isolation procedures were completed. Fresh plant materials were used for isolation work to reduce the chance of contamination.

### Isolation and Identification of Endophytic Fungi

The samples were washed thoroughly with sterile distilled water. The materials were then surfaces sterilized using ethanol 75% (1 min), 0.5% sodium hydrochloride (3 min) and rinsed thoroughly with sterile distilled water. The effect of the surface sterilization procedure was ascertained for every segment of tissue by imprint method[15]. The samples were ground using sterile mortar. 1 g of samples was put in 9 mL Nafis and vortexed. Furthermore, 1 ml of aliquot was plated onto potato dextrose agar (PDA; 12 g Difco potato dextrose broth, 20 g agar/L, with streptomycin 100 mg/L) using spread plate technique. The plates were then incubated at room temperature until fungal growth appeared (1-3 weeks). Each fungal colony was transferred into PDA for purification of fungal strain and mounted on a clean slide with Lactophenol cotton blue[16]. The fungal isolates were identified based on their morphological and reproductive characters (Arunachalam and Gayathri, 2010) and with the help of the Standard Manual[17&18].

## RESULTS AND DISCUSSION

The Discovery of endophytic fungi in plant tissues opened up new possibilities in the search for metabolically active compounds. Recently studies have been carried out about endophytic biodiversity, taxonomy, reproduction, host ecology and their effort on host[19&20]. The endophytes were isolated using a mycological medium namely Potato dextrose agar (PDA). In our study, a total of sixteen strains were obtained from the leaves, stems and roots of *Avicennia marina* (plate:1;Fig:1). Endophytic fungi can be found in almost all plant parts. Each part of the plant generally is found at least two colonized with different endophytic fungi species (Table 1). The total number of colonies of the endophytic fungal stem are less than leaf or root. Root showed in great quantities of fungal colonies. The endogenous fungal population is much in roots may reflect the fact that the root is the major site where microorganism access to the plants. The presence of *Penicillium* sp. in root mentioned that the soil fungal could distribute to new habitat such as plants because the origin habitat of *Penicillium* is the soil. The occurrence of fungal endophytes such as *Aspergillus* sp. and *Penicillium* sp. from *Avicennia marina* which is early revealed by authors[21].The root is part of the plant that showed the transition of microorganism as its root has more fungal species. This result was related to plant protection from diseases caused the extreme environment. Endophytes with mangrove roots confer protection from adverse environmental conditions and allow them to compete with saprophytic fungi that decompose aging roots.

## CONCLUSION

Mangrove endophytic fungi continue to be a prolific source of unique secondary metabolites with engrossing structural features a significant number of which reveal promising biological activities. A rich fungal species is yet to be discovered and investigated over the coming years. Extending the biological screens used in natural product studies to extend over the commonly tested antimicrobial activities will increase the chance to discover bioactive drug leads with medicinal potential. This could provide a framework for future natural product production.

## ACKNOWLEDGMENT

We dedicate this paper to my Research Supervisor who persisted in studying endophytes and allured me to the topic.

**Table .1 Shows Endophytic fungi isolated from *Avicennia marina*.**

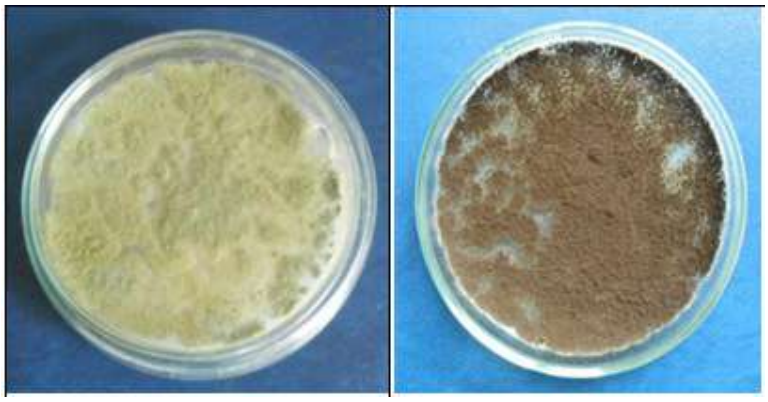
| S.No. | Name of the fungi | Parts of plant | | |
|---|---|---|---|---|
| | | Root | Stem | Leaf |
| 1. | *Aspergillus flavus* | + | - | + |
| 2. | *Aspergillus niger* | - | + | + |
| 3. | *Aspergillus terreus* | + | + | + |
| 4. | *Aspergillus sydowii* | - | - | + |
| 5. | *Aspergillus sulphurus* | + | - | + |
| 6. | *Aspergillus awamori* | + | + | + |
| 7. | *Penicillum citrum* | + | + | + |
| 8. | *Penicillus crsogenum* | - | + | + |
| 9. | *Penicillum lanosome* | + | - | + |
| 10. | *Cladosporiums p* | + | + | - |
| 11. | *Fusarium oxysporum* | + | + | + |
| 12. | *Fusarium solani* | + | - | + |
| 13. | *Daldinia* sp. | + | + | - |
| 14. | *Rhizopus* sp. | + | - | + |
| 15. | *Mucor* sp. | + | + | - |
| 16. | *Verticillium* sp. | + | + | - |

**Table. 2 Shows cultural characteristic on PSA of the isolated species**

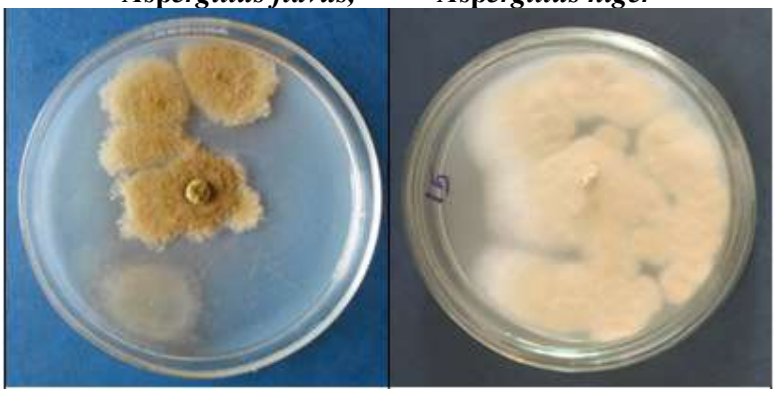
| S.No. | Name of the fungi | Upper surface | | | Lower surface | Observations |
|---|---|---|---|---|---|---|
| | | Cultural aspects | Density | Colour | | |
| 1. | *Aspergillus flavus* | Effuse, floccose | Medium | Conidial heads yellow to green | Idem to upper face | Hyphae, septate with Conidiophore |
| 2. | *Aspergillus niger* | Effuse, globose/flococcos | High | Blackish brown; Phialides | Idem to an upper face | Hyphae, septate with ascospore |
| 3. | *Aspergillus terreus* | Effuse, floccose | High | Pinkish-Cinnamon, deeper brown shades | Idem to upper face except for the colour (pale or bright yellow to deep brown) | Hyphae, septate with Conidiophore |
| 4. | *Aspergillus sydowi* | Effuse, floccose | Medium | Velvety, Bluish-green | Idem to upper face except for the color (orange to red later black) | Hyphae, septate with Conidiophore |
| 5. | *Aspergillus sulphurus* | Effuse, floccose | Medium | Dirty white with yellow spore at the centre | Idem to upper face except for the color(slightly radially furrowed colour) | Hyphae, septate with ascospore |
| 6. | *Aspergillus awamori* | Effuse floccose High Dull yellow-brown Fading of the textile color Hyphae | Septate with conidiophore | Effuse floccose High Dull yellow-brown Fading of the textile color Hyphae | Septate with conidiophore | Hyphae, septate with ascospore |
| 7. | *Penicillum citrum* | Effuse globose | High | Bluish-green to clear green, becoming olive to brownish-olive | Dull blue-green to yellow-green | Aerial hyphae with conidiophores |
| 8. | *Penicillum chrysogenum* | Effuse globose | High | Bluish-green with Yellowish pigment | Idem to an upper face | Branched conidiophores, relatively short phialides and smooth to finely roughened conidia |
| 9. | *Penicillum lanosum* | Effuse, floccose | High | Lanose, white with center and form gray-green | Idem to the upper face (slightly yellow) | Conidiophore, compact vertical of Phialides |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10. | *Cladosporiums* sp. | Effuse, globose to ellipsoid | Medium | olivaceous-brown in colour | Idem to the upper face | Conidiophore, branched, septate, and dark |
| 11. | *Fusarium oxysporum* | Effuse, globose | High | Brownish white to violet | Idem to upper face except for the colour (Carmine to yellow) | Oval to reniform chlamydospores |
| 12. | *Fusarium solani* | Effuse low floccose | High | White to cream | Idem to upper face (chocolate colour in center) | Conidiophores branch into thin, elongated monophialides that produce conidia. |
| 13. | *Daldinia* sp. | Effuse, globose | Medium | Purple, brown, or silvery-black inside | Black – gray colour | Septate conidiophores irregularly branched into mononymous, dichotomous or trichotomous structure |
| 14. | *Rhizopus* sp. | Effuse, cotton like | Light | White at first, become bluish black at maturity | Idem to an upper face | Rhizoids rare; sporangiophores and stolons branched. |
| 15. | *Mucor* sp. | Effuse, globose | High | Grey to brown | Idem to the upper face | Sparsely septate, sporangiospores, branched and form apical, globular sporangia |
| 16. | *Verticillium* sp. | Effuse low floccose | Medium | Yellowish, red, pinkish-brown or green | White or brown (rust color) | Conidiophores are hyaline, simple or branched |

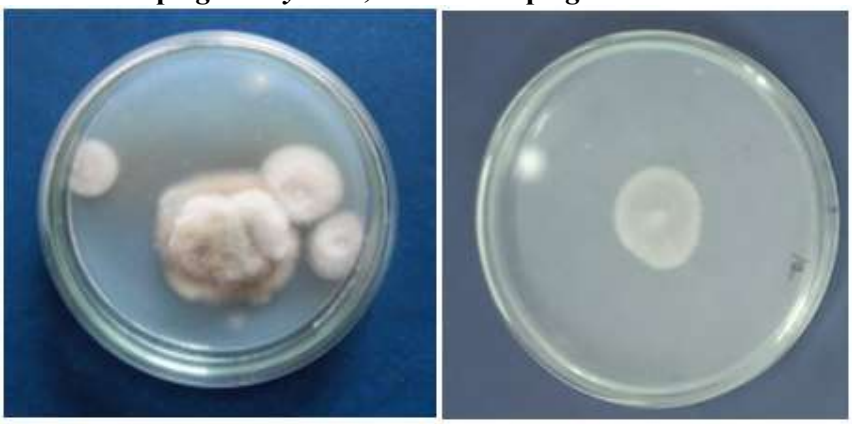
**Plate 2: Pure culture of endophytic fungi**



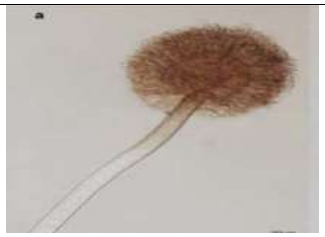
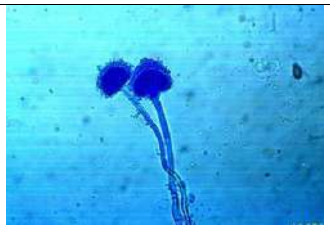
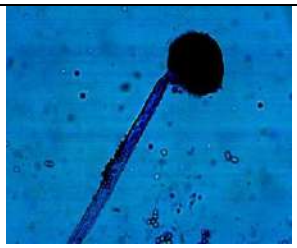
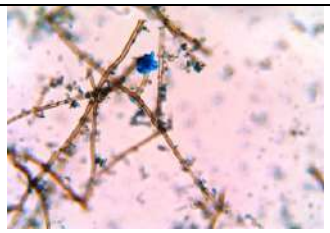
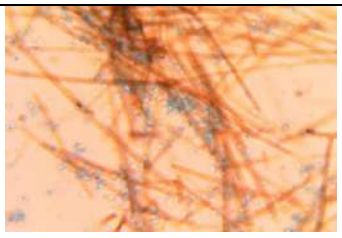
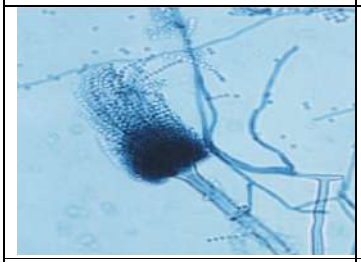
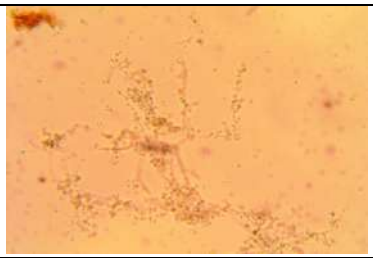
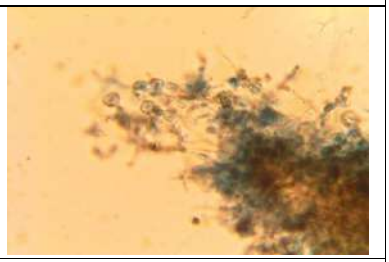
***Aspergillus flavus,*** ***Aspergillus niger***



***Aspergillus sydowii,*** ***Aspergillus terreus***



***Aspergillus sulphureus,*** ***Aspergillus awamorie***

| | | |
|---|---|---|
|  |  |  |
| *Aspergillus* sp | *A.awamori* | *A.flavus* |
|  |  |  |
| *A.niger* | *A.terreus* | *Alveophoma caballeroi* |
|  |  |  |
| *Cladosporium* sp | *Cylindroxyphium virginianum* | *Cytosporella sycina* |

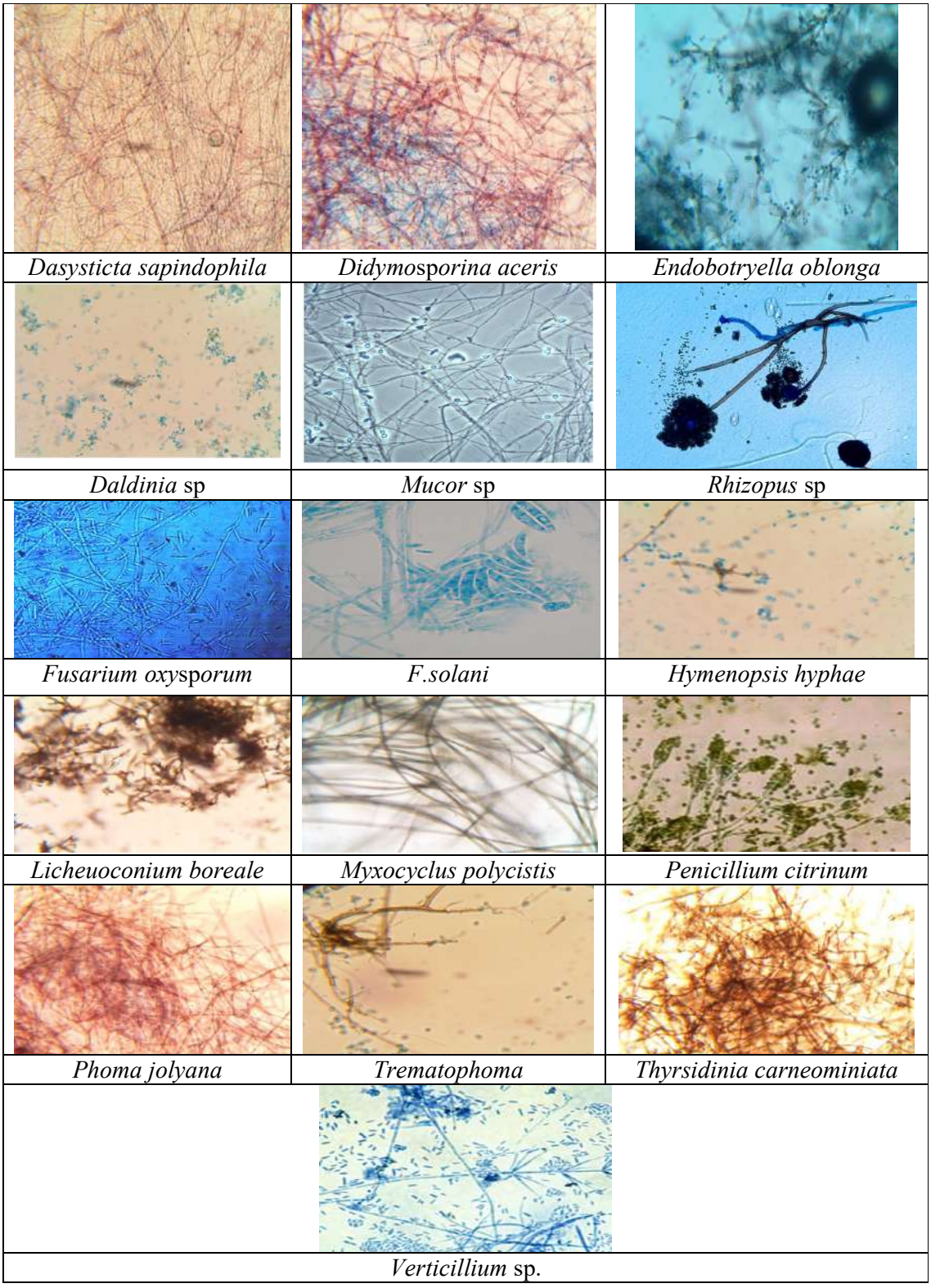



**Plate 3: Microphotography of endophytic fungi**

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SP-59

# BIOCONTROL OF AFALOTOXIGENIC *Aspergillus flavus* IN THE FEED MAIZE BY VARIOUS PLANT ESSENTIAL OIL

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## ABSTRACT

Today, the industry caters mainly to the dairy and poultry sectors. Most fungal contaminants in stored feed materials usually arise from infestations that began in the field, although some can directly infest storage grains as well when conditions are right. In the present investigation includes evaluating the incidence of *Aspergillus flavus* and multitoxin in different maize sample and study the effect of essential oils against the aflatoxigenic*Aspergillus flavus*. A total of 10 maize samples were collected from different Poultry mills in Namakkal District, Tamilnadu. In the maize samples, *A.flavus*was isolated in high number, followed by *A.niger, A. fumigatus*.. Among the 15 *Aspergillus flavus*isolates, 8 isolates were identified as toxigenic *A. flavus* which produce bluish-green fluorescent area surrounding colonies. The practical importance of botanical pesticides based on essential oils shows a rising tendency every year, by the development of environmental trends and integrated management, gaining ever more ground in the control of harmful fungi.

**KEYWORDS**: Afalotoxigenic, *AspergillusFlavus* , Poultry feed, Maize , Various Plant Essential Oil

## INTRODUCTION

The term poultry used as synonymous with chicken includes several avian species such as chicken, duck, turkey, geese, and guinea-fowl domesticated for economic purposes. Poultry is now a very important and widespread agricultural industry in the tropics[1]. Poultry provides protective food in human nutrition in the form of egg, meat and employment opportunities at various [2]. Poultry is also efficient converters of feed into the animal protein of high biological value. Poultry's feeding is one of the most important branches of poultry science since feed cost alone accounts for 60-65 % of the total farm expenses [3]. Most fungal contaminants in stored feed materials usually arise from infestations that began in the field, although some can directly infest storage grains as well when conditions are right [4]. The term mycotoxin means poison from fungi. Among the thousands of species of fungi, only about 100 are known to produce mycotoxins. These fungal toxins are chemically diverse representing a variety of chemical families and range in molecular weight from about 200 to 500. There are three major genera of fungi that produce mycotoxins: *Aspergillus*, *Fusarium* and *Penicilium*. and although between 300 and 400 mycotoxins are known, those mycotoxins of most concern, based on their toxicity and occurrence, are aflatoxin, deoxynivalenol (DON or vomitoxin), zearalenone, fumonisin, T-2 toxin, and T-2-like toxins (trichothecenes), ochratoxin and citrinin. Mould contamination is widespread in tropical countries where poultry production and processing are expanding rapidly[5]. Poultry are highly susceptible to mycotoxicoses caused by aflatoxins, trichothecenes, ochratoxins and some fusariotoxins. [6]

## MATERIALS AND METHODS

### SOURCE OF COLLECTED SAMPLE:

The samples used for the research work are different kinds of maize that were used in the poultry feed industry. These samples were collected from the different poultry feed mill in Namakkal district. The ingredient was collected separately, packed in a polythene bag and brought to the laboratories.

## DETERMINATION OF MOISTURE ESTIMATION [7]

Ten g of the sample was added to the preweighed Petri plate. The container was placed in a hot air oven at 120˚C for approximately 2 hours. The dish was removed from the oven, covered, cooled in a desiccator, and weighed.

$$\text{Percentage of moisture} = \frac{100\ (P-A)}{P} \times 100$$

P = weight in g of sample; A = weight in g of dried sample

## ESTIMATION OF pH[8]

For the measurement of pH, a 1:10 sample: distilled water suspension for each sample was prepared and stirred for 24 h in 200 ml beaker. Each sample was analyzed in triplicate. The pH of the suspension was measured using a digital pH meter.

## ISOLATION OF *Aspergillus* spp. FROM MAIZE

International Seed Testing Association (ISTA) techniques were used to detect the seed-borne mycoflora. The grains of each sample was tested by using the agar plate method. The untreated grains and were placed on sabourad's dextrose agar plates. Fifteen grains were placed in each Petri dishes were incubated at 24 ± 1˚C under 12h of the alternating cycle of light and darkness for 7 days. The grain borne fungi which grew out from the seeds on the medium in the form of fungal colonies were subculture and macro and microscopic characteristics of each fungi growth were studied to the identification of the fungi.

## MAINTENANCE OF CULTURE

The isolated strains of A. flavus isolates were maintained on Czapek Dox agar and Sabouraud's Dextrose Agar (SDA) slants.

## IDENTIFICATION OF AFLATOXIN PRODUCING ISOLATES.

An inoculum with a loopful of the spores of each *A. flavus* isolates was placed at the center of the dish containing the media with 0.3% of pharmaceutical- grade of β- cyclodextrin and incubated at 30ºC for 10 days. The presence or absence of fluorescence in the agar surrounding the colonies assayed was determined under UV radiation (365nm) and expressed as positive or negative. All the experiments were replicated three times.

## THIN LAYER CHROMATOGRAPHY FOR DETECTION OF MULTITOXIN[9]

### PROCEDURE FOR SEPARATION OF EXTRACT

About 10g of the ground sample was taken and blended at high speed for 3 min with 36 ml of Acetonitrile, 4 ml of 4% KCl and 0.8 ml of 5N HCl. The extract was filtered using Whatman No1. 20ml filtrate was transferred into 250ml separating funnel and added 20ml of water and added 20ml of hexane and shake well. The hexane layer is discarded. The lower layer was collected and again added 20ml of hexane and shake well and collected acetonitrile phase. 10ml of chloroform was added into the acetone nitril phase and collected chloroform layer and evaporated to dryness. The residue is dissolved in 0.2 ml chloroform.

### DEVELOPMENT OF TLC PLATES

For examination of extracts; aluminum- packed Slica gel 60 $F_{254}$ (Merck, type 1.05554.0007) was used as supplied for 20 x 20 $cm^2$ plates according to A.O.A.C. (1984). Plates were spotted along 1.5 cm from the bottom with 40 µL aliquots of extract and different volume (2, 4, 6, 8 and 10 µL) of aflatoxin B1 and B2 standard (4 µg / ml) and ochratoxin (5 µg / ml) standard were spotted on different TLC plates. The plate was developed in chloroform: acetone (CA) (9:1) in one direction and toluene: ethyl acetate: Formic acid (TEF) (5:4:1) in the second direction perpendicular to the first direction in equilibrated, TLC chrome tanks at room temperature until the solvent front had reached a line marked 2 cm from the top of the plate. The standard aflatoxins were developed by CA solvent and ochratoxin in the TEF solvent system. After development, plates were removed and air-dried in a fume- cabinet and then examined in a long UV (wavelength 364 nm) light in a UV cabinet.

## DETECTION OF MULTITOXIN

Developed chromatograms were examined under UV light, since aflatoxins B1, B2 and Ochratoxin A visualized blue fluorescence without treatment.

## IDENTIFICATION AND QUANTIFICATION OF MULTITOXIN

The chemical confirmation of aflatoxin was performed by spraying the chromatophore with 25% sulphuric acid. The presence of ochratoxin A on the TLC plate was confirmed by treatment with ammonia [10]. The citrinin was confirmed by spraying with an acidic solution of p- anisaldehyde [11] and dried at 110ºC in a hot air oven and viewed under long UV light. Each toxin was also confirmed based on RF values and standard toxins that were given below:

| S.No | Toxin name | Rf values | | Colour under UV light | Confirmation Test | Confirmation colour |
|---|---|---|---|---|---|---|
| | | Sol I | Sol II | | | |
| 1 | Aflatoxin B1 | 0.73 | 0.22 | Blue | 25% $H_2SO_4$ acid spray | Yellow colour |
| 2 | Aflatoxin B2 | 0.71 | 0.20 | Blue | 25% $H_2SO_4$ acid spray | Yellow colour |
| 3 | Ochratoxin A | 0.00 | 0.46 | Blue | Exposure to ammonia | Blue colour |

The amount of each toxin was calculated after visualization on TLC plate by comparing the known concentration of the standard solution.

## CALCULATION

$$\text{Multitoxins (ppb)} = \frac{S \times C \times D \times 1000}{T \times E}$$

S = Standard volume which matches with test volume in fluorescence intensity
C = Concentration of standard
D = Dilution factor
T = Test volume which matches with standard volume in fluorescence intensity
E = Effective weight of the sample= 4.9

## ANTIFUNGAL ASSAY OF ESSENTIAL OILS AGAINST AFLATOXIGENIC *A.flavus*

Agar diffusion test or well diffusion assay was performed by the method of [12] with slight modification. The aflatoxigenic *A. flavus* spore was swabbed on Sabouraud's dextrose agar plates using a sterile cotton swab. Agar wells were prepared with the help of a sterilized cork borer with 6 mm diameter. Using a micropipette, 20ul of different essential oil was added to the wells in the plate. The plates were incubated in an upright position for 3 days at room temperature and the zones of inhibition were determined.

## RESULTS AND DISCUSSION

## COLLECTION OF SAMPLES

A total of 10 maize samples were collected from different Poultry mills in Namakkal District, Tamilnadu from January 2018.

## DETERMINATION OF MOISTURE ESTIMATION AND pH

Moisture content and hydrogen ionic strength (pH) for all maize samples are shown in table-1 and figure-1. In all maize samples, the moisture content ranged from 9.8-15.0%. Samples- 2,5, 6 and 10 had the highest moisture content between 12.3-15% and also had the highest *A.flavus* fungal flora. The remaining samples had the lowest moisture content below 12% had the lowest population of *A.flavus.* The hydrogen ion concentration (pH) of maize samples fall in the magnitude of the acidic condition. In all the maize samples the pH ranged from 6.0-6.5pH which indicated acidic nature.(Table-1)

## ISOLATION AND IDENTIFICATION OF *Aspergillus* spp. INDIFFERENT MAIZE SAMPLES

The isolates of *Aspergillus* spp. were tabulated in table-2. In the maize samples, *A.flavus* was isolated in high number, followed by *A.niger, A. fumigatus*. The colony morphology of different isolates. The colonies of *A.flavus* on Sabourad's Dextrose Agar (SDA) at 25°C were yellow to lush/bright green with white to

yellow reverse. *A.flavus* was rapid growth, texture woolly to cottony and somewhat granular. Hyphae were septate and hyaline. Conidial heads are radiate to loosely columnar with age. Conidiophores were coarsely roughened, uncoloured. Vesicles are globose to subglobose, metulae covering nearly the entire vesicles in biseriate species. Conidia are smooth to very finely roughened, globose to subglobose. The colony morphology of *A.niger* was found as when immature, they are covered with white fluffy aerial mycelia and when mature they are covered with black spores. They produce septate hypae and globose shaped conidial head with large black to brownish-black. Conidiophores were hyaline and the conidia were globose to echinulate with 4-5 m in diameter. The colonies of *A.fumigatus* on Sabourad's Dextorse Agar (SDA) at 25°C were smoky gray green with a slight yellow reverse. Some isolates may display a lavender diffusible pigment. Very mature colonies turn slate gray. Rapid growth, the texture is woolly to cottony and somewhat granular. Hypae were septate and hyaline. Conidial heads were strongly columnar in an undisturbed culture. Conidiophores were smooth-walled, uncolored, up and terminate in a dome-shaped vesicle. These species were uniseriate with closely compacted phillades. Conidia are smooth to finely roughened, subglobose.

**Table – 1-Estimation of moisture content and pH from different maize samples**

| S.No | Sample name | Moisture (%) | pH |
|---|---|---|---|
| 1. | Maize-1 | 9.12 | 6.1 |
| 2. | Maize-2 | 14.5 | 6.5 |
| 3. | Maize-3 | 11.2 | 6.3 |
| 4. | Maize-4 | 10.8 | 6.2 |
| 5. | Maize-5 | 12.9 | 6.3 |
| 6. | Maize-6 | 15.0 | 6.5 |
| 7. | Maize-7 | 9.8 | 6.1 |
| 8. | Maize-8 | 11.6 | 6.2 |
| 9. | Maize-9 | 10.0 | 6.0 |
| 10. | Maize-10 | 12.3 | 6.3 |

**Table – 2-Isolation of *Aspergillus.*spp from different maize samples**

| S.No | Sample name | *A.niger* | *A.flavus* | *A.fumigatus* | Other *Aspergillus.*spp | Total |
|---|---|---|---|---|---|---|
| 1. | Maize-1 | 2 | 2 | 0 | 1 | 5 |
| 2. | Maize-2 | 2 | 6 | 1 | 0 | 9 |
| 3. | Maize-3 | 2 | 3 | 0 | 0 | 5 |
| 4. | Maize-4 | 2 | 2 | 0 | 1 | 5 |
| 5. | Maize-5 | 2 | 5 | 0 | 0 | 7 |
| 6. | Maize-6 | 4 | 7 | 2 | 0 | 13 |
| 7. | Maize-7 | 0 | 2 | 1 | 1 | 4 |
| 8. | Maize-8 | 1 | 3 | 1 | 1 | 5 |
| 9. | Maize-9 | 2 | 5 | 0 | 2 | 9 |
| 10. | Maize-10 | 2 | 4 | 0 | 0 | 6 |

**Table – 3 Estimation of multitoxin from different maize sample**

| S.No | Sample Name | Aflatoxin - B1 (ppb) | Aflatoxin - B2 (ppb) | Ochratoxin – A (ppb) |
|---|---|---|---|---|
| 1. | Maize-1 | 32.65 | 16.32 | - |
| 2. | Maize-2 | 32.65 | - | - |
| 3. | Maize-3 | - | - | - |
| 4. | Maize-4 | 24.48 | - | - |
| 5. | Maize-5 | 65.30 | 24.48 | - |
| 6. | Maize-6 | 81.50 | 40.81 | 12.33 |
| 7. | Maize-7 | 24.48 | - | - |

| | | | | |
|---|---|---|---|---|
| 8. | Maize-8 | 24.48 | - | - |
| 9. | Maize-9 | - | - | - |
| 10. | Maize-10 | 16.32 | - | - |

**Figure – 1. Estimation of moisture content and pH from different maize samples**

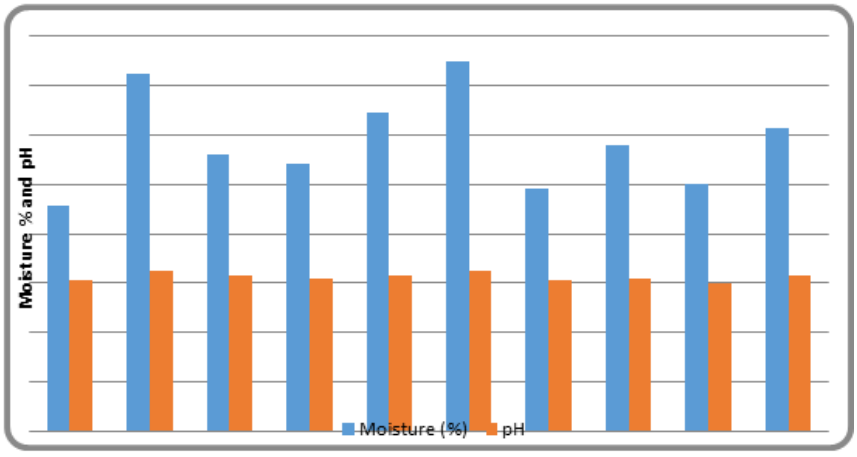
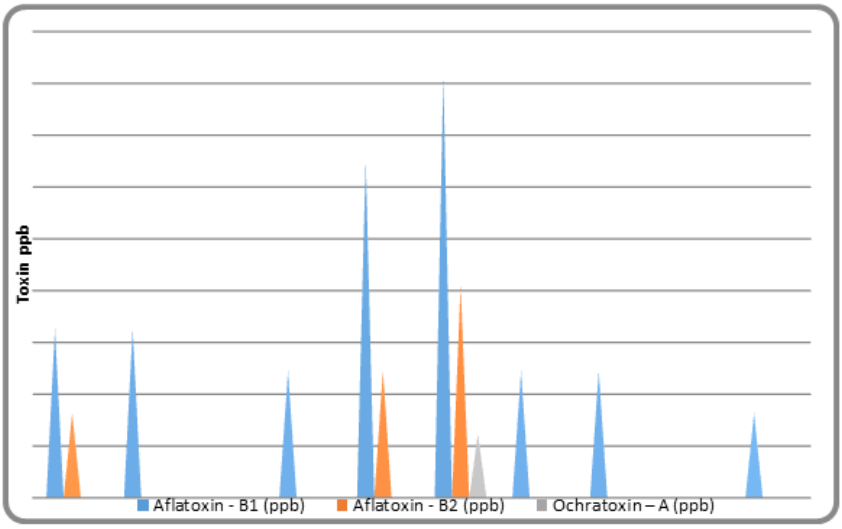



**Figure-2 Estimation of multitoxin from different maize sample**




In nature, mycotoxins rarely occur as a single contaminant since many fungal species that produce mycotoxins grow and produce their toxic metabolites under similar conditions. Furthermore, a typical poultry ration is made up of several grain sources; each of which may be contaminated with a different mycotoxin or more than one mycotoxin. There is an increasing awareness of the hazards imposed on both human and animal health by mycotoxins present in food and feed. In the present investigation, a study on the occurrence of multitoxin and aflatoxigenic fungi in different maize samples collected in different poultry mills around the Namakkal in January 2018. (Table-3). In maize samples, altogether 3 types of *Aspergillus* fungi such as *A.flavus, A.niger, A.fumigatus* only were recorded. He reported that 8 genera of fungi such as *Aspergillus, Penicillium, Rhizopus, Fusarium, Peacilomyces, Scopulariopsis, Alternaria*, *Candia* were found in maize. Corn is susceptible to *A.flavus* infection via the silks and these stress conditions at the time of anthesis (pollination) lead to preharvest aflatoxin contamination in corn[13]. In all the maize samples, the moisture content ranged from 9.8-15.0%. The samples which had high moisture content had the highest fungal flora. The samples which had low moisture content favored lowest fungal flora. The present results were confirmed with previous finding .[14]. In all the maize samples, the hydrogen ion concentration (pH) ranged from 6.0-6.5pH, which indicates the acidic condition. *Aspergillus is* most commonly found in stored cereal grains, they are ubiquitous, can cause food spoilage and biodeterioration, and are capable of producing many different mycotoxins. *Aspergillus* species *A. flavus, A. parasiticus, A. nomius, A.*

*fumigatus, A. versicolor* and *A. ochraceus)* are some of the more common toxigenic species[15] .In this present study, 15 isolates of *A.flavus* were isolated from maize sample collected from Namakkal District. In general *A.flavus* has been described as yellow to green with white to yellow reverse on SDA plates at 25°C. In microscopic examination, *A.flavus* appeared as septate hyphae and hyaline. Conidial heads are radiate to loosely columnar with age. Conidiophores are coarsely roughed, uncolored. Conidia are smooth to very finely roughed, globose and subglobose, 3-6 µm in diameter. Crops can be contaminated by fungi during production, storage, processing and transportation when temperature and humidity condition was suitable.In the present investigation, all samples were agricultural commodities that may be contaminated with fungi. Aflatoxigenic *A.flavus* displayed fluorescence as a ring around its colonies cultured on medium with β cyclodextrin as additive[16]. In this study, among 15 A.flavus isolates, 8 isolates produce bluish-green fluorescent areas surrounding colonies when observed under a long wavelength (365nm) UV light after 3 days of incubation at 30˚C in SDA medium incorporated with 0.3% of the pharmaceutical grade of beta-cyclodextrin. The aflatoxigenic isolates showed fluorescence in the medium and non-toxigenic isolates did not show the fluorescence. This may be due to the presence of beta-cyclodextrin. These results were confirmed with those of [17.] Maize was an excellent substrate for mold growth and mycotoxin production[18] (Fig-2). He found aflatoxin B1 level up to 14µg/kg and aflatoxin G1 level up to 58µg/kg in stored grains. In the present study, among the 10 maize samples, sample-6 showed the highest concentration of AFB1 (81.50ppb), AFB2 (40.81ppb), and ochratoxin-A (12.33ppb), followed by sample-5 showed AFB1 (65.3ppb), AFB2 (24.8ppb). The remaining samples showed that the 16.32 ppb of AFB2 toxin. The sample-6 only was contaminated with Ochratoxin- A.

## CONCLUSION

This work elucidates the potential of botanicals and essential oils as natural preservatives in seed, food and feeds against *A. flavus*, the well known casual agents of farm product diseases and food poisonings. More research on toxigenic fungi control with natural products should be undertaken; provision of appropriate processing facilities and some of the marketing strategies for the products should be carefully planned. Plant fungicide production could be sequentially integrated into a sustainable crop protection system in developing countries. Integrated Disease Management strategy of prevention and control of toxigenic fungi and aflatoxins should be considered.

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SP-60

# COMPARATIVE STUDIES ON ANTIBACTERIAL EFFICACY OF NATURAL & ARTIFICIAL HONEY ON ISOLATED PATHOGENS FROM BURNT WOUND SAMPLE

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## ABSTRACT

The present study dealt with the Isolation and Characterization of a bacterial pathogen from a burnt wound sample. The burnt wound infection is a major complication in patients. Burnt wounds showed significantly higher bacterial counts compared with the normal wound. The burnt wound sample was inoculated using nutrient agar medium and medium and incubated at 37°C for 24 hrs. After incubation pure cultures of organisms were made in respective agar mediums. The pure culture of bacteria was identified and characterized as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* These organisms are dangerous pathogens penetrating the wound producing a lot of pain and exudates. The development of burnt wound infection is not a new phenomenon and is an ongoing problem for many people. Open wounds serve as an entry route for contamination 28 with invading microorganisms. Besides delayed wound healing of infected burn wounds, it is associated with increased discomfort, hospital stay and health care costs. The present work on the honey showed antibacterial activity against the burnt wound pathogenic Bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* It will meet the requirements of essential products required in humans, animals as well as in the pharmaceutical industry. The natural honey is more effective and safer than Artificial honey and the antibiotics against the burnt woundpathogens.

**KEYWORD:** Antibacterial activity, Antibiotics, Honey, Burnt wound, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

## INTRODUCTION

Honey is created by bees as a food source. In cold weather or when food sources are scarce, bees use their stored honey as their source of energy. By contriving for bees swarms to nest in artificial hives, people have been able to semi-domesticated the insects and harvest excess honey. In the hive, the bees use their "HONEY STOMACH" to ingest and regurgitate the nectar several times until it is partially digested[1]. Bees store honeycombs and there use it for food in winter. Since it is a natural sweetener because it is high glucose and fructose content; it is widely used in candies, cereals, and baked goods. There are more than 300 types of honey's varying in flavor and colours (from pale yellow to dark amber), depending on the type of blossoms visited by the honey bee. Honey has been known to humans for centuries to have antimicrobial potential[2]. Most microorganisms do not grow in honey because of its low water activity of 0.6%[3]. The development of wound infection is not a new phenomenon and is an ongoing problem for many people. Open wounds serve as an entry route for contamination 28 with invading microorganisms. Besides delayed wound healing of infected wounds, it is associated with increased discomfort, hospital stay, and health care costs[4]. Infected wounds scar more severely and are associated with prolonged restoration. The risk of systemic infection and even death is also associated with infected wounds[5].

## MATERIALS AND METHODS

### COLLECTION OF WOUND SAMPLE[6]

The Clinical sample (Burnt Wound) collected aseptically was from The Government Hospital from

Kumbakonam, Tanjore Dt, (Tamilnadu). The sterile swab was introduced deeply enough to obtain a moist specimen and the swab was replaced in the transport media. The sampled swab was taken immediately to the laboratory for processing of the sample. If delayed inoculated in the nutrient broth.

**PREPARATION OF MEDIA AND PLATING METHOD[7]**

Isolation of clinical samples was streaked over the nutrient agar plates and incubated at 30°C for 48 hours. After the incubation period, selected colonies of clinical samples were transferred from the mixed culture of the plate onto respective agar plates and incubated at 30°C for 48 hours. Slants containing pure culture were stored at 4°C for further examination.

**MORPHOLOGICAL AND PHYSIOLOGICAL TEST**

Cultural characteristics of pure isolates in nutrient agar media were recorded after incubation period for $30^0$c of 48 hrs. Morphological methods consist of macroscopic and microscopic methods. The microscopic characterization was done by light microscopy. By using the microscopic method appearance of organisms was identified as colour, shape and morphology.

**GRAM STAINING[8]**

Gram staining technique was first discovered by a Danish physician named Hans Christian gram in 1884. This technique divides bacterial cells into two major groups, gram positive and gram negative bacteria, this makes it an essential tool for clarification and differentiation of microorganisms. A thin smear was prepared on a clean grease-free glass slide. The smear was flooded with crystal violet and allowed to stand for 1 minute. Then the slide was washed with water and then flooded with gram's iodine and left for 1 minute. The stain was drained and decolorised with 95% ethanol which was then washed with water. The smear was counterstained with safranin for 1 minute. The slide was blot dried and examined under a microscope.

**BIOCHEMICAL TEST[9]**

Imvic test (Indole test, Methyl red test, Voges proskauer test), Citrate utilization test, Triple sugar iron test, Urease test, Carbohydrate fermentation test.

**COLLECTION OF HONEY SAMPLE**

Honey samples were collected from two different regions., Natural (paruthicheri), and Artificial (shop), in sterile containers and kept at room temperature (24-$26^0$ c) for 30 days before experimentation. Each sample was diluted to various concentrations (25%, 50%, 75% and 100% in v/v) in order to test their antibacterial potential.

**PREPARATION OF HONEY AT DILUTION**

**NATURAL HONEY & ARTIFICIAL HONEY**

The preparation of Natural & Artificial honey in various concentration (100%, 75%, 50%, 25%) In 100% Natural & Artificial honey consist of pure honey without dilution. 75% Natural & Artificial honey consists of 7.5 ml of Natural & Artificial honey diluted with 2.5 ml of distilled water. 50% Natural & Artificial honey consists of 5 ml of honey diluted with 5 ml of distilled water. 25% Natural & Artificial honey consists of 2.5 ml of honey diluted with 7.5 ml of distilled water.

**DETERMINATION OF ANTIBACTERIAL EFFECT OF HONEY SAMPLE: PREPARATION OF DISC[10]**

Whatman No.1, 6mm filtered paper disc was prepared and sterilized by autoclaving. These discs were plated and each disc was impregnated with 15 ml diffusion concentration of honey and dried overnight at 31°C. This was carried out under sterile conditions inside a laminar flow.

**NATURAL HONEY & ARTIFICIAL HONEY**:

Muller Hinton agar plates were prepared and the surface of the plate was allowed to dry. Each Muller-Hinton agar plate was inoculated with the standard inoculums like *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa* by soaking a swab and rotating it over the agar plate. The Natural Honey under the concentration of 25%, 50%, 75% and 100% was dipped into the disc. The Natural honey disc was placed over the inoculated agar. After 24 hours of incubation at 37°C, the Zone of inhibition was measured and recorded. A triplicate plate was also carried out.

## ANTIBIOTIC SENSITIVITY TEST[11]

### DISC DIFFUSION METHOD

The chemotherapeutic agents are known as antibiotics. Each antibiotic do possess different antimicrobial activities concerning different microorganisms. Some of them have a short spectrum some with broad spectrum against wide range of microbes. The diameter of zone of inhibition, resulting from diffusion of drug into the medium (Muller Hinton agar) To compare the activity of the test material, the synthetic antibiotics such as Ampicillin(10 g), Ceftriazone(10 g), Erythromycin(10 g), Penicillin(10 g) and Tetracyclin(10g) discs were used. They were placed (incubated) in Muller Hinton agar containing isolated pathogens *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa.* After overnight incubation at 37$^{o}$C in the incubator, the zones of inhibition were measured. Disks are, Ampicillin(A), Ceftriaxone(CI), Erythromycin(E), Penicillin(P), Tetracycli(T)

## RESULT AND DISCUSSION

### PATHOGENIC BACTERIA

The pathogenic bacteria were isolated from the Burnt wound infection by using the swab technique and isolated in nutrient agar medium (Fig.1 & 2). Then the isolated bacteria were identified through a gram staining method (Fig-3) (Table-1) and some various biochemical tests (Fig – 4A, B, C, D) (Table-2).

### IDENTIFICATION OF PATHOGENIC BACTERIA

The isolated organisms were identified by using the grams staining method and using the selective medium for the organisms. The result showed Two gram-negative organisms and one gram-positive organism. The organisms were confirmed by using the Eosin methylene agar medium, Blood agar medium, cetrimide agar medium. In Eosin methylene agar medium on ***E.coli*** (Fig. 5A) shows metallic green colonies in the Blood agar medium on *Staphylococcus aureus* (Fig. 5B) shows pink colour colonies. In cetrimide, agar medium on *Pseudomonas aeruginosa* (Fig. 5C) shows green colour colonies.

### ANTIBACTERIAL ACTIVITY OF HONEY SAMPLE

### EFFECT OF NATURAL HONEY ON *Escherichia coli* (Fig. 7A)

- Natural honey showed the inhibitory action against ***Escherichia coli*** by 25%,50%,75%,100% concentration are 10mm, 12mm, 14mm,18mm

***Staphylococcus aureus*** (Fig. 7B)

- Natural honey showed the inhibitory action against ***Staphylococcus aureus*** by 25%,50%,75%,100% concentration are 9mm,11mm,13mm,16.5mm.

***Pseudomonas aeruginosa*** (Fig. 7C)

- Natural honey showed the inhibitory action against ***Pseudomonas aeruginosa*** by 25%,50%,75%,100% concentration are 9mm, 10mm, 13mm, 16mm.

write given in Table 3.

### EFFECT OF ARTIFICIAL HONEY ON *Escherichia coli* (Fig. 7A)

- Artificial honey showed the inhibitory action against ***Escherichia coli*** by 25% 50% 75% concentration is 0mm. But in 100% concentration honey shows 11 mm of inhibition.

***Staphylococcus aureus*** (Fig. 7B)

- Artificial honey showed the inhibitory action against ***Staphylococcus aureus*** by 25% 50% 75% concentration is 0mm. But in 100% concentration honey shows 9 mm of inhibition

***Pseudomonas aeruginosa***(Fig. 7C)

- Natural honey showed the inhibitory action against ***Pseudomonas aeruginosa*** by 25%50% 75% concentration is 0mm. But in 100% concentration honey shows 8 mm of inhibition.

Write given in Table 4. The comparative result of Natural Honey and Artificial Honey were illustrated as bar diagram in figure 9.

### ANTIBIOTIC SENSITIVITY TEST

### EFFECT OF ANTIBIOTICS ON *Escherichia coli* (Fig. 8A)

- The antibiotic disc showed the inhibitory action against ***Escherichia coli*** by Ampicillin is 8mm, Ceftriaxone is 11mm, Erythromycin is 16mm, Penicillin is 13mm, Tetracyclin is 8mm.

***Staphylococcus aureus*** (Fig. 8B)

- The antibiotic disc showed the inhibitory action against ***Staphylococcus aureus*** by Ampicillin is 9mm, Ceftriaxone is 11mm, Erythromycin is 13mm, Penicillin is 12mm, Tetracyclin is 10mm

***Pseudomonas aeruginosa*** (Fig. 8C)

- The antibiotic disc showed the inhibitory action against ***Pseudomonas aeruginosa*** by Ampicillin is 5mm, Ceftriaxone is 10mm, Erythromycin is 13mm, Penicillin is 12mm, Tetracyclin is 15mm.

The comparative result of antibiotic discs were illustrated as bar diagram in figure 10.

In recent times this has been rediscovered and honey is in fairly wide spread use as a topical antibacterial agent for the treatment of wounds, burns and skin ulcers, there being many reports of its effectiveness. The present study higher activity of honey against *Staphylococcus aureus, Escherichia coli and P.aeruginosa*. The present study higher activity of honey against *Staphylococcus aureus, Escherichia coli and P.aeruginosa*. The antibacterial activity was demonstrated on both gram-positive and gram-negative bacteria. Gram-negative bacteria were more susceptible to honey. This agrees with a study by that honey has more activity against both gram-positive than gram negative organism. The high antimicrobial activity found support in this study against the gram – negative bacteria tested along with the control strains. This was evident in the percentage levels of bacterial sensitivity as high as 100% for *E.coli*. In my present study, the antibacterial activity of honey shows a higher inhibition compared with synthetic antibiotics. The synthetic antibiotics like Ampicillin, Ceftriaxone, Erythromycin, Penicillin and Tetracyclin.

**FIG-1. BURNT WOUND**

**FIG-2. MOTHER CULTURE**

**SAMPLE** **CONTROL**


**FIG-3. BIOCHEMICAL TEST**

**WOUND SAMPLE**

***E.coli***

**FIG-4. BIOCHEMICAL TEST**

***S.aureus***

***P.aeruginosa***

FIGURE - 5A. ISOLATION OF TEST ORGANISMS

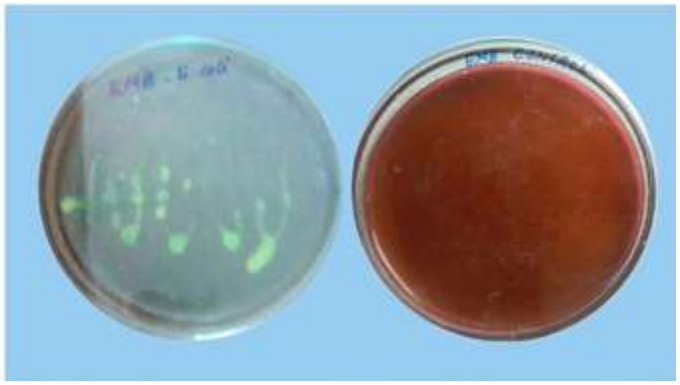


Plate -1 Plate 2

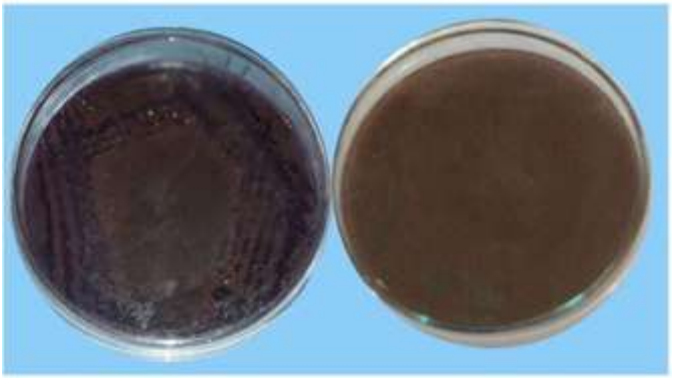


Plate -1 Plate 2

FIGURE – 5B.

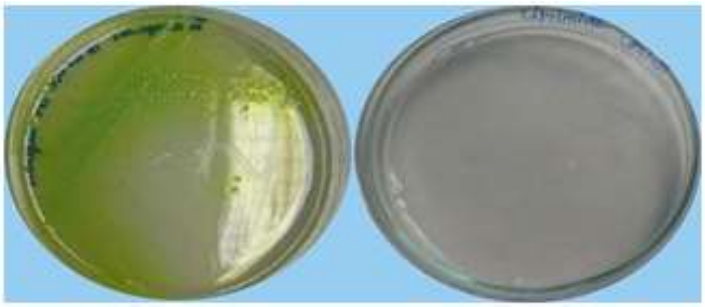
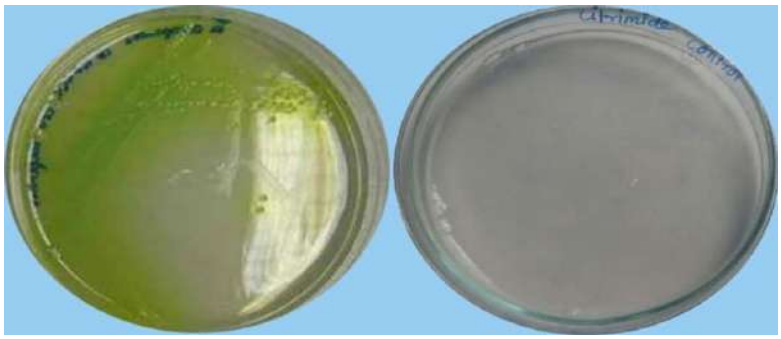


FIG - 5C. ISOLATION OF TEST ORGANISM



**IG -6 TWO DIFFERENT TYPESOF HONEY**

NATURAL HONEY

ARTIFICIAL HONEY

**FIG - 7A. INHIBITION ZONE OF HONEYAGAINST**

*E.coli*

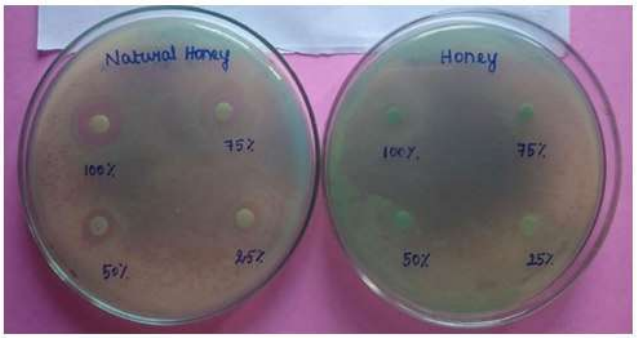



PLATE -1 PLATE -2

PLATE - 3

**FIG - 7B. INHIBITION ZONE OF HONEY AGAINST**

*Staphylococcus aureus*

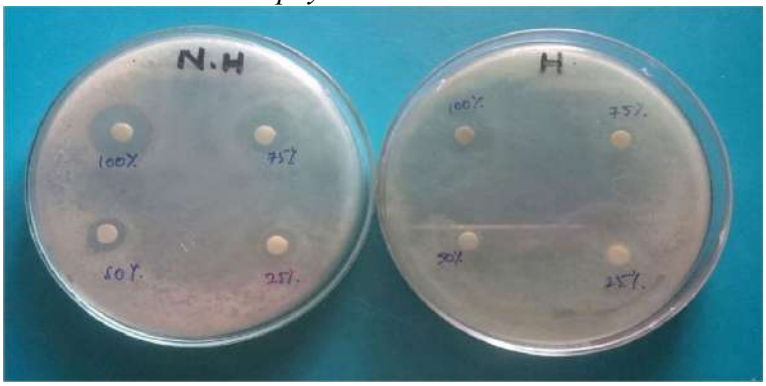



PLATE -1 PLATE -2

PLATE - 3

**Fig - 7c. Inhibition Zone Of Honey Against**

*Pseudomonas aeruginosa*


PLATE -1 PLATE -2

PLATE - 3

FIG - 8A. ANTIBIOTIC SENSITIVITY TEST

PLATE -1

PLATE -2

ZONE OF INHIBITION

AGAINST *E.coli*

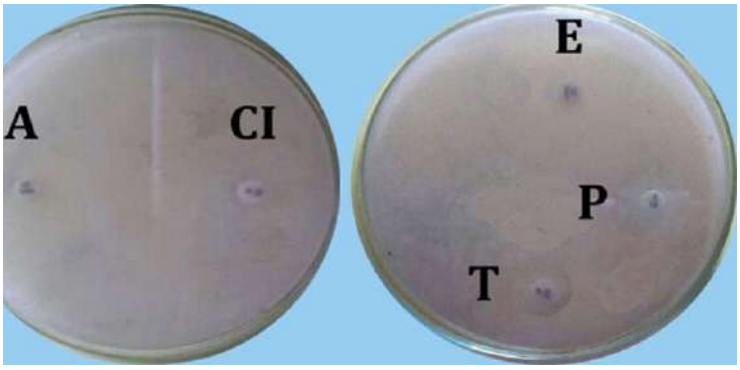



PLATE - 3 PLATE - 4

**FIG - 8B ANTIBIOTIC SENSITIVITY TEST**

**ZONE OFINHIBITION AGAINST *S.aureus***

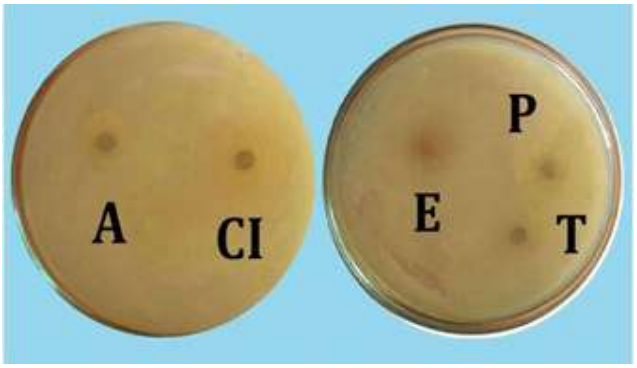



**PLATE -1 PLATE -2**

PLATE -3

**FIG - 8C. ANTIBIOTIC SENSITIVITY TEST**

**ZOñiE OF Ih!HIBITIOñi AGAINST**
***P.aeruginosa***

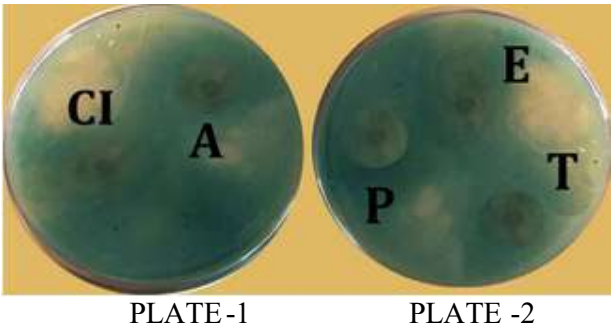



PLATE -1 PLATE -2

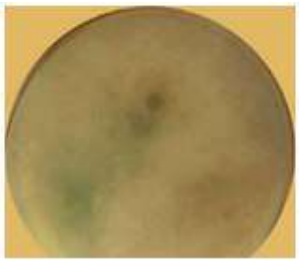


PLATE - 3

**Table – 1. MORPHOLOGICAL IDENTIFICATION TESTS FOR ISOLATED ORGANISMS**

| Tests | *P.aeruginosa* | *S.aureus* | *E.coli* |
|---|---|---|---|
| COLONY MORPHOLOGY | Circular, convex and smooth and translucent colonies. | Round smooth raised and glistening, usually from gray to deep golden yellow colour | Circular, convex and smooth colonies with distinct edges grayish-white colour colonies |
| GRAM REACTION | Gram-negative. | Gram-positive cocci and appears as grape-like cluster | Gram-negative Rods |
| MOTILITY | Motile | Nonmotile | Motile and Non Motile |

**Table – 2. BIOCHEMICAL IDENTIFICATION TESTS FOR ISOLATED ORGANISMS**

| Biochemical Tests | *P.aeruginosa* | *S.aureus* | *E.coli* |
|---|---|---|---|
| **INDOLE** | - | - | + |
| **MELTHYL RED** | + | + | + |
| **VOGAS PROSKAUER** | - | - | - |
| **CITRATE** | + | - | - |
| **UREASE** | - | - | - |
| **CHO TEST** | + | - | - |
| **SUCROSE** | A&G | A | A |
| **LACTOSE** | A&G | A | A&G |
| **GLUCOSE** | A&G | A | A&G |

**POSITIVE (+) , NEGATIVE (-), A – ACID, A&G - ACID AND GAS**

| NAME OF THE HONEY | *Staphylococcus aureus* | | | |
|---|---|---|---|---|
| | **25%** | **50%** | **75%** | **100%** |
| **NATURAL HONEY** | 9 mm | 11 mm | 13 mm | 16.5 mm |
| **ARTIFICIAL HONEY** | 0 | 0 | 0 | 9 mm |

| NAME OF THE HONEY | *Escherichia coli* | | | |
|---|---|---|---|---|
| | **25%** | **50%** | **75%** | **100%** |
| **NATURAL HONEY** | 10 mm | 12 mm | 14 mm | 18 mm |
| **ARTIFICIAL HONEY** | 0 | 0 | 0 | 11 mm |

| NAME OF THE HONEY | *Pseudomonas aeruginosa* | | | |
|---|---|---|---|---|
| | **25%** | **50%** | **75%** | **100%** |
| **NATURAL HONEY** | 9 mm | 10 mm | 13 mm | 16 mm |
| **ARTIFICIAL HONEY** | 0 | 0 | 0 | 8 mm |

**Figure-9**

**COMPARATIVE STUDY OF ANTIBACTERIAL POTENTIAL OF ARTIFICIAL AND NATURAL HONEY**

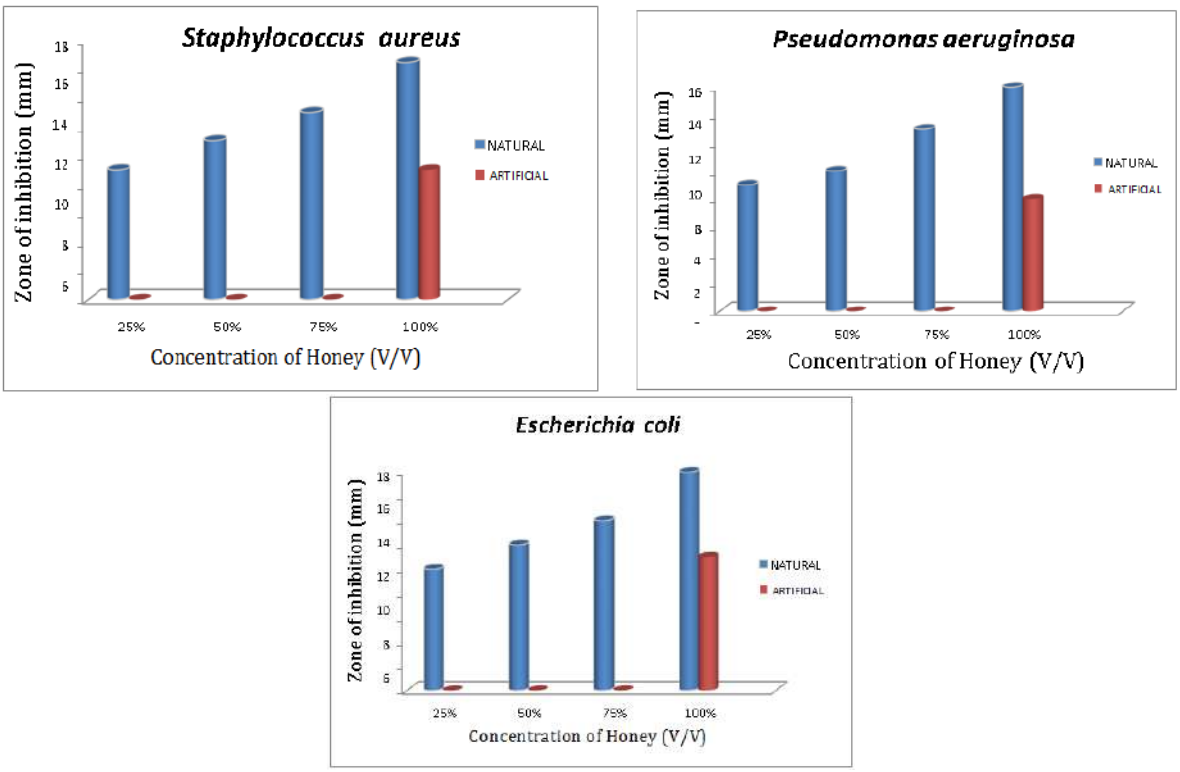



**Figure : 10 A ANTIBIOTICS SENSITIVITY TEST ON *Escherichia coli***

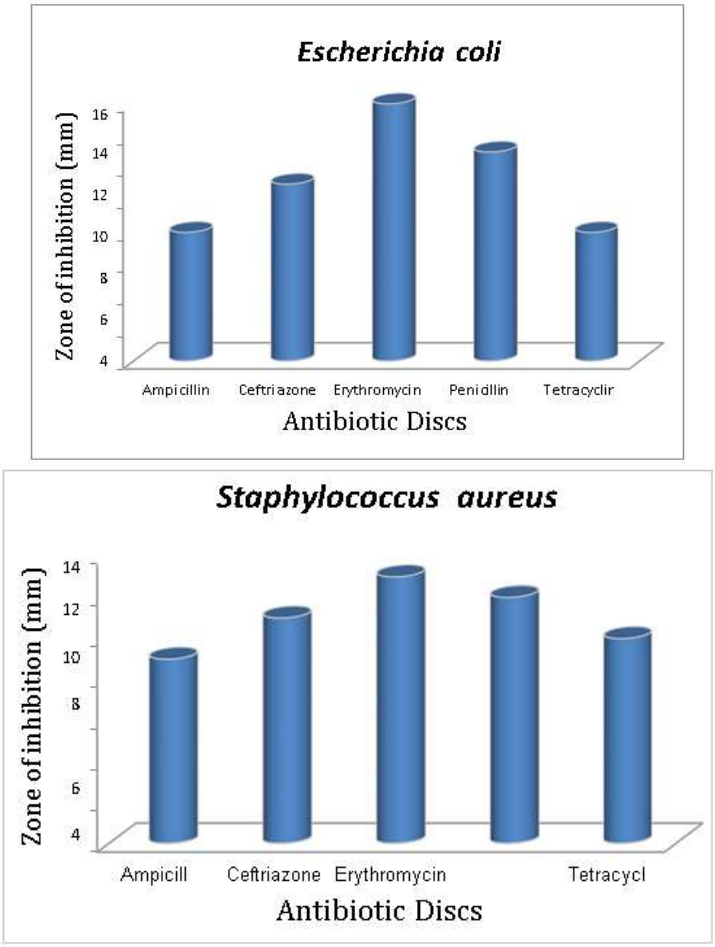
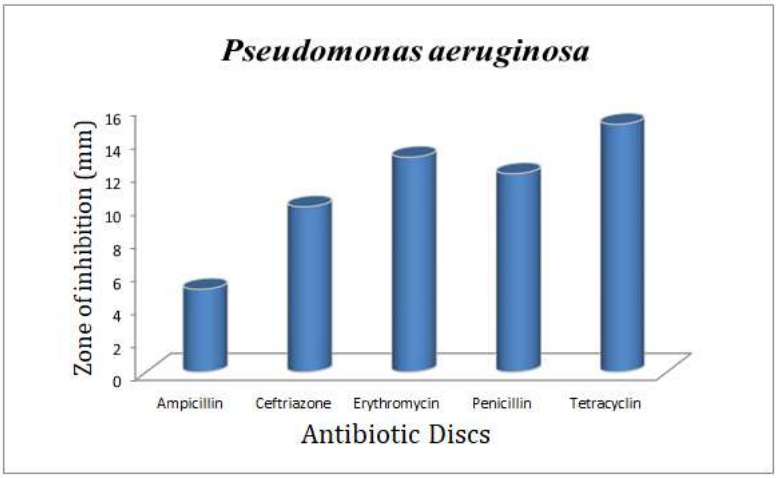


**Figure : 10 B. ANTIBIOTICS SENSITIVITY TEST ON *Staphylococcus aureus***




| S.No. | Symbol | Name of the Antibiotics | Zone of Inhibition |
|---|---|---|---|
| 1 | A | Ampicillin | 8 mm |
| 2 | CI | Ceftriaxone | 11 mm |
| 3 | E | Erythromycin | 16 mm |
| 4 | P | Penicillin | 13 mm |
| 5 | T | Tetracyclin | 8 mm |

| S.No. | Symbol | Name of the Antibiotics | Zone of Inhibition |
|---|---|---|---|
| 1 | A | Ampicillin | 9 mm |
| 2 | CI | Ceftriazone | 11 mm |
| 3 | E | Erythromycin | 13 mm |
| 4 | P | Penicillin | 12 mm |
| 5 | T | Tetracyclin | 10 mm |

**Table : 10 C. Antibiotics Sensitivity Test On *Pseudomonas aeruginosa***

| S.No. | Symbol | Name of the Antibiotics | Zone of Inhibition |
|---|---|---|---|
| 1 | A | Ampicillin | 5 mm |
| 2 | CI | Ceftriaxone | 10 mm |
| 3 | E | Erythromycin | 13 mm |
| 4 | P | Penicillin | 12 mm |
| 5 | T | Tetracyclin | 15 mm |

## CONCLUSION

The present study dealt with the Isolation and Characterization of a bacterial pathogen from a burnt wound sample. The burnt wound infection is a major complication in patients. Burnt wounds showed significantly higher bacterial counts compared with the normal wound. The burnt wound sample was inoculated using nutrient agar medium and medium and incubated at $37^0$C for 24 hrs. After incubation pure cultures of organisms were made in respective agar mediums. The pure culture of bacteria was identified and characterized as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* These organisms are dangerous pathogens penetrating the wound producing lot of pain and exudates. The development of burnt wound infection is not a new phenomenon and is an ongoing problem for many people. Open wounds serve as an entry route for contamination 28 with invading microorganisms. Besides delayed wound healing of infected burn wounds, it is associated with increased discomfort, hospital stay and health care costs. This

investigation has opened up the possibility of the use of this natural honey in drug development for human consumption possibly for the treatment of various infections caused by microbes. These are novel, nature, and economic sources of antimicrobials, which can be used in the prevention of disease caused by pathogenic microbes. Therefore this study will open up and scope for future utilization of the waste for therapeutic purposes. The results also indicate that selective extraction from natural materials important for obtaining fractions with high antimicrobial activity. The present work on the honey showed antibacterial activity against the burnt wound pathogenic Bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*Natural honey and Artificial honey showed maximum inhibition zone was formed in 100% honey against pathogens. When compared with the zone of inhibition against pathogens for natural and artificial honey, the natural honey showed higher effective than artificial honey against the burnt wound pathogens. The antibacterial activity of the antibiotics like Ampicillin, Erythromycin, Tetracycline, Penicillin, Ceftriaxone. The antibiotic Erythromycin shows the maximum inhibition against *Escherichia coli* than other antibiotics. The tetracyclin shows the maximum inhibition against *Pseudomonas aeruginosa* than other antibiotics. It will meet the requirements of essential products required in humans, animals as well as in the pharmaceutical industry. The natural honey is more effective and safe than Artificial honey and the antibiotics against the burnt wound pathogens. Finally from my study, I concluded that natural honey generally good medicine for burn wound infections.

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SP-61

# ISOLATION OF ANTAGONISTIC ACTINOMYCETES FROM MUTHUPET MANGROVE FOREST SOIL AND THEIR ANTIBACTERIAL ACTIVITY

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## ABSTRACT

The development of new antimicrobial agents, preferably naturally occurring ones with novel mechanisms of action, is an urgent medical need. The soil in particular is an intensively exploited ecological niche, the inhabitants of which produce many useful biologically active natural products, including clinically important antibiotics. In the present study, actinomycetes species were isolated from Muthupet mangrove forest soil samples and analyzed their antagonistic effect. The positive antagonistic actinomycetes colony was identified and antibacterial activity of positive actinomycetes strains was analyzed against antibiotic-resistant bacterial strains. Actinomycetes are prolific producers of antibiotics and important suppliers to the pharmaceutical industry can produce a wide variety of secondary metabolites. *Streptomyces parvulus* fermentation antibiotic compound was extracted using ethyl acetate by centrifugation and the antibacterial compound containing disc were prepared separately, the disc was used for assay of antibacterial activity against antibiotic-resistant bacterial strains. Finally concluded that the isolated actinomycetes *Streptomyces parvulus* was highly recommended for antibiotic production in the industrial level, it create a new sector in the pharmaceutical field.

**KEYWORDS:** Actinomycetes, *Streptomyces parvulus,* Antagonistic activity

## INTRODUCTION

Now a day become most informant problem for the clinical filed is antibiotic-resistant bacteria. In developing country like India face this problem from last two decants. The reason of the problem is the overuse and misuse of antibiotics. Antibiotic-resistant bacteria are affected anyone, of any age in any country. The various studies on antibacterial resistance showed that extended-spectrum β-lactamases (ESBL), Metallo- β- lactamases (MBL) and Methicillin-Resistant *Staphylococci aureus* (MRSA) have become very common in India[1-5]. In this situation we do two courses of action, one is antibiotic uses awareness for inhabitants and another one discovers new antibiotics against antibacterial resistance strain from natural sources because the resistant power usually not present in the environmental habit of bacteria but that strain transfer or infected to human become antibacterial resistant power.

## MATERIALS AND METHODS

### Sample collection

Soil samples were collected from Muthupet Mangrove forest (Latitude of 10°46'N Longitude of 79.51'E), Tamilnadu, India in sterile airlock polythene bags and transported to the laboratory according to a previously described method[6]. Collected samples were stored at 4ºC until do further use.

### Preparation of soil samples

The collected soil samples were subjected to pre-treatment of dry heat at 56°C for 10 minutes to increase the number of mycelium-forming actinomycetes relative to the non-actinomycetal heterotrophic microbial flora. After that one gram dried soil samples were added to 10 ml sterile water and further diluted up to $10^{-6}$ dilution in sterile water.

**Isolation of actinomycetes**

0.1 ml of each diluted sample was inoculated by spreading with a sterile glass rod on actinomycetes solation agar medium separately[7]. The media were supplemented with antibiotics of cycloheximide (40 µg/ml), nystatin (30 µg/ml) and nalidixic acid (10 µg/ml) after autoclave to inhibit the fungal and nonfilamentous bacterial growth. The inoculated plates were incubated at 30ºC for 7 to 9 days or until the appearance of colonies with a tough leathery texture, dry or folded appearance and branching filaments with or without aerial mycelia.

**Test Antibiotic-resistant bacterial Strains**

Four antibiotic-resistant bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* were used in this study and obtained from Microbial Type Culture Collection Centre at Chandigarh, India.

**Primary Screening of antibacterial activity**

**Screening of Antagonistic effect**

The log phase antibiotic resistant bacteria cultures were swabbed on prepared muller-Hinton agar plates separately. The ESBL and AmpC detection Ezy MICTM Strip was placed on bacteria cultures swabbed plates. It is a unique phenotypic ESBL and AmpC detection strip which is coated with a mixture of 4 different antibiotics with and without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefotaxime, Cefepime and Cloxacillin (Mixture) + Clavulanic acid with the highest concentration tapering downwards, whereas the lower half is similarly coated with Ceftazidime, Cefotaxime, Cefepime and Cloxacillin (Mixture) in a concentration gradient in the reverse direction. The bacteria cultures swabbed plates top corner were inoculated isolated actinomycetes. All plates were incubated and an antagonistic effect was observed.

**Identification of Actinomycetes**

The positive antagonistic actinomycetes colonies were identified based on the cultural and morphological characteristics[8].

**Preparation of Inoculum**

The isolates actinomycetes were grown on starch casein agar slant at 30˚C for 7 days for complete sporulation. 5ml of sterile water was added to the slant and the spores were scraped and transferred into a 100ml Erlenmeyer flask containing 50ml of broth medium. After inoculation, the flask was incubated at 30˚C in a shaker for 48 hours. The microorganisms were harvested and washed with sterile saline solution and the cells were resuspended in 25ml sterile saline solution. This cell suspension was used as inoculums.

**Fermentation and Extraction of Antibiotic Compounds**

The selected antagonistic actinomycetes were inoculated into starch casein nitrate broth medium separately and incubated at 28ºC on a rotary shaker at 220 rpm for 7 days. After incubation, the broth was filtered through Whatman no1filter paper and then Millipore filter. The filter was transferred aseptically into a conical flask and stored at 4˚C for further study. The culture filtrate mixed with an equal value of ethyl acetate separately and centrifuged at 5000rpm for 10min to extract antimicrobial compounds.

**Disc preparation**

The 6 mm (diameter) discs were prepared from Whatman no.1 filter paper. The discs were sterilized by autoclave at 121˚C. After sterilization, the moisture discs were dried on hot air oven at 50˚C. The discs were impregnated with each extract and left to dry on hot air oven at 40˚C.

**Assay of antibacterial activity**

The antibacterial activity of actinomycetes extracts was analyzed against antibiotic-resistant bacteria by the Disc diffusion assay method[9]. The sterile Muller Hinton agar plates were prepared and the test organisms such as *Escherichia coli, Staphylococcus aureus, Streptococcus pyrogens* and *Pseudomonas aeruginosa* were spread over the Muller Hinton agar plates by using sterile cotton swaps separately. After the bacterial swapping, the prepared extract disk was placed on each plate. All the plates were incubated at 37˚C for 24hours. After incubation, the plates were observed for the zone of inhibition.

## RESULT AND DISCUSSION

In the present study, actinomycetes species were isolated from Muthupet mangrove forest soil samples and analyzed their antagonistic effect. The positive antagonistic actinomycetes colonies were identified and antibacterial activity of positive actinomycetes strains was analyzed against a clinical pathogen. In this study six actinomycetes species were isolated from the mangrove forest soil sample. In this starch casein nitrate agar medium actinomycetes, colonies showed in powdery white colour colonies, which are named as ISA1, ISA2 upto ISA6. Sivakumar[10] reported that the characteristics can be used as markers by which an individual strain can be recognized. Particularly, chemotaxonomy plays a major role in the identification of actinomycetes to generic levels. The West Coast of India, especially from Ernakulam to Kannur has a wide range of salinities and was selected as an ecosystem for studying the diversity of actinomycetes and their antimicrobial properties. The isolated actinomycetes colonies antagonistic effects were analyzed against some antibiotic-resistant bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogens*. Among the 6 Actinomycetes isolates positive antagonistic effect only noted in ISA1 (Table – 1). The positive antagonistic effect results were viewed in Fig.-I. The present study revealed that among the isolates *Streptomyces* was the dominant genera. Frequency and dominance of *Streptomyces* among actinomycetes in various soil types were reported by several workers[11-12]. Alexander[13] reported that about 20-45% of marine actinomycetes exhibited antimicrobial activity; whereas actinomycetes isolated from marine sediments of Visakapatnam, exhibited only 18% of antimicrobial activity as stated by Ellaiah and Reddy[14]. After screening positive antagonistic isolates were identified at a generic level based on cultural and morphological characteristics. Based on the observation, the positive antagonistic isolate ISA1 was confirmed as *Streptomyces parulus*. Tartora *et al.,*[15] reported normally, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhimurium* are even capable of growth in some antibiotics and their resistance to more antibiotics has also been a medical concern. In this study maximum antibacterial activity were noted *Streptococcus pyogenes* (14mm) compare to other test organisms (Table – 2). Marine *Streptomyces sp* exhibited the highest antibacterial activity against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhimurium*. It is interesting to note that this response represents antibiotic potential competing microorganisms against *Pseudomonas aeruginosa, Salmonella typhimurium* and *Klebsiella pneumoniae* in the environment.

**Table – 1 Screening of Antagonistic effect**

| S. No. | Isolated Actinomycetes | Antagonistic effect |
|---|---|---|
| 1 | ISA1 | + |
| 2 | ISA2 | - |
| 3 | ISA3 | - |
| 4 | ISA4 | - |
| 5 | ISA5 | - |
| 6 | ISA6 | - |

***+ Positive; - Negative***

**Fig. -1 Antagonistic effect against antibiotic-resistant bacteria *Streptococcus pyogens***

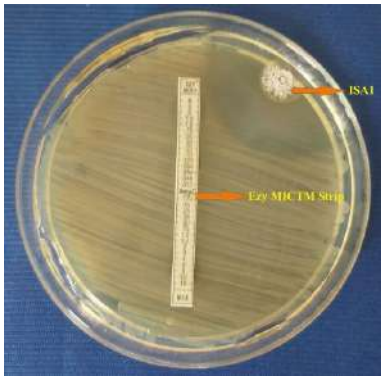

**Table – 2. Antibacterial activities of *Streptomyces purvulus* fermented extracts against some clinical antibiotic-resistant bacteria**

| S. No. | Bacterial Strains | Zone of Inhibition (mm in diameter) (M±SD) n=3 |
|---|---|---|
| 1 | *Escherichia coli* | 10±0.81 |
| 2 | *Pseudomonas aeruginosa* | 08±0.78 |
| 3 | *Staphylococcus aureus* | 12±0.29 |
| 4 | *Streptococcus pyogens* | 14±0.80 |

**Values are expressed Mean ± Standard Deviation; n=3.**

## CONCLUSION

The isolated actinomycetes colonies antagonistic effect was analyzed against some antibiotic-resistant bacteria. The positive antagonistic actinomycetes colony ISA1 was identified based on the cultural and morphological characteristic and confirmed as *Streptomyces parvulus*. After fermentation, the antibiotic compound was extracted using ethyl acetate by centrifugation. Antibacterial compound containing disc was prepared separately, the disc was used for assay of antibacterial activity against clinical pathogens. Among various pathogens, the highest antibacterial activity recorded against *Streptococcus pyogenes* compared the other test bacteria. Finally concluded that the isolated *Streptomyces parvulus,* were highly recommended for antibiotic production at the industrial level, it creates a new sector in the pharmaceutical field. In my further study optimize the productivity of antimicrobial compounds and analyzed the chemical nature of the product.

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# *INVITRO* ANTIBACTERIAL SCREENING OF FUNGI ISOLATED FROM MARINE SOILS OF ANDAMAN ISLANDS

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## ABSTRACT

In the search of bioactive potentials of marine fungi, 16 fungal strains were isolated from Andaman marine habitats. *Invitro* antibacterial efficacy of fungal isolates was evaluated by agar well diffusion method. The aqueous extract of *Aigialus grandis* exhibited a broad spectrum of antibacterial activity against tested pathogens. The maximum inhibition was observed in diethyl ether extract of *Gliocladiopsis* sp. against *Escherichia coli* (21.7 mm). *Penicillium chermesinum* extracts exhibited minimum to moderate activity against the tested pathogens (Inhibition zone ranging 1.0 – 12.7 mm). The standard antibiotic tetracycline has moderate antibacterial activity and the least activity was observed in ampicilin.

**KEYWORDS**: antibacterial efficacy, pathogens, moderate activity, standard antibiotic

## INTRODUCTION

Fungi are one of the important microbial components of the soil. Among the three major habitats of the biosphere, the marine realm which covers 70% of the earth's surface provides the largest inhabitable space for living organisms, particularly microbes. In the oceans, fungi live as saprophytes, parasites and symbionts on various matrices such as sand, logs, water, soil bubbles as well as algae and other microorganism (9 and 16). Marine fungi have proven to be a rich and promising source of novel anticancer,antibacterial, antiplasmodial, anti-inflammatory and antiviral agents [1&4]. Many of these fungi have been proven to be rich source of structurally novel and biologically active secondary metabolites, which are emerging as a significant new chemical resource for drug discovery[2]. The productions of these unique secondary metabolites by marine fungi are possible because of adaptation to a very distinct set of environmental pressures[7].Only very little information is available concerning antimicrobial activities of isolates of marine-derived fung [3]. compared the antimicrobial activities from 1500 cultures of marine isolates to 1450 cultures of terrestrial fungi. It was concluded that a higher number of antimicrobial active species were found among the marine isolates than the terrestrial. The production of antimicrobial metabolites from slow-growing marine fungi was investigated by [11]. Moreover, since marine species are not well investigated more new biologically active compounds were secured than from terrestrial. This study therefore focuses on the investigation of marine fungi isolated from Chidiya Tapu, Andaman Islands, India and antibacterial activities against various strains of bacteria were investigated.

## MATERIALS AND METHODS

### Description of a sampling site

The Andaman Islands are a group of Indian Ocean archipelagic islands in the Bay of Bengal between India to the west, and Burma (also known as Myanmar), to the north and east. It is located between 12°30 ′N to 12.5°N and 92°45 ′E to 92.75°E. Chidiya Tapu in Andaman and Nicobar Islands is 25 km away from the capital, Port Blair. It is located in the southernmost tip of South Andaman. Chidiya Tapu is worldwide famous for its thick, green vegetations, enchanting beaches, collections of corals, colorful butterflies and most importantly, the view of the sun setting in the wine red horizon.

### Collection of Samples

Soil samples were collected at a depth within 10 cm using a metal spatula. The spatula was sterilized every time with 70 percent alcohol. 5 to 7 soil samples were collected randomly and were pooled together. The samples were kept in new polythene bags, sealed and transported to the laboratory for the mycological

examination.

**Dilution plating method**

The dilution plating technique described by (15) was used to isolate the fungi from soils. Soil sample weighting 1g was diluted in 10 ml of 50% seawater (1:1 v/v seawater (30 ppt): distilled water). One ml of the diluted sample ($10^{-2}$ and $10^{-3}$) was poured and spread on petri plates containing a sterilized PDA medium. The inoculated plates were incubated in a dust-free cupboard at room temperature (24±2°C) for 3 - 5 days.

**Preparation of potato dextrose agar medium**

| | | |
|---|---|---|
| Potato | - | 200 gms |
| Dextrose | - | 20 gms |
| Agar | - | 15 gms |
| Distilled water | - | 1000 ml |
| pH | - | 5.6 |

The potato tubers were peeled and weighed for about 200 gms. The tubers were chopped into small pieces with the help of a sterile knife. The chopped potatoes were transferred into a conical flask containing about 1000 ml of distilled water. The contents were boiled for 20 minutes. The supernatant was decanted and filtered through a muslin cloth and the filtrate was collected. To this filtrate dextrose and agar were added and shaken well to dissolve the ingredients and made up to 1000 ml by addition of distilled water. Finally, the medium was autoclaved at121ºC for 20 mins at 15 lbs pressure. Streptomycin sulfate (50µg/ml) was added and mixed well to prevent the bacterial contamination.

**Observations**

The colonies growing on PDA plates with different morphology were counted separately. The fungal cultures were then transferred, subcultured and the pure cultures were maintained on PDA medium. A portion of the growing edge of the colony was picked up with the help of a pair of needles and mounted on a clean slide with lactophenol cotton blue stain. The slide was gently heated in a spirit lamp to facilitate the staining and remove air bubbles if any. The excess stain was removed with the help of tissue paper and then the coverslip was sealed with transparent nail polish. The slide was observed under a compound microscope. Microphotography of the individual fungal species was also taken using a Nikon phase contrast microscope (Nikon, Japan).

**Identification**

Colony colour and morphology were noted besides hyphal structure, spore size, shapes and spore-bearing structures. They were compared with the standard works of Manual of Soil fungi[6]; A Manual of Penicillia[13]; Manual of Aspergillus (12) and Soil fungi[5]

**Screening of fungi for Antibacterial efficacy[3]**

The selected fungal organisms were screened for antibacterial efficacy by the agar well diffusion method. The bacterial pathogens such as *Bacillus subtilis, Enterobacter aerogens, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas putida, Salinicoccus* sp*., Staphylococcus aureus, Salmonella typhi* and *Vibrio cholerae* were obtained from the Microbial Germ Plasm Culture Collection Unit (MGPCCU) at Sri Gowri Biotech Research Academy, Thanjavur, Tamilnadu, India.

**Solvent extraction of fungal isolates [10]**

Three conical flasks were taken and 150 ml of PDB was prepared in each of the three conical flasks using seawater and distilled water mixture in a ratio of 1:1. The selected fungal were inoculated in each of the conical flasks separately and incubated at 28°C for 5 days. After incubation, the fungal mats were taken from each of the flasks and put into each of the four beakers. To this, each of the solvents (diethyl ether, ethyl acetate and distilled water) was added separately and subjected to sonication for 15 mins. The fungal mat extracts were tested against human pathogenic bacteria.

**Assay**

Diethyl ether, ethyl acetate and distilled water extracts were tested for their antibacterial efficacy against the

bacterial pathogens.

**Preparation of nutrient agar medium**

| | | |
|---|---|---|
| Beef extract | - | 3 gms |
| Peptone | - | 5 gms |
| Sodium chloride | - | 5 gms |
| Agar | - | 15 ms |
| Distilled water | - | 1000ml |
| pH | - | 7 |

All the ingredients were weighed and put into the conical flask containing 1000 ml distilled water. The flask was sterilized by using an autoclave at 121°C for 20 min at15 lbs pressure.The nutrient agar medium was poured into the sterile petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using a sterile cork borer. 100 µl fungal extracts were transferred into separate wells. The standard antibiotics (Ampicillin, Penicillin and Tetracyclin) and solvents (ethyl acetate and diethyl ether) were used as positive and negative controls respectively. Then the plates were incubated at 37ºC for 24 hrs. After the incubation, the plates were observed for the formation of a clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

## RESULTS

In the present investigation 16 fungal species belonging to 9 genera were isolated from Chidyatapu, Andaman Islands (Table 1). Besides the above, the maximum number of species diversity was encountered with the fungal species belonging to the class Deuteromycetes (7 genus and 14 species), Ascomycetes (1genus and 1 species) and Phycomycetes (1genus and 1 species).Among the 9 genera recorded, the genus *Aspergillus* (6 species) was dominant followed by *Penicillium* (3 species) and all other genera were represented by one species each. Based on the abundance *Aigialus Grandis, Gliocladiopsis* sp. and *Penicillium chermesinum* were selected for further investigation

**Table 1. Fungal isolates from Chidya Tapu**

| S.No | Fungal Isolates |
|---|---|
| 1. | *Absidia glauca* |
| 2. | *Aigialus grandis* |
| 3. | *Aspergillus awamori* |
| 4. | *A. flavus* |
| 5. | *A. luchensis* |
| 6. | *A. niger* |
| 7. | *A. oryzae* |
| 8. | *A. terreus* |
| 9. | *Cladosporium herbarum* |
| 10. | *Gliocladiopsis* sp. |
| 11. | *Fusarium oxysporum* |
| 12. | *Penicillium chermesinum* |
| 13. | *P. luteum* |
| 14. | *Penicillium* sp. |
| 15. | *Spicaria elegans* |
| 16. | *Verticillium terrestre* |

**Antibacterial efficacy of selected fungal organisms**

The antibacterial efficacy of the selected fungal cultures such as *Aigialus grandis, Gliocladiopsis* sp. and *Penicillium chermesinum* was evaluated by agar well diffusion method. Diethyl ether, ethyl acetate and

distilled water were used as a solvents system for mycelial mats. The tested bacterial pathogens were *Bacillus subtilis, Enterobacter aerogens, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumonia,Pseudomonas putida, Salinicoccus* sp., *Salmonella typhi, Staphylococcus aureus* and *Vibrio cholera.*

**Antibacterial efficacy of *Aigialus Grandis***

The aqueous extract has maximum activity against *Klebsiella oxytoca* (20.7 mm), moderate activity against *Escherichia coli* (16.7 mm), *Pseudomonas putida* (10.3 mm), and least activity against *Bacillus subtilis* (3.3 mm). Diethyl ether extract exhibited minimum to moderate activity against the tested pathogens (Inhibition zone ranging 1.3 – 15.7 mm). Ethyl acetate extract of *Aigialus grandis* showed significant antibacterial activity against *Salmonella typhi* (18.3 mm), *Klebsiella oxytoca* (17.3 mm) and *Escherichia coli* (17.0 mm) and no activity was observed against *Bacillus subtilis, Enterobacter aerogens, Staphylococcus aureus* and *Vibrio cholera* (Table 2).

**Table 2. Antibacterial efficacy of *Aigialus grandis***

| S. No | Bacterial pathogens | Distilled water | Diethyl ether | Ethyl acetate |
|---|---|---|---|---|
| | | Zone of inhibition (Dia in mm) | | |
| 1. | *Bacillus subtilis* | 3.3±2.9 | 4.3±1.2 | - |
| 2. | *Enterobacter aerogens* | 4.0±1.0 | 3.3±2.9 | - |
| 3. | *Escherichia coli* | 16.7±2.9 | 15.7±2.1 | 17.0±2.6 |
| 4. | *Klebsiella oxytoca* | 20.7±2.1 | 11.7±7.6 | 17.3±2.5 |
| 5. | *Klebsiella pneumoniae* | 6.7±2.9 | 5.0±0.0 | 6.7±2.9 |
| 6. | *Pseudomonas putida* | 10.3±2.5 | 7.0±2.6 | - |
| 7. | *Salinicoccus* sp. | 6.7±2.9 | 7.3±2.5 | 6.7±2.9 |
| 8. | *Salmonella typhi* | - | 4.3±1.2 | 18.3±2.9 |
| 9. | *Staphylococcus aureus* | - | 4.0±1.7 | - |
| 10. | *Vibrio cholerae* | 0.7±1.2 | 1.3±2.3 | - |

***Expressed as Mean±SD (n=3); - - no inhibition zone***

**Antibacterial efficacy of *Gliocladiopsis* sp.**

The remarkable activity was exhibited by aqueous extract of *Gliocladiopsis* sp. against *Escherichia coli* (21.7 mm) and *Klebsiella oxytoca* (20.3 mm). The aqueous extract showed weak activity against *Bacillus subtilis* (4.3 mm), *Pseudomonas putida* (6.7 mm), *Salmonella typhi* (4.3 mm) and *Staphylococcus aureus* (3.3 mm). The maximum inhibition was observed in diethyl ether extract against *Escherichia coli* (21.7 mm) followed by *Klebsiella oxytoca* (18.3 mm), *Klebsiella pneumonia* (16.3 mm) and *Pseudomonas putida* (13.3 mm). The diethyl ether extract did not show any activity against *Bacillus subtilis, Enterobacter aerogens* and *Vibrio cholera.* The growth of *Klebsiella pneumonia* and *Pseudomonas putida* (16.7 mm) was strongly inhibited by ethyl acetate extract (Table 3).

**Table 3. Antibacterial efficacy of *Gliocladiopsis* sp.**

| S. No | Bacterial pathogens | Distilled water | Diethyl ether | Ethyl acetate |
|---|---|---|---|---|
| | | Zone of inhibition (Dia in mm) | | |
| 1. | *Bacillus subtilis* | 4.3±1.2 | - | - |
| 2. | *Enterobacter aerogens* | - | - | 3.3±2.9 |
| 3. | *Escherichia coli* | 21.7±2.9 | 21.7±2.9 | 11.7±2.9 |
| 4. | *Klebsiella oxytoca* | 20.3±2.5 | 18.3±2.9 | 13.0±2.0 |

| | | | | |
|---|---|---|---|---|
| 5. | *Klebsiella pneumoniae* | 16.7±2.9 | 16.3±1.5 | 16.7±2.9 |
| 6. | *Pseudomonas putida* | 6.7±2.9 | 13.3±5.8 | 16.7±2.5 |
| 7. | *Salinicoccus* sp. | 10.0±5.0 | 7.3±2.5 | 8.3±2.9 |
| 8. | *Salmonella typhi* | 4.3±1.2 | 3.3±2.9 | 4.3±1.2 |
| 9. | *Staphylococcus aureus* | 3.3±2.9 | 2.7±2.5 | 5.0±0.0 |
| 10. | *Vibrio cholerae* | 16.7±2.9 | - | 1.7±2.9 |

**Expressed as Mean±SD (n=3); - - no inhibition zone**

**Antibacterial efficacy of *Penicillium chermesinum***

Aqueous extract of *Penicillium chermesinum* exhibited moderate activity against *Klebsiella pneumonia* (12.7 mm), *Enterobacter aerogens* (9.3 mm) and *Bacillus subtilis* (8.3 mm). The aqueous extract has no activity against *Escherichia coli, Salinicoccus* sp., *Salmonella typhi* and *Vibrio cholera.* Diethyl ether extracts showed a zone of 13.3 mm and 10.0 mm of inhibition against *Klebsiella pneumonia* and *Enterobacter aerogens* respectively. Antibacterial activity of ethyl acetate extract was maximum against *Klebsiella pneumonia* (12.7 followed by *Enterobacter aerogens* (11.7 mm), *Staphylococcus aureus* (11.0 mm), *Klebsiella oxytoca* (7.7 mm), *Bacillus subtilis* (6.7 mm), *Pseudomonas putida* (6.7 mm), *Salinicoccus* sp. (6.7 mm), *Salmonella typhi* (6.7 mm), and there is no zone of inhibition in *Escherichia coli* and *Vibrio cholera* (Table 4).

**Table 4. Antibacterial efficacy of *Penicillium chermesinum***

| S. No | Bacterial pathogens | Distilled water | Diethyl ether | Ethyl acetate |
|---|---|---|---|---|
| | | Zone of inhibition (Dia in mm) | | |
| 1. | *Bacillus subtilis* | 8.3±2.9 | 8.3±2.9 | 6.7±2.9 |
| 2. | *Enterobacter aerogens* | 9.3±1.2 | 10.0±2.0 | 11.7±2.9 |
| 3. | *Escherichia coli* | - | - | - |
| 4. | *Klebsiella oxytoca* | 6.7±2.9 | 6.7±2.9 | 7.7±2.1 |
| 5. | *Klebsiella pneumoniae* | 12.7±2.5 | 13.3±2.9 | 12.7±2.5 |
| 6. | *Pseudomonas putida* | 6.7±2.9 | 7.7±2.5 | 6.7±2.9 |
| 7. | *Salinicoccus* sp. | - | 2.3±2.5 | 6.7±2.9 |
| 8. | *Salmonella typhi* | - | 1.0±1.7 | 6.7±2.9 |
| 9. | *Staphylococcus aureus* | 6.7±2.9 | 5.0±0.0 | 11.0±1.7 |
| 10. | *Vibrio cholerae* | - | - | - |

***Expressed as Mean±SD (n=3); - - no inhibition zone***

The antibiotic sensitivity test using standard Ampicillin, Penicillin and Tetracyclin were tested against tested bacteria strains. The results of the antibiotic sensitivity test were presented in table 5. Tetracycline antibiotics exhibited higher antibacterial activity compared with Ampicillin, and Penicillin. The zone of inhibition was ranging between 5 -15 mm. The result of the antimicrobial effect of five solvents revealed no activity against the tested pathogens.

**Table 5. Antibacterial efficacy of Standard antibiotics (Positive control)**

| S. No | Bacterial pathogens | Ampicillin | Penicillin | Tetracyclin |
|---|---|---|---|---|
| | | Zone of inhibition (Dia in mm) | | |
| 1. | *Bacillus subtilis* | - | 5 | 7 |
| 2. | *Enterobacter aerogens* | 3 | 5 | 10 |
| 3. | *Escherichia coli* | 3 | - | 13 |
| 4. | *Klebsiella oxytoca* | - | - | 10 |
| 5. | *Klebsiella pneumoniae* | - | - | 5 |
| 6. | *Pseudomonas putida* | 5 | - | 15 |
| 7. | *Salinicoccus roseus* | - | - | 5 |
| 8. | *Salmonella typhi* | - | - | 5 |
| 9. | *Staphylococcus aureus* | - | - | 12 |
| 10. | *Vibrio cholerae* | - | - | 15 |

***- - no inhibition zone***

## DISCUSSION

The marine fungi investigated in this study were isolated from Chidiya Tapu, Andaman Islands. Among the 16 fungal isolates *Aigialus Grandis, Gliocladiopsis* sp. and *Penicillium chermesinum* were selected for antibacterial efficacy based on the abundance.The aqueous extract of *Aigialus grandis* showed maximum activity against *Klebsiella oxytoca* (20.7 mm), moderate activity against *Escherichia coli* (16.7 mm), *Pseudomonas putida* (10.3 mm), and least activity against *Bacillus subtilis* (3.3 mm). Antibacterial activity of aqueous extract of *Gliocladiopsis* sp. exhibited promising activity against *Escherichia coli* (21.7 mm) and *Klebsiella oxytoca* (20.3 mm). Diethyl ether extracts of *Penicillium chermesinum* showed a zone of 13.3 mm and 10.0 mm of inhibition against *Klebsiella pneumonia* and *Enterobacter aerogens* respectively. Recently[14] reported that the antibacterial activity of 11 fungal isolates from Indonesian marine habitats. *Aspergillus* sp. was the most active fungus against *Bacillus subtilis* and *Staphylococcus aureus*. Similarly (8) studied that the most active fungal isolate was identified as *Penicillium viridicatum* against the tested bacterial strains.

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SP-63

# STUDIES ON ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF MARINE SEAWEEDS *Ulva reticulate* AND *Ulva lactuca*

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## ABSTRACT

The bacterial cultures *viz., Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes, Bacillus subtilis, Bacillus aureus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera, Salmonella typhi, Klebsiella pneumonia, Enterobacter aerogenes and the fungal cultures viz., Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Candida albicans, Candida glabrata* and *Saccharomces cetevisiae* were procured rom MTCC, Chandigarh. The highest mean zone of inhibition was observed methanol extract of *Ulva lactuca* against all tested bacterial and fungal pathogens when compared to *Ulva reticulate* and maximum antioxidant activity also observed. The GC-MS study revealed the presence of nine different bioactive components *viz.,* Hexadecanem 1,10-Decanediol, 2-Hepatone, 5-methyl, 2,4-Dimethylcyclopentanol, Pentanal, 2- methyl, S(2,-3S)–(-)-3-Propyloxiranemethanol, 3,4-Hexanediol, 2,5-dimethyl-, Octanem 3,4,5,6-tetramethyl-and Diazoprogesterone. Their results showed the use of Seaweeds as antimicrobial agents for pharmacology or as a health promoting food for aquaculture. The screening results confirmed that these Seaweeds can be further studied and used as possible source of antimicrobial compounds.

**Keywords:** Antioxidant, Marine seaweeds, *Ulva reticulate*, *Ulva lactuca*

## INTRODUCTION

Marine algae are one of the largest producers of biomass in the marine environment[1]. Active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids are produced by several species of marine macro and micro algae and have antibacterial, antialgae, anti macrofouling and antifungal properties which are effective in the prevention of biofouling and have other likely uses, as in therapeutics[2]. More than 10,000 species of marine algae have been reported all over the world. In India, about 220 genera and 740 species of marine algae were recorded of which 60 species are of economic value. Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with antiviral, antifungal, antibacterial and antifungal activities[3]. The antibacterial activity of seaweeds was generally assayed using extracts in various orangic solvents viz., acetone, methanol-toluene, petroleum ether and chloroform- methanol several extractable compounds, such as cyclic polysulfides and halogenated compounds are toxic to microorganisms and therefore, responsible for the antibiotic activity of some seaweeds. The various red algae particularly *Corallina officinalis, coralline rubens* and *Alsidium helminnthocorton* were employed as vermifuges in ancient times. Dulse is a laxative and also used to reduce fever. Several red algae such as *Chodurus crispus, Guacilaia sp., Gelidium sp.,* and *Pterrocladia sp.,* have been used to treat various stomach and intestinal disorders. The stipes of *Laminaria cloustoni* have been used aiding child birth by distending the uterus during labour. A number of marine algae have been found to have anticoagulant and antibiotic properties. *Carrageenan* was used in ulcer therapy and alginates are found to prolong the rate of activity of certain drugs.

***Antioxidant Activity***

Antioxidants in biological systems have multiple functions such as to protect for oxidative damage. The major action of antioxidant in cells is to prevent damage caused by the action of reactive oxygen species and reactive nitrogen species. Those compounds cause extensive oxidative damage to cells leading to age related diseases, cancer and wide range of other human disease[4].

## MATERIALS AND METHODS

***Collection of Seaweeds***

The seaweeds *viz., Ulva reticulate* and *ulva lacuca* (Plate. 1) were collected form mandapam coast of Tamil Nadu, India (Fig. 1) that is situated in $9^0$17N latitude and $79^0$07'E longitude and having 9 m MSL in Tamil Nadu. The seaweed was taxonomically identified at the Centre for Advanced Studies in Marine Biology, Annamalai University.

***Taxonomy an occurrence of collected seaweeds***

**Occurrence and distribution of *Ulva reticulate***

*Ulva reticulate* is widely distributed in the coastal area of India (Andaman & Nicobar Islands), Saudi Arabia, Yemen, Sri Lanka, Pakistan, Japan, Australia and New Zealand. Green to dark green in colour, this species in the Phylum Chlorophyta is formed of two layers of cells irregularly arranged, as seen in cross section, the chloroplast is cup-shaped with 1 to 3 pyrenoids.

***Preparation of seaweed extracts***

The collected seaweeds samples were cleaned and the necrotic parts were removed. The seaweed materials (1.0Kg) were ground ta a fine powder using electrical blender. Forty grams of powdered seaweeds were extracted successively with 200 ml of solvents (methanol, acetone, chloroform, hexane and ethyl acetate) in Soxhlet extractor (Fig 1) until the extract was clear. The extracts were evaporated to dryness by reduced pressure using rotary vacuum evaporator and the resulting pasty from extracts were stored in a refrigerator at $4^0$C for future use[5].

***Collection of test bacterial cultures***

Eleven different bacterial cultures of Gram positive and Gram negative bacteria were procured from Microbial Type culture Collection (MTCC), Chandigarh. (plate 2).

***Gram positive bacteria***

a) *Staphylococcus aureus* (MTCC 3160)
b) *Streptococcus epidermis* (MTCC 889)
c) *Streptococcus pyogenes* (MTCC 1926)
d) *Bacillus subtilis* (MTCC 1427)
e) *Bacillus cereus* (MTCC 7417)

***Gram negative bacteria***

a) *Escherichia coli* (MTCC 1195)
b) *Pseudomonas aeruginosa* (MTCC 7093)
c) *Vibrio cholerae* (MTCC 3904)
d) *Salmonella typhi* (MTCC 3215)
e) *Klebsiella pneumonia* (MTCC 4032)
f) *Enterobacter aerogenes* (MTCC 6804)

***Collection of test fungal cultures***

Fungal cultures were purchased from MTCC, Chandigarh.

a) *Aspergillus flavus* (MTCC 1883)
b) *Aspergillus niger* (MTCC 4285)
c) *Aspergillus fumigatus* (MTCC 4964)
d) *Saccharomyces cerevisiae* (MTCC 2627)
e) *Candida glabrata* (MTCC 3983)

**Cultures maintenance and inoculum preparation**

***Maintenance of test bacterial cultures***

The test bacterial isolates were sub-cultured and maintained on suitable media for plate and stored in refrigerator.

**Inoculum preparation**

Standard methodology was followed for inoculum preparation.

**Disc preparation**

The seaweeds crude extracts (2.5 mg/ml and 5mg/ml) obtained using solvents (methanol, acetone, chloroform, hexane and ethyl acetate) were mixed with 1ml of 5% Dimethyl sulfoxide (DMSO).

***Preparation of algal disc for antifungal activity***

6 mm diameter disc were prepared using sterile Whatman No. 1 filter paper. The seaweeds crude extracts (10 mg/ml) obtained using solvents (methanol, acetone, chloroform, hexane and ethyl acetate) were mixed with 1ml of 5% Dimethyl sulfoxide (DMSO).

***Antibacterial Assay***

***Disc diffusion method***

The antibacterial activity of seaweed extracts was determined by disc diffusion method[6]. Mueller Hinton agar was used for antibacterial activity. Ampicillin (5 ug/disc) was used as positive control.

***MIC for bacteria***

MIC was determined with the help of Mueller Hinton broth by broth macro dilution method[7].

***Antifungal Assay***

***Disc diffusion method***

The flucanozole (100 units/disc) was used as positive control and the 5% DMSO was used as a blind control in these assays. The zone of inhibition was observed and measured in millimetres. Each assay in these experiments was repeated three times for concordance.

***Analysis of the phytocompounds in Ulva lactuca using gas chromaography (GC-Ms) technique***

Three micro liter of the filtrate of *Ulva lactuca* methanol extract was injected into the GC column. There, the sample got evaporated and carried away by the carrier gas, helium and it got segregated into individual compounds. The samples fraction coming out of the column was let into the mass detector and the mass spectrum of each component was recorded. The mass spectrum of the unknown component was compared with known spectrum of NIST library and the components were identified.

**RESULT**

The bioactivity of two different marine seaweeds crude extracts viz., *Ulva retifulate* and *Ulva lactuca* were evaluated against pathogenic bacteria.

***Antibacterial activity of crude seaweeds extracts***

***Ulva reticulate***

The antibacterial activity of marine seaweed crude extracts of *Ulva reticulate* was investigated against Gram Positive and Gramnegative bacteria and the results were given in Table-1. The zone of inhibition of *Ulva reticulate* crude extracts against Gram Positive and Gram negative bacterial was ranged between 7 mm to 16mm at 5.0 mg/ml. The minimum inhibitory concentration (MIC) value of *Ulva reticulate* against tested bacteria was ranged between 2.50 mg/ml to 80 mg/ml and the results were showed in table 2. The lowest MIC (2.50 mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia* and *Enterobacter aerogenes.*

**Table-1: Antibacterial activity of crude extracts of *Ulva lactuca***

| Concentration of the seaweed extracts (mg/ml) and Zone of inhibition (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Microorganisms*** | **Methanol** | | **Acetone** | | **Chloroform** | | **Hexane** | | **Ethyl acetate** | | **Positive Control*** |
| | 2.5 | 5 | 2.5 | 5 | 2.5 | 5 | 2.5 | 5 | 2.5 | 5 | |
| *Staphylococcus aureus* | 11±0.5 | 13±0.6 | 10±0.8 | 13±0.3 | 7±0.3 | 9±0.4 | 6±0.2 | 10±0.7 | 7±0.4 | 10±0.3 | 16±0.5 |
| *Streptococcus pyogenes* | 11±0.3 | 10±0.3 | 11±0.3 | 13±0.5 | 6±0.4 | 10±0.3 | 5±0.6 | 8±0.6 | 8±0.6 | 11±0.5 | 18±0.3 |
| *Streptococcus epidermidis* | 10±0.5 | 12±0.3 | 10±0.4 | 13±0.7 | 7±0.3 | 11±0.5 | 6±0.8 | 9±0.5 | 8±0.2 | 12±0.4 | 14±0.8 |
| *Bacillus cereus* | 13±0.6 | 16±0.2 | 10±0.8 | 13±0.4 | 7±0.4 | 11±0.4 | 7±04 | 10±0.3 | 7±0.3 | 9±0.6 | 19±0.6 |
| *Escherichia coli* | 13±0.5 | 16±0.3 | 10±0.5 | 14±0.2 | 6±0.5 | 9±0.5 | 6±0.5 | 8±0.4 | 6±0.5 | 9±0.5 | 17±0.5 |
| *Pseudomonas aeruginosa* | 11±0.2 | 15±0.2 | 11±0.8 | 14±0.4 | 8±0.5 | 12±0.6 | 7±0.6 | 10±0.3 | 7±0.4 | 10±0.4 | 17±0.3 |
| *Vibrio cholera* | 8±0.6 | 10±0.2 | 8±0.3 | 11±0.3 | ±8±0.5 | 12±0.4 | 8±0.3 | 11±0.4 | 6±0.3 | 9.5 | 16±0.5 |
| *Salmonella typhi* | 10±0.5 | 13±0.3 | 9±0.2 | 12±0.4 | 6±0.4 | 9±0.3 | 7±0.5 | 9±0.6 | 6±0.6 | 9±0.6 | 19±0.6 |
| *Klebsiella pneumoniae* | 12±0.6 | 15±0.6 | 12±0.5 | 14±0.6 | 8±0.6 | 13±0.2 | 7±0.6 | 9±0.3 | 8±0.6 | 11±0.7 | 20±0.8 |
| *Enterobacter aerogenes* | 11±0.2 | 15±0.7 | 11±0.2 | 13±0.3 | 8±0.4 | 12±0.5 | 6±0.5 | 9±0.5 | 8±0.5 | 12±0.8 | 17±0.4 |

Mean±SD,*Ampicillin (5µg)

**Table-2: Minimum inhibitory concentration of curde extracts of *Ulva reticulate***

| Minimum inhibitory concentration (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **Microorganisms** | **Hexane** | **Methanol** | **Acetone** | **Chloroform** | **Ethyl acetate** | **Positive control*** |
| *Staphylococcus aureus* | 40 | 2.50 | 5 | 20 | 10 | 5 |
| *Streptococcus pyogenes* | 80 | 5 | 10 | 40 | 10 | 10 |
| *Streptococcus epidermidis* | 80 | 5 | 10 | 20 | 10 | 10 |
| *Bacillus subtilis* | 40 | 2.50 | 5 | 10 | 5 | 10 |
| *Bacillus cereus* | 20 | 2.50 | 5 | 10 | 10 | 10 |
| *Escherichia coli* | 20 | 2.50 | 5 | 10 | 5 | 5 |
| *Pseudomonas aeroginosa* | 20 | 5 | 10 | 20 | 10 | 5 |
| *Vibrio cholera* | 40 | 10 | 20 | 40 | 40 | 20 |
| *Salmonella typhi* | 80 | 20 | 20 | 80 | 40 | 20 |
| *Klebsiella pneumonia* | 20 | 1.25 | 2.50 | 10 | 5 | 10 |
| *Enterobacter aerogenes* | 20 | 2.25 | 2.50 | 10 | 5 | 10 |

Mean±SD,*Ampicillin (5µg)

**Table-3: Antibacterial activity of crude extracts of *Ulva lactuca***

| Concentration of the seaweed extracts (mg/ml) and Zone of inhibition (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Microorganisms** | **Methanol** | | **Acetone** | | **Chloroform** | | **Hexane** | | **Ethyl acetate** | | **Positive Control*** |
| | 2.5 | 5 | 2.5 | 5 | 2.5 | 5 | 2.5 | 5 | 2.5 | 5 | |
| *Staphylococcus aurueus* | 11±0.5 | 13±0.6 | 10±0.8 | 13±0.3 | 7±0.3 | 9±0.4 | 6±0.2 | 10±0.7 | 7±0.4 | 10±0.3 | 16±0.5 |
| *Streptococcus pyogenes* | 11±0.3 | 10±0.3 | 11±0.3 | 13±0.5 | 6±0.4 | 10±0.3 | 5±0.6 | 8±0.6 | 8±0.6 | 11±0.5 | 18±0.3 |
| *Streptococcus epidermidis* | 10±0.5 | 12±0.3 | 10±0.4 | 13±0.7 | 7±0.3 | 11±0.5 | 6±0.8 | 9±0.5 | 8±0.2 | 12±0.4 | 14±0.8 |
| *Bacillus cereus* | 13±0.6 | 16±0.2 | 10±0.8 | 13±0.4 | 7±0.4 | 11±0.4 | 7±04 | 10±0.3 | 7±0.3 | 9±0.6 | 19±0.6 |
| *Escherichia coli* | 13±0.5 | 16±0.3 | 10±0.5 | 14±0.2 | 6±0.5 | 9±0.5 | 6±0.5 | 8±0.4 | 6±0.5 | 9±0.5 | 17±0.5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 11±0.2 | 15±0.2 | 11±0.8 | 14±0.4 | 8±0.5 | 12±0.6 | 7±0.6 | 10±0.3 | 7±0.4 | 10±0.4 | 17±0.3 |
| *Vibrio cholera* | 8±0.6 | 10±0.2 | 8±0.3 | 11±0.3 | ±8±0.5 | 12±0.4 | 8±0.3 | 11±0.4 | 6±0.3 | 9.5 | 16±0.5 |
| *Salmonella typhi* | 10±0.5 | 13±0.3 | 9±0.2 | 12±0.4 | 6±0.4 | 9±0.3 | 7±0.5 | 9±0.6 | 6±0.6 | 9±0.6 | 19±0.6 |
| *Klebsiella pneumoniae* | 12±0.6 | 15±0.6 | 12±0.5 | 14±0.6 | 8±0.6 | 13±0.2 | 7±0.6 | 9±0.3 | 8±0.6 | 11±0.7 | 20±0.8 |
| *Enterobacter aerogenes* | 11±0.2 | 15±0.7 | 11±0.2 | 13±0.3 | 8±0.4 | 12±0.5 | 6±0.5 | 9±0.5 | 8±0.5 | 12±0.8 | 17±0.4 |

Mean±SD,*Ampicillin (5µg)

**Table-4: Minimum inhibitory concentration of crude extracts of *Ulva lactuca***

| Minimum inhibitory concentration (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **Microorganisms** | **Hexane** | **Methanol** | **Acetone** | **Chloroform** | **Ethyl Acetate** | **Positive Control*** |
| *Staphylococcus Aureus* | 40 | 2.50 | 5 | 20 | 10 | 5 |
| *Streptococcus pyogenes* | 80 | 5 | 10 | 40 | 10 | 10 |
| *Streptococcus epidermidis* | 40 | 5 | 10 | 20 | 10 | 10 |
| *Bacillus subtilis* | 20 | 2.50 | 5 | 10 | 5 | 10 |
| *Bacillus cereus* | 20 | 2.50 | 5 | 10 | 5 | 10 |
| *Escherichia coli* | 20 | 2.50 | 5 | 10 | 10 | 10 |
| *Pseudomonas Aeruginosa* | 40 | 5 | 10 | 20 | 10 | 5 |
| *Vibrio cholerae* | 80 | 10 | 20 | 40 | 40 | 20 |
| *Salmonella typhi* | 80 | 20 | 20 | 80 | 40 | 20 |
| *Klebsiella pneumoniae* | 20 | 1.25 | 2.50 | 10 | 5 | 10 |
| *Enterobacter aerogenes* | 20 | 2.50 | 2.50 | 10 | 5 | 10 |

Mean±SD,*Ampicillin (5µg)

***Ulva lactuca***

The antibacterial activity of marine seaweeds crude extracts of *Ulva lactuca* was investigated against Gram Positive and Gram-negative bacteria.

***Antifungal activity of crude extracts of seaweeds***

***Ulva reticulate***

The antifungal activity of marine seaweed crude extracts of *Ulva reticulate* was investigated against fungal Pathogens (*Asergillus niger, Aspergillus flavus, Aspergillus fumigatus, Saccharomyces cerevisiae, Candida albicans* and *Candida glabrata*) and the results were given in Table-5.

***Ulva lactuca***

The antifungal activity of marine seaweed crude extracts of *Ulva lactuca* was investigated against fungal isolates (*Aspergillus niger, Aspergills flavus, Aspergilluss fumigatus, Saccharomyces cerevisiae, Candida albicans* and *Candida glabrata*). Minimum inhibitory concentration (MIC) value of *Ulva lactuca* against tested fungi was ranged between 4 mg/ml to 64 mg/ml and the results were showed inTanle-8. The lowest MIC (4mg/ml) value of methanol crude extract was recorded against *Candida albicans* and *Candida glabrata.*

**Table-5: Antifungal activity of crude extracts of *Ulva reticulate***

| **Microorganisms** | **Hexane** | **Ethyl Acetate** | **Acetone** | **Methanol** | **Chloroform** | **Positive Control*** |
|---|---|---|---|---|---|---|
| *Aspergillus flavus* | 6±0.3 | 8±0.5 | 10±0.3 | 13±0.6 | 8±0.3 | 15±0.6 |
| *Aspergillus niger* | 6±0.3 | 7±0.6 | 8±0.5 | 8±0.5 | 7±0.4 | 13±0.4 |
| *Aspergillus fumigatus* | 5±0.5 | 6±0.3 | 8±0.4 | 10±0.3 | 6±0.5 | 14±0.7 |
| *Saccharamyces cerevisiae* | 5±0.4 | 6±0.7 | 6±0.4 | 7±0.6 | 5±0.6 | 10±0.6 |
| *Candida albicans* | 6±0.5 | 8±0.4 | 9±0.4 | 9±0.4 | 6±0.4 | 12±0.7 |
| *Candida glabrata* | 6±0.6 | 8±0.3 | 8±0.5 | 9±0.6 | 7±0.3 | 13±0.6 |

**Table-6: Antifungal activity of crude extracts od *Ulva lactuca***

| Concentration of the seaweed extracts (10mg/ml) and Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|
| **Microorganisms** | **Hexane** | **Ethyl Acetate** | **Acetone** | **Methanol** | **Chloroform** | **Positive Control*** |
| *Aspergillus flavus* | 6±0.3 | 10±0.3 | 10±0.3 | 12±0.5 | 8±0.4 | 15±0.6 |
| *Aspergillus niger* | 6±0.6 | 8±0.5 | 8±0.4 | 9±0.5 | 7±0.3 | 13±0.4 |
| *Aspergillus fumigatus* | 6±0.3 | 8±0.5 | 9±0.5 | 11±0.3 | 8±0.3 | 14±0.7 |
| *Saccharomyces cerevisiae* | 6±0.4 | 7±0.4 | 7±0.4 | 8±0.2 | 7±0.1 | 10±0.6 |
| *Candida albicans* | 7±0.5 | 10±0.5 | 11±0.3 | 12±0.6 | 8±0.3 | 12±0.7 |
| *Candida glabrata* | 9±0.3 | 7±0.4 | 11±0.6 | 13±0.5 | 7±0.5 | 13±0.6 |

***Flucanozole(100 units/disc)**

**Table-7: Minimum inhibitory concentration of crude extracts of *Ulva lactuca***

| Minimum inhibitory concentration (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **Microorganisms** | **Hexane** | **Methanol** | **Acetone** | **chloroform** | **Ethyl Acetate** | **Positive Control*** |
| *Aspergillus flavus* | 32 | 8 | 8 | 32 | 16 | 8 |
| *Aspergillus niger* | 64 | 8 | 16 | 32 | 32 | 8 |
| *Aspergillus Fumigutus* | 32 | 8 | 8 | 32 | 16 | 8 |
| *Saccharomyces Cerevisiae* | 64 | 16 | 32 | 32 | 16 | 32 |
| *Candida albicans* | 32 | 4 | 8 | 16 | 8 | 4 |
| *Candida glabrata* | 32 | 4 | 8 | 16 | 16 | 4 |

***Flucanozole**

***Antioxidant activity***

The active fractions were lyophilized and used for antioxidant and anticancer activities. In the present study, *Ulva reticulate* and *Ulva lactuca* was evaluated for its Radical scavenging activity using DPPH. The effects of *Ulva reticulate* on the antioxidant activity in vitro, the DPPH scavenging rate of the purified antibiotic compound hypothetical protein was examined. When the compound concentration was increased from 2.0 to 10 mg/ml, the DPPH scavenging rate was 25%, 40%, 65%, 80% and 95.4% respectively (Table -9). The compound concentration was increased from 2.0 to 10 mg/ml, the DPPH scavenging rate was 25.3%, 48%. 69%, 84.7% and 96.1% respectively (Table-10).

**Table – 8: Scavenging effect on DPPH radicals by *Ulva reticulate***

| S.No | Sample concentration (mg/ml) | DPPH scavenging rate (%) |
|---|---|---|
| 1 | 2 | 25 |
| 2 | 4 | 40 |
| 3 | 6 | 65 |
| 4 | 8 | 80 |
| 5 | 5 | 95.4 |

**Table -9: Scavenging effect of DPPH radicals by *Ulva lactuca***

| S.No | Sample concentration (mg/ml) | DPPH scavenging rate (%) |
|---|---|---|
| 1 | 2 | 25.3 |

| | | |
|---|---|---|
| 2 | 4 | 48 |
| 3 | 6 | 69 |
| 4 | 8 | 84.7 |
| 5 | 10 | 96.1 |

***Gc – Ms analysis of bioactive compounds***

Among the extracts obtained from two seaweeds, *Ulva lactuca* showed highest antibacterial activity. Hence, *Ulva lactuca* was selected for compound identification by using GC-MS analysis. This study reveals the presence of following nine different compounds.

## DISCUSSION

Seaweeds are the eukaryotic organisms that lives in salty water of the ocean and recognized as a potential source of bioactive natural products[8]. They contain compounds ranging from sterols, terpenoids to brominated phenolics, which show bioactivity against microorganisms. Seaweeds are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharamaceutical agents[9&10]. The crude extract of *Ulva pertusa* was more active against *Staphylococcus aureus* and *Bacillus subtilis* but less active against *Escherichia coli*[11&12]. *Ulva spp*., have been reported as antibacterial agents, antifungal agents, antiviral and anti mycoplasma agents[13]. However, their antioxidant activity and anticancer properties were not dealt widely. There has been an expanding tendency of the genus Ulva and a species called as *U. laetevirens* has been[14] reported. off the northeast coast of the United States. Also, [15]palmitic acid as the major component of the total fatty acids in U. reticulata, and stated that these fats and fatty acids from marine algae may play an important role in the formation of many other bioactive secondary metabolites which exhibit their inherent antibacterial activity. So, in our study also support with above discussion.

## CONCLUSION

These results suggest the possibility of using marine algal extracts in therapy as natural alternatives to antibiotics currently in the market and has clearly showed the seaweeds from the coast of Mandapam in Tamil Nadu are valuable source of biologically active compounds. Further, this research paves way to determine the structure and nature of these bioactive components from marine species which constitutes approximately a half of the total global biodiversity.

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SP-64

# CHARACTERIZATION AND ANTI BIOGRAM OF MICROBES ON BURN WOUND SAMPLE

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## ABSTRACT

A major burn can define as any burn that requires intravenous fluid resuscitation (10% body surface area (BSA) in a child, 15% in an adult) and/or a burn to the airway. Practical thickness burns can be further divided into superficial (1st degree) and deep (2nd degree), which refers to the depth at which the dermal layer is injured. Sensation is preserved and healing of the skin more likely. Persistence of a multi drug resistant *Pseudomonas aeruginosa* clone in an intensive case burn unit. Hospital infection may occur sporadically or as outbreaks. Etiological diagnosis is by the routine bacteriological methods of smear culture, identification and sensitivity testing. When an outbreak occurs, the source should be identified and eliminated. Typing of isolate phage, bacteriocin, antibiogram or bio typing – from cases and sites may indicate a causal connection. Obvious examples of sources of hospital outbreaks are nasal carriage of *Staphylococci* by surgeons or *Pseudomonas* growing in hand lotions.

**KEYWORDS :** Biogram, wound sample, *Staphylococci*, *Pseudomonas*, burns, injury

## 1.INTRODUCTION

The potential wound pathogens like bacteria are categorized according to differences in their shape and cell wall. Cocci (spherical shaped cells) Bacilli (rods) and Sprirochaetes (spirals). They can be visualized using a bacteriological staining process called Gram staining. The growth and survival of all bacteria is dependent upon environmental factors. Strict aerobes require oxygen whereas anaerobes are rapidly killed by oxygen. The *Staphylococcus aureus* "Super bug" J.Clin. Investin.[19]. *Staphylococcal septicaemia* in burns.[21] emergence of vancomycin resistance during therapy against methicillin – resistant *Staphylococcus aureus* in burn patient importance of low–level resistance to vancomycin.[22] Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other Aerobic Bacteria on Human skin. Coagulase negative *Staphylococci* as a cause of symptomatic urinary infections in children. A classification of *Micrococc*i and *Staphylococci* based on physiological and biochemical tests[4]. Reduction in *Staphylococcus*. Systemic reviews of wound care management Dressings and topical agents used in the healing of chronic wounds[1]. Silver[2] toxicity in mammals and low its products aid wound repair regulation of wound healing from a connective tissue perspective[14]. The effect of Ultrasonic and thermal treatment of wounds[29]. Inflammatory changes in the skin adjacent to the burn are typical of burn wounds. However, areas that have progressive erythema or become more painful indicate cellulites. Cellulites may not be associated with fever, tachycardia, or leukocytosis. Prehospital and emergency department burn care. Role of the gastrointestinal tract in burn sepsis[3]. Advances in burn wound care[22]. Early excision and grafting of face and neck burns in patients over 20 years[7]. *Stenotrophomonas Maltophilia*: a serious and rare complication in patients suffering from burns[8]. Burn injury and pulmonary sepsis: development of clinically relevant model[9]. Hypertrophic burn scars: analysis of variables[11]. The use of a laminar airflow isolation system for the treatment of major burns[12]. A major burn can be defined as any burn that requires intravenous fluid resuscitation (10% body surface area (BSA) in a child, 15% in an adult) and/or a burn to the airway. Beyond simple erythema. Burns are either partialor full thickness depending on whether the basement membrane has been lost. Quantitative bacteriologic study of the burn wound surface[5]. Practical bacteriologic monitoring of the burn victim[13].

Bacteria and wound healing[15]. Utility of Gram stain for the microbiological analysis of burn wound surfaces[17]. Changes of microbial flora and wound colonization in burned patients[8]. *Pseudomonas* are the dominant spoilage psychograph species include *Pseudomonads aeruginosa, P.frag*i and *P. lundensis* which is fluorescent *Pseudomonads* are common in soil and water and are part of the natural surface flora of many plants. Some species of *Pseudomonads* produce pyoverdine or fluorescein. Which are ware soluble fluorescent pigments. These pigments can be observed in spoiled food by using ultraviolet light.

## 2. MATERIALS AND METHODS

### 2.1 SAMPLING AREA

The samples for bacteriological analysis were collected from Kumbakonam Government Hospital, Thanjavur District in Tamilnadu that particular lady was burned entire body and the infections wound is more Ulcertative. The sample collected by careful collection methods. It is collected by swabbing methods. The swab kept on sterile container and it was taken to the laboratory safely for further bacteriological and fungal analysis.

### 2.2 TRANSPORT OF SAMPLES

The sample was carried to the laboratory in a sterile, culture vessel and were kept in aseptically it was sent to the laboratory for microbial analysis. The sample collected on sterile cotton – tipped applicator stick in Stuart transport medium. The applicator stick was touched in the wound material collected in a sterile tube.

## 3. MICROSCOPIC EXAMINATION[16]

### 3.1 HANGING DROP METHOD

Motility of bacteria is identified using a loopful of overnight broth culture of the isolate by hanging drop method.

### 3.2 GRAM'S STAIN

A smear of suspended colony was made on a clean glass slide and heat fixed it was flooded with crystal violet solution and allowed to remain for one minute. Then it washed with water flooded with iodine solution and allowed to stand for one minute. It was then drained and decolorized with 95% ethanol for 15-30 seconds and then washed and counter stained with safranin for one minute then examined under microscope.

## 4. BIOCHEMICAL TEST[14]

Indole Production Test
Methyl Red Test
VP – Test
Citrate Utilization Test
Starch Hydrolysis
Catalase Test
Urease Test
Lipid Hydrolysis
Antibiotic Sensitivity Test

Sterile Muller Hinton agar plates were prepared and the plated were swabbed nearly with the isolated culture. A few antibiotic discs were selected and placed on the media using sterile forceps. Care was taken that the distance between the two discs placed on the media should be at least 25mm apart. Hence the zone produced by the two adjacent antibiotics disc cannot coincide each other. All the plates were incubated at 370C for 28 hours.

## 4. RESULT

The Bio-chemical test for the organisms isolated from various wound samples. *P.aeruginosa* shows gram negative motile. *S.aureus* is gram positive cocci non motile organisms. Showing antibiotic sensitive pattern of wound samples. The *S.aureus* is sensitive to ciprofloxacin, gentamycin, chloramphenicol, lincomycin and vancomycin. They are resistance to Co-trimoxazole and Ceftaxime and Norfloxacin. *P.aeroginosa* is

sensitive to norfloxacin, amikacin, gentamycin, norfloxacin, tobramycin and co-trimoxazole. They are resistance to Chloramphenicol, lincomycin, vancomycin and Cefotaxime. *E.coli* is sensitive to ciprofloxacin, amikacin, gentamycin, norfloxacin and to tobramycin co-trimoxazole. They are resistance to chloramphenicol, lincomycin, vancomycin and cefotaxime.

## 5. DISCUSSION AND SUMMARY

The appearance of visible pus in the wound depend on the interaction of bacteria, host factors and foreign bodies, Neonates, the elderly, the malnourished, and the obese all have increased susceptibility. *Pseudomonas aeruginosa* is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen the bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Number of studies showed that *Staphylococcus aureus* to be a predominance etiological agent in burn wound infection. However, in India, incidence of *S. aureus* infection was quite significant but was next only to *Pseudomonas* sp. [25] Nosocomial infection in the burnt patients is major challenge for a clinician. It has been estimated that 75% of all deaths in burnt patients were associated with infections[26]. Species include *pseudomonas aeruginosa, P. fragi,* and *P.lundensis* which is fluorescent. *Pseudomonades* are common in soil and water and are part of the natural surface flora of many plants[26]. In contrast, in the present observation *Staphylococcus aureus* produce enterotoxin. *S.epidermidis* causes only minor lesions, expect in patients who have surgically inserted protheses or are immunodeficient. *S.aureus*, named for its golden – yellow colonies, on blood agar produces coagulase (i.e., "Coagulase positive")

## CONCLUSION

Strains of *E.coli* can express siderophores, such as enterobacter, which readily removes iron from mammalian iron transport proteins such as transferring; lactoferrin. Some strains also express another siderophores, aerobactin, which may be plasmid-mediated. The ability of strains of *E.coli* to acquire ferric ions is a recognized pathogenic mechanism. Expression of the aerobactin-mediated iron uptake system is a common feature of strains isolated from patients with septicemia, pyelonephritis and lower urinary tract infection.

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SP-65

# ISOLATION OF ENDOPHYTIC ANTAGONISTIC BACTERIA FROM *ALLIUM SATIVUM* AS BIOCONTROL AGENTS OF BLACK MOLD DISEASE

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## ABSTRACT

The increased concern for the use of chemical agents and the quest to recognize a potent biological pesticide has stimulated research to develop and implement the use of biological agents. The study was conducted to isolate the endophytic bacteria from *Allium sativum* against the black mold disease causing pathogen, *Aspergillus niger*. The main objective of the study was to isolate the endophytic bacteria from the garlic and detect its potentiality as a biological agent for which detection of biocontrol activities were performed. From the surface sterilized healthy garlic samples in Tryptic soy agar, 14 strains were isolated and were then screened for *in vitro* antagonism against the pathogen causing the black mold disease. Six isolates showed significant activity against the *Aspergillus spp*. Highest percentage of Inhibition was shown by the isolates ASEB 01 with 77.7%. The identification and characterization were done based on their morphological and biochemical characteristics in which they were identified to be of *Bacillus sp and Pseudomonas sp.* which showed great *in vitro* antifungal mechanism. The biocontrol activities of endophytic bacteria were also evaluated for the production of allelochemicals like HCN, volatile and non-volatile organic antimicrobial compounds and sequestration of iron resulting in iron deficient conditions for pathogens. As part of biocontrol mechanism the microorganisms were also evaluated for the use their ability of hydrolytic enzyme for the production of enzymes such as cellulase, amylase, pectinase, chitinase and lipase. The study puts forward the biocontrol ability of the native endophytic antagonistic bacteria from garlic cloves against the black mold disease causing pathogen *Aspergillus niger.*

**KEYWORDS**: *Allium sativum,* Black mold disease, Dual culture, Endophytes

## INTRODUCTION

The widespread use of toxic agricultural chemical has been a huge concern in this modern age and hence an increased recognition of the biological agents against the pests should be developed and implemented in the fields[1]. The potential use of microbe – based agents in control of pests has gained more importance and also has been addressed in many reports[2, 3] .Microorganisms that live in the intercellular space of a healthy plant tissues are called endophytes which generally does not cause any ill effects to the plants or cause any sort of disease symptoms[4]. Plants and microorganisms, especially endophytes exhibit symbiotic association which is well known to man and in particular, the plant protects, feeds and favors the growth of endophytes within the plants to which the endophytes favor back by producing biologically active substances that not only enhance the growth but also gives the plants to tackle abiotic and biotic stresses[5]. Moreover, some endophytic microorganisms are characterized also to have the capability of synthesizing the same metabolites as the host plant which will enhance the growth of the plant and also develop resistance within against pathogenic microbial attack[6]. Therefore, it is essential to undergo systematic investigation of endophytic microbes which is necessary, and will not only provide genetic information, but also pave way for new natural products with greater biological activity which can be developed as a potential agent in the field of medicine, agriculture and industry[7, 8] . *Allium sativum* the scientific name for the common "Garlic" is a species in the onion genus. In Liliaceae family, garlic serves as a member which have immense amount of active compounds showing antimicrobial, anticancer and antioxidant properties. Various reports on the isolation and identification of endophytic bacteria, fungi and Actinomycetes from Garlic plants has also been documented but all these isolates were assessed for their antimicrobial, antioxidant, anti-inflammatory

and cytotoxic properties and their synthesis of secondary metabolities production[9] . But there are only seldom reports on the endophytic microbes, targeting at the plant growth promotion and the synthesis of allelochemicals compounds. One of the major agents of loss of crops is the *Aspergillus niger* causing the Black mold disease. Hence, different strategies have been applied for the suppression of black mold disease, including treatments with fungicides, preventive and detection of infection before seeding etc. but it was not very easy to control the disease[10] . The alternative control strategies, mainly the use of biological pesticides, has been extensively increased due to hazards from chemical pesticides caused to the environment and public health. However, due to the absence of data about biological control of garlic pathogens is evident in literature. Considering the increasing occurrence of black mold disease of garlic, it is necessary to investigate the possibilities of using native endophytic microbes of garlic as potential biocontrol agents of the disease. Hence a study has been designed to find out ecofriendly measures for alleviating the yield loss caused by black mold disease of garlic.

## MATERIALS AND METHODS

### *Collection of samples*

Healthy and fresh garlic bulbs were collected from Calicut, Kerala and were brought to the laboratory and finally processed for the endophytic bacterial isolation.

### *Isolation of endophytic bacteria from Allium sativum.*

### *Pretreatment and surface sterilization*

The garlic cloves were initially subjected to thorough washing under running tap water to remove any dust or soil particles followed by a careful vigorous washing in the detergent Tween 20. The samples were then subjected to serial washing in sterile tap water and kept immersed in sterile phosphate buffered saline (pH 7) for 10 min to equilibrate osmotic pressure and prevent passive diffusion of sterilizing agents. The sterilization of garlic cloves were done[11]. The cloves were then surface sterilized with 1% sodium hypochlorite for 2 min and the samples were subjected to washing for five times with sterilized distilled water. Then the samples were treated using 70% ethanol for approximately 30 seconds for another five times serial washing using sterilized distilled water. The samples were blot dried on sterile filter papers and were excised with autoclaved scalpel and forceps in the laminar air flow chamber, then air dried in laminar flow. To confirm that the surface of garlic tissue was effectively sterilized, 1 ml of the sterile distilled water that was used in the final rinse of surface sterilization procedures were planted onto Tryptic Soy Agar (TSA) media and incubated at room temperature.

### *Isolation and Purification of endophytic bacteria*

Isolation of endophytic bacteria were done according to the following procedures. The surface sterilized samples were transferred to sterile petriplates and carefully cut into small pieces of approximately one cm. The samples were then transferred carefully to Tryptic Soy Agar (TSA) plates. All plates including the sterility control were incubated at 28±2° C for 5 days and observed periodically for bacterial growth. Morphologically distinct colonies were selected, purified and kept at 4°C in TSA slants.

### *Identification of the endophytic bacteria*

The Identification of the isolated endophytes were done based on the Morphological, biochemical and cultural characteristics for the 14 endophytic bacterial isolates[12].

### *Detection of Biocontrol Activity of endophytic bacteria*

### *Isolation and Identification of the fungal pathogen Aspergillus niger*

The pathogenic fungus responsible for black mold disease was isolated from garlic cloves with the same disease. The garlic cloves were surface sterilized and cut into two pieces as transverse sections. The pieces were transferred aseptically to sterile Potato Dextrose agar (PDA) plates. The fungal growth was observed after incubation at room temperature for 7 days. The fungal growth obtained from the garlic cloves with disease was subjected to macroscopic and microscopic observations to study the colony morphology, shape and arrangement of hyphae, conidia etc.

### *In vitro* antagonism (Dual culture test)

The 14 isolated endophytic bacterial culture were tested for antagonistic activity against the pathogenic

fungus, *Aspergillus niger*. The endophytic bacteria was streaked onto a sterilized plate containing 20ml of combination media, ie. of nutrient agar and potato dextrose agar in the ratio 1:1. A fungal culture disc of the plant pathogen *Aspergillus niger* was placed adjacently to the endophytic bacteria. Control plates with *Aspergillis niger* disc was also maintained. The percentage of inhibition was calculated after the incubation for 7 days using the formula as shown below.

Percentage reduction in the growth of *Aspergillus niger* =

$$\frac{\text{Growth in control (C) (dm)} - \text{Growth in Antagonist (T) (dm)}}{\text{Growth in control}} \times 100$$

Putative endophytes with promising antagonistic activity were selected for further experiments to explore the biocontrol mechanisms of the selected endophytic isolates against *Aspergillus niger.*

***Detection of Biocontrol Mechanisms***

***Siderophore production***

The production of siderophore by the endophytic bacteria was done using the ferric chloride [13]. One ml of the actively growing bacterial cultures were inoculated to 10 ml King's B broth. After the incubation for 3 days at 28±2°C using a rotary shaker at 120 rpm, the inoculated broth were centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected in sterile test tube. Equal volume of 2% aqueous ferric chloride solution was added and kept undisturbed. The appearance of orange to red brown coloration indicates the presence of siderophores.

***HCN production***

Hydrocyanic acid (HCN) production by the endophytic bacteria were analyzed [14], by inoculating them onto Tryptic Soy Agar (TSA) medium supplemented with 4.4 g glycine. Sterile paper strips were dipped in the solution of picric acid which was then drained and placed on the lid of the petriplate. Petri plates were then sealed with parafilm and were kept for incubation for 48 hrs at 28±2°C. If the colour change was observed from yellow to brown indicated on the filter paper strips containing picrid acid then that results in the production of HCN production. Control plate was kept with uninoculated TSA plates bearing filter paper soaked in picric acid solution.

***Volatile organic compound production***

A lawn culture with one ml of standard inoculum of ($2x10^8$ CFU/ml) endophytic bacterial isolates was made on NA. Meanwhile, PDA plates with 5 days old actively growing inoculum of fungal pathogen *Aspergillus niger* was also prepared. Fungal sclerotia were carefully transferred to sterile PDA plates and used as test pathogen inoculum. The lids of both the bacterial and fungal cultures were removed aseptically in a laminar airflow chamber and the bottom plates were soon set one on another and sealed with the parafilm to make it airtight and was incubated for 4-7 days. Control plates were also maintained with fungal sclerotium and NA plates without antagonistic endophytic bacteria. The production of volatile organic compound was monitored by absence of sclerotium germination and mycelial expansion.

***Non volatile organic compound production***

The cell free culture filtrate (CFCF) of the bacterial endophytes studied were used for the detection of non volatile and/or diffusible organic compounds by adopting well diffusion assay. One ml of the standard inoculum was added to 5 mL Tryptic Soy Broth (TSB) and kept for 7 days incubation in rotary shaker at 28±2°C. The centrifugation was performed at 10000 rpm for 10 minutes and the cell free supernatant was collected in sterile test tubes. About 100 µl of culture filtrate was added to wells made in sterile NA: PDA medium containing centrally placed agar block of *Aspergillus niger,* the test pathogen from a 5 days old actively growing mother culture. Observations were made for sclerotium germination and mycelial expansion of *Aspergillus niger* in the control and test plates.

***Lytic enzyme production***

The extracellular lytic enzyme production was assessed by inoculating bacterial endophytes onto the respective media and incubated for 48 hours at 28±2°C. The zone of inhibition surrounding the bacterial colony was considered as the zone of enzyme activity [15].

***Amylase production***

**T**he test endophytic bacteria was grown on Nutrient Agar (NA) medium supplemented with 2% soluble starch. After 24 hrs of incubation, the plates were then flooded with 1 ml freshly prepared 2% potassium iodide solution. The zone of inhibition surrounding the bacterial colony was considered to be positive for amylase synthesis.

***Cellulase production***

The test endophytic bacteria were cultured on Luria-Burtani(LB) agar medium which was supplemented with 0.5% Na-carboxymethyl cellulose (CMC). After incubation, the plates were flooded with 0.2% aqueous Congo red solution and kept for 30 minutes undisturbed and then thoroughly rinsed with sterilized distilled water. Destaining was then done by washing it twice with 1M NaCl. Surrounding the colony if a clear yellow halo area was found against a red background then cellulose synthesis was indicated.

***Chitinase production***

The test endophytic bacteria were grown on the colloidal chitin agar medium and after incubation 28±2°C for 48 hours if a clear zone is seen surrounding the colony the chitinase synthesis was considered positive.

***Protease production***

Skim milk agar was used to detect the synthesis of protease by the test endophytic bacteria. After incubation, plates were visualized with clear digested area around the colony gives a positive result.

***Lipase production***

Lipase activity was analyzed using special media containing calcium chloride added to NA again supplemented with 1% tween 20 after sterilization. Clear zone around bacterial colony after incubation at 28±2°C for 48 hrs will show positive enzyme production.

## RESULTS AND DISCUSSIONS

There are a lot of antagonists in nature to be applied in many fields such as agricultural defense and in medical domain. The control of plant fungal diseases is difficult which may possibly lead to a huge economic loss. Thus, strategies should be ensured by plants to avoid fungal infection, especially with the development and use of biologically active agents. Endophytic bacteria residing inside the plants are well known for plant growth promotion as well as for their biocontrol activities. Garlic (*Allium sativum* L) has been termed as the "antibiotics grown out of the land" [16]. In our current study, fourteen endophytic strains have been isolated from surface sterilized leaves of garlic cloves and screened to find antifungal activity against *Aspergillus niger,* the pathogen of the black mold disease of garlic. A dual culture test is a widely recognized and authoritative *in vitro* test used for the preliminary screening of biocontrol agents against different plant pathogens[17].This study, also utilizes the same classic methodology to assess the 14 endophytes *in vitro* analyses against soil borne pathogenic fungus *Aspergillus niger* in which 6 isolates showed antagonistic activity against the pathogen. The endophyte ASEB01, gram positive *Bacillus* sp. showed highest and significant inhibition *in vitro* with 77.7% followed by other gram positive *Bacillus s*p. and ASEB05 being *Pseudomonas sp*. with 55.5 to 66.6% as showed in the Table 1. Many reports are available with biocontrol abilities of endophytic bacteria isolated from diverse hosts majorly belonging to *Bacillus* sp. [18, 19, 20, 21] The identification studies based on morphological, biochemical and cultural characteristics revealed that the bacteria belongs to *Bacillus sp* and *Pseudomonas sp.*

**Table 1. *In vitro* screening of endophytic bacteria against *Aspergillus niger***

| Endophytic isolates | Zone of Inhibition (mm) | *In vitro* antifungal activity (%) |
|---|---|---|
| **ASEB 01** | 2.5 | 77.7 |
| **ASEB 02** | 3 | 66.6 |
| **ASEB 03** | 3 | 66.6 |
| **ASEB 04** | 4 | 55.5 |
| **ASEB 05** | 2.5 | 72.2 |
| **ASEB 09** | 4 | 55.5 |

*Bacillus sp*. and *Pseudomonas sp.* are attractive for research due to their possible utilization in the biological control of fungal diseases[22,23]. They offer several preferences over other microorganisms because of their ability to form endospores and tolerate adverse environmental conditions[24]. Furthermore, their long term viability and rapid growth in a liquid medium simplify the production of commercial preparations[25]. The biocontrol activities of endophytic bacteria are due to the production of various antibiotics or allelochemicals like HCN, volatile and non-volatile organic antimicrobial compounds, sequestration of iron resulting in iron deficient conditions for pathogens and their establishment inside suitable niche therefore causes competition of space for pathogens [26, 27, 28]. The Table 2 shows the biochemical mechanisms of the selected 6 strains by demonstrating the production of Siderophores, Volatile and non-volatile organic compounds for ASEB 01 and ASEB 05, whereas none of the strains could produce HCN. The role of induced systemic resistance due to the induction of plant defense mechanism with the colonization of endophytic bacteria may be the reason for the inhibition of *Aspergillus niger* under *in vitro* conditions due to one or a combination of these mechanisms.

**Table 2. *In vitro* Biocontrol mechanisms of the selected antagonistic endophyte against *Aspergillus niger***

| Endophytic isolates | Siderophore | Volatile organic compounds | Non Volatile organic compounds | HCN |
|---|---|---|---|---|
| **ASEB 01** | + | + | + | - |
| **ASEB 02** | + | - | + | - |
| **ASEB 03** | + | - | + | - |
| **ASEB 04** | + | - | + | - |
| **ASEB 05** | ++ | + | + | - |
| **ASEB 09** | + | + | - | - |
| **Control** | - | - | - | - |

The microorganisms use the ability of hydrolytic enzyme for the production of enzymes such as cellulase, amylase, pectinase, chitinase, lipase etc. primarily as part of biocontrol mechanism. In Table 3, we can see that the selected antagonistic endophytes have the capability of producing the enzymes like amylase, cellulase, and protease, but none produced chitinase and lipase. Studies have revealed that lytic enzymes such as amylase, chitinases, cellulases, lipases, and proteases, degrade fungal and bacterial cell wall and prevent plant pathogens to cause infection. As far as endophytes are considered, the production of lytic enzyme production [29, 30, 31] and production of pectinolytic and cellulolytic enzymes[32] can result in vertical spreading and secondary cell wall lysis of host plants enabling colonization and endophytic establishment.

**Table 3. Lytic enzyme activities of the selected antagonistic endophytes**

| Endophytic isolates | Amylase | Cellulase | Chitinase | Protease | Lipase |
|---|---|---|---|---|---|
| **ASEB 01** | + | + | - | + | - |
| **ASEB 02** | + | + | - | + | - |
| **ASEB 03** | + | + | - | + | - |
| **ASEB 04** | + | + | - | + | - |
| **ASEB 05** | - | + | - | + | - |
| **ASEB 09** | + | + | - | + | - |

## CONCLUSION

To conclude, the present study puts forward the biocontrol ability of the native endophytic antagonistic bacteria from garlic cloves against the black mold disease causing pathogen *Aspergillus niger.* In our study to screen for the promising strains capable as a biocontrol agent was found to be ASEB 01 and ASEB 05 which were identified as *Bacillus sp.* and *Pseudomonas sp.* with excellent *in vitro* antifungal mechanisms. The future perspectives include the molecular and phylogenic analyses as well as purification of secondary antifungal metabolites.

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SP-66

# Production of Protease by *Penicillium roqueforti* through Optimization of environmental conditions

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## ABSTRACT

A fungal isolate with significant proteolytic activity was isolated from Mumbalai mangrove forest soil at Pudukkottai district, Tamilnadu, India. The isolate was identified as *Penicillium Roqueforti* based on morphological, biochemical and molecular characterization. Factors influencing the maximum production of protease by *P.roqueforti* were optimized. The maximum protease enzyme yield was achieved when the incubation temperature was 24°C in the growth medium with a pH 5 for 72 hours. *P.roqueforti* exploited sources of carbon and nitrogen for its proteases production, Cassava flour was the best source of carbon for maximum production of protease. The maximum production of protease during the course of present studies was13±0.36 U/ ml/mg. Molecular weight was determined by SDS-PAGE electrophoresis gave 20-30 KDa protein. Based on our results protease from *P.roqueforti* mainly used for industrial application.

**KEYWORDS:** Protease, *Penicillium roqueforti*, Mumbalai, Mangrove forest soil, SDS-PAGE.

## INTRODUCTION

Proteases are acidic, neutral, or alkaline proteases[9]. Proteases enzymes function is to hydrolyze peptide bonds of proteins called as proteolytic enzymes or proteinases. These enzymes are widely present in all plants, animals and microorganisms. Fungal proteases are used in the food, dairy, detergent, leather and pharmaceutical industries. They are also used for bioremediation and production of therapeutic peptides[21,23.] Fungal enzymes are more suited for industrial applications as fungi are easily cultured and are fast growing; many fungal enzymes are extracellular and are amenable to manipulations. Furthermore, fungal proteases are more diverse and exhibit wider substrate specificity[6].It is worth noting, because it relates to *P. roqueforti*, that their proteolytic enzyme makes them suitable for biotechnological applications [1]. Proteolytic enzymes have the ability to carry out certain modification of proteins by limited cleavage such as activation of zymogenic enzymes, blood clotting and fibrin clots lysis, processing and secretory proteins transport across the membranes. The enzymatic conversion of industrial raw materials also occurs through the use of proteases. In biochemical terms, *P.roqueforti* presents a complex proteolytic system generally classified as endoenzymes (acid protease and metalloprotease) or exoenzymes (acid carboxypeptidase and alkaline aminoprotease), on the basis of their site of action on protein substrates which are commonly used in the dairy industry[11,2,3,8,14,4]. They are further classified as serine proteases, aspartic proteases, cysteine proteases or metallo proteases depending on their catalytic mechanism. They are also classified into different families and depending on their amino acid sequences and evolutionary relationships the objective of the present work was the optimization of different environmental factors such as pH, temperature, carbon and nitrogen source of medium for protease production by *P.roqueforti*. There is a renewed interest in proteases as targets for improving therapeutic agents against fatal diseases such as cancer, malaria and AIDS. The development of recombinant rennin and its commercialization is an excellent example of the successful application of modern biology to biotechnology. Advances in biotechnology and microbiology have bring a favorable environment for the development of proteases and will continue to facilitate their applications to provide a sustainable niche for mankind and to increase the quality of human life.

## MATERIALS AND METHODS

### *Microorganism and maintenance*

The *P. roqueforti* culture was isolated from rhizosphere soil samples such as *Avicennia marina*, *Suaeda monica* and *Salicornia depressa*. The soil samples were collected from Mumbalai mangrove forest at Pudukkottai district, Tamilnadu, India, using potato dextrose agar plates. The cultures showing larger zones of casein hydrolysis on the test plates were picked up and transferred to PDA slants. The culture was maintained by weekly transfer onto fresh slants of potato-dextrose-agar (PDA) and was stored in refrigerator at 4°C.

### *Molecular Identification and Phylogenetic Analysis of P. roqueforti*

*P. roqueforti* DNA was extracted using a DNA-Easy Plant Mini kit (QIAGEN, Basel, Switzerland). 18S rRNA gene was amplified by PCR using universal primers the methods[20]. The universal primers were NS1 Forward (GTAGTCATATGCTTGTCTC) and NS1 Reverse (CTTCCGTCAATTCCTTTAAG). Resulting PCR products were sequenced using ABI 3500XL Genetic Analyzer and deposited in NCBI. Fungi was identified based on the percentage of homology to sequences available in the database. The sequences were compared against the sequences available from genbank using BLASTN program. Phylogenetic analysis was constructed using the Neighbour-joining method [15].

### *Protease production*

*Penicillium roqueforti* culture was inoculated in sterile 100 ml of fermentation broth containing (% w/v): yeast extract 1.0, $MgSO_4$ 0.02, glucose 2.0, $K_2HPO_4$ 0.1, pH 7.0. Flasks, inoculated were incubated at 28˚C for 5-6 days in a rotary shaker. At the end of incubation, the contents were filtered through Whatmann filter paper No. 1 and the filtrates were centrifuged at 8,000 rpm at 4°C for 10 minutes. Pellet was discarded after centrifugation and supernatant was used as source of protease enzyme. The supernatant of crude enzyme was further used for subsequent studies [5].

### *Optimization studies*

Production of protease from *P. roqueforti* was optimized by controlling physicochemical parameters like incubation temperature ranging from 20 to 36°C, pH medium ranging from 3 to 11,Carbon source (Corn steep liquor) and Nitrogen(Cassava flour) was estimated at incubation times (72 hrs).

### *Purification of enzymes*

Purification and characterization of Protease Crude extract was precipitated by 70% saturation with ammonium sulphate [13]and then dialyzed against 50mM phosphate buffer (pH 7.0) for 24 hours. The filtrate was loaded onto a column chromatography. The protein content was estimated by the method[10]. All experiments were conducted in triplicates and their mean values represented. SDS-PAGE electrophoresis was carried out and molecular weight was determined[7].

## RESULT AND DISCUSSION

The wide use of protease in biotechnology and industrial purpose which was attracted researches to obtain protease enzyme from *Penicillium roqueforti.* According to our results, *P. roqueforti* has best producer of protease and selected for further process in submerged fermentation. However, Maximum protease production of 88±0.38 mg/ml was seen at pH 5.0 for *P.roqueforti* (Table.1). This is in similar with the optimum pH 7.0 reported for *A.flavus* by **[16]**. These results denoted that alkalinophilic nature of *A.niger*. Production of Protease increased with increase in temperature from 24°C to 37°C. Maximum production of protease (78±0.65mg/ml) was obtained at 24°C (Table.2). Our results accordance with[12] for *A. usami*. It was observed that environmental temperature not only affects growth of organism but also revealed the influencing of protease production. It is well known that fungal protease production is dependent on the availability of both carbon and nitrogen sources. In the current study, sources of carbon and nitrogen were investigated in order to find the maximum protease production by *P. roqueforti*. The significant effect of sources of carbon (Cassava flour) either on protease activity or on enzyme production is shown in Table1- 3.

**Table 1: Protease production by *Penicillium roqueforti* at various pH**

| S.No | pH | Enzyme activity (IU/ml) | Protein level (mg/ml) |
|---|---|---|---|
| 1. | 3 | 140±11.96 | 12±0.49 |
| 2. | 5 | 920±26.91 | 88±0.38 |
| 3. | 7 | 340±13.65 | 29±0.95 |
| 4. | 9 | 140±15.83 | 17±0.94 |
| 5. | 11 | 080±11.94 | 09±0.96 |

**Table 2: Protease production by *Penicillium roqueforti* at various Temperatures**

| S.No | Temperature (°C) | Enzyme activity (IU/ml) | Protein level (mg/ml) |
|---|---|---|---|
| 1. | 20 | 220±12.59 | 20±0.59 |
| 2. | 24 | 880±23.94 | 78±0.65 |
| 3. | 28 | 310±14.42 | 31±0.85 |
| 4. | 32 | 180±13.93 | 18±0.95 |
| 5. | 36 | 140±15.82 | 11±0.55 |

**Table 3: Protease production by *Penicillium roqueforti* with Carbon and Nitrogen sources**

| S.No | Fungal Species | Various sources | Enzyme assay (IU/ml) | Protein level (mg/ml) |
|---|---|---|---|---|
| 1. | *Penicillium roqueforti* | Corn steep liquor | 520±20.58 | 50±0.53 |
| | | Cassava flour | 960±27.63 | 89±0.54 |

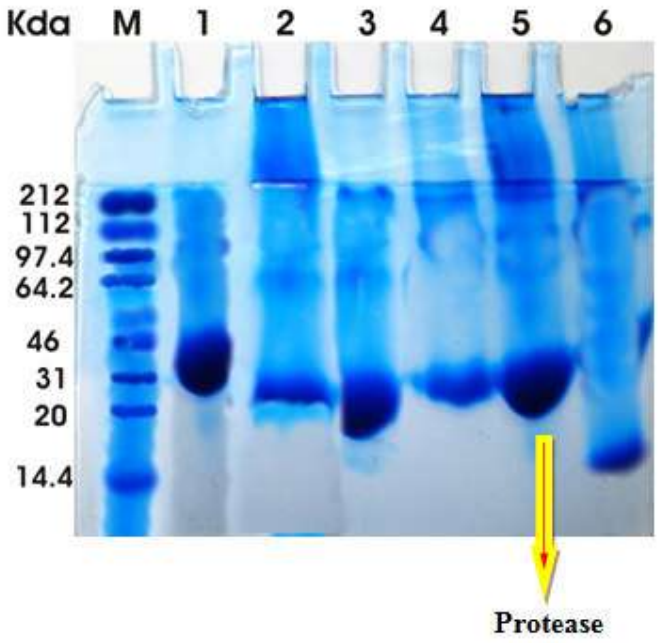
Our findings are in occurrence with the results[17, 23] had protease production by *Rhizopus microspores* NRRL 3671 and *Alternaria alternata*, respectively, was highly influenced by sources of carbon and nitrogen. In the present study, *P.roqueforti* gave maximum activity of protease enzyme in presence of cassava flour as carbon source. At the end of purification process protease showed specific activities of 13±0.36 U/mg-1 protein, recovery of 14%, purification folds were 3.8 (Table 4).

**Table 4: Purification and recovery of protease from *Penicillium roqueforti***

| Methods of purification | Enzyme volume unit | Protease production (IU/ml) | Total protein (mg) | Specific activity (U/ml/mg protein | Purification (fold) | Recovery (%) |
|---|---|---|---|---|---|---|
| Culture supernatant | 100 | 680±21.94 | 113±10.93 | 1.2±0.08 | 1 | 100 |
| Ammonium sulphate | 10 | 450±17.84 | 75±0.94 | 08±0.04 | 1.5 | 67 |
| Dialysis | 10 | 230±11.58 | 31±0.44 | 4.1±0.11 | 6.2 | 32 |
| Chromatography | 10 | 140±10.94 | 09±0.54 | 13±0.36 | 3.8 | 14 |

Similar results were agreement with[15]. SDS-PAGE analysis of *Penicillium roquofortii* strain protease. M-indicates molecular weight markers and P-shows purified protease band of molecular weight approximately 33 kDa. Stability at wide range of pH is desirable properties of any enzyme for industrial applications. In our case the optimum temperature of enzyme activity was 50°C, while the optimum pH for its activity was recorded as pH 7.0. The molecular mass of protease was estimated to be about 33kDa as analyzed by SDS-PAGE (Plate II) has also been reported for the enzyme from *Malbranchea inichella*[16].

**Figure 1: Protein profile of the *Penicillium roqueforti***




## CONCLUSION

The present study is the first to produce protease from a newly isolated fungus from the soil of mangrove forest. Further analysis on PDA medium revealed that *Penicillium roqueforti* (GenBank accession for 18S rRNA sequence: KP419693) has the maximum level of protease production. In future, industrial application of protease from this promising strain, are in progress.

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SP-67

# A STUDY ON SEASONWISE VARIATION IN THE DIVERSITY OF PLANKTON AND BENTHIC ORGANISMS IN GREAT VEDARANYAM SWAMP, POINT CALIMERE WILDLIFE SANCTUARY, SOUTHERN INDIA.

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## ABSTRACT

The seasonal variation in the diversity of plankton and benthos were assessed across the period between 2004 and 2006 at Great Vedaranyam Swamp of Point Calimere Wildlife Sanctuary, Southern India. Plankton were identified in foraging areas of Greater Flamingo (*Phoenicopterus ruber roseus*). Totally 38 species of phytoplankton comprising of 28 species of diatoms, 5 species of blue green algae, 3 species of green algae, stoneworts and 1 species of dinoflagelloids were recorded. These phytoplankton belonged to 12 families under 8 orders viz., Nostocales, Oscillatoriales, Chlorellales, Zygnematales, Centrales, Pennales, Dinophysiales and Charales. Totally 27 species of zooplankton were identified. Among them 18 species belonged to class Maxillipoda and 9 species were in class Malacostracea. Totally 28 species of benthic organisms belonged to 5 classes viz., Insecta, Spionida, Malacostra, Ostrocoda and Adenophorea were identified in different habitats during the study period.

**Keywords:** Phytoplankton, Zooplankton Benthic organisms, Nostocales, Spionida,

## INTRODUCTION

Phytoplankton constitute the basis of nutritional cycle of an aquatic ecosystem as they form bulk of the food for zooplankton, fishes and other aquatic organism, and play a key role in maintaining proper equilibrium between abiotic and biotic components and biodiversity aquatic of the ecosystem[1]. Biotic factors that play a major role in influencing water bird densities in a habitat include the availability of planktons, macro fauna such as fish, crabs, prawn etc., and benthic invertebrates[2,3&4]. Specific visitations of water birds[5] of the Garaha wetlands of Jammu, India, were correlated to the seasonal variations in the nektons, planktons and benthos. So, the water birds which depend on them should also show population fluctuations in response to benthic substrate qualities[6&7]. Phytoplankton constitute the basis of nutritional cycle of an aquatic ecosystem as they form bulk of the food for zooplankton, fishes and other aquatic organism, and play a key role in maintaining proper equilibrium between abiotic and biotic components and biodiversity aquatic of the ecosystem[1]. Zooplanktons are major herbivores as well as important predators in aquatic ecosystems[8]. Furthermore, the Flamingos[9] feed on plankton rich areas of swamp in Point Calimere Wildlife and Bird Sanctuary, Southern India. Hence in this paper the result of the survey on the plankton and benthos were assessed in the foraging areas of flamingo across the year between 2004 and 2006 are discussed.

## STUDY AREA

Point Calimere Wildlife & Bird Sanctuary is located along the Palk Strait in three districts of Tamil Nadu: Nagapattinam, It lies in between 79.399 E & 79.884 E longitudes and 10.276 N & 10.826 N latitudes. The Point Calimere Wildlife and Birds Sanctuary which was declared as a Ramsar Site on 19th August 2002 has three divisions. The extensive mud flats of swamp area have many variations of water quality depending upon the season, during monsoon time the whole swamp area experience wide variation of water quality depending upon the seasons.

## MATERIALS AND METHODS

### *Study Period and Seasons*

Data were collected from January 2004 to December 2006. Four seasons namely Post-monsoon (January-March), Summer (April-July), Pre-monsoon (August and September) and Monsoon (October-December) of three successive years were classified to analyze the data.

### *Collection and analysis of Plankton and benthos*

During the study period both plankton and benthos were collected and analyzed in different season. phyto planktons were collected by a using planktonic net No. 30 and the mesh size was 48µm and zooplankton net No.10 and the mesh size was 150µm (upper aperture 22cm, depth 34cm and lower aperture 6cm). The planktons were collected at least from three different stations in each site and also it was collected by dragging the net for 0.9 meters[7]. The planktons were collected and preserved using 5% formaldehyde for later identification (Michael, 1986). Identification of both phytoplankton and zooplankton were done[10, 11 & 12]. Benthos was collected by using quadrates of length 15cm, width 15cm and depth 15cm. The mud collected was sieved through a 0.5mm sieve and animals filtrated were preserved in 5% formaldehyde[13]. Polycheate worms and chironomous larva were identified. Molluscan forms were weighed (wet weight) after removing excess of water with a blotting paper. The benthic animals viz., Polycheate and molluscs were identified by using manuals[14 &15].

## RESULT

### *Analysis of Seasonal Variations of Plankton and benthos*

We identified Totally 38 species of phytoplankton comprising of 28 species of diatoms (Bacillariophyceae), 5 species of blue green algae (Cyanophyceae), 3 species of green algae (Chlorophyceae), 1 species of stoneworts (Charophyceae), and 1 species of dinoflagelloids (Dinophyceae) were recorded. These planktons belonged to 12 families under 8 orders viz., Nostocales (1), Oscillatoriales (4), Chlorellales (2), Zygnematales (1), Centrales (19), Pennales (10), Dinophysiales (1) and Charales (1). Totally 27 species of zooplankton were identified. Among them 18 species belonged to class Maxillipoda and 9 species were in class Malacostracea. They belonged to 12 families viz. Acartiidae (3), Paracalanidae (2), Calanidae (8), Eucalanidae (1), Oithonidae (1), Miraciidae (2), Temoridae (1), Euphausiidae (1), Cumacidae (2), Idoteidae (1) Penaeoidae (7) and Amphipodae (1) which included in 8 different orders. Totally 28 species of benthic organisms were identified in different habitats during the study period. They belonged to 5 classes viz., Insecta, Spionida, Malacostra, Ostrocoda and Adenophorea and 5 orders viz., Diptera, Spioniformia, Decapoda, Myodocopida and Rhabditida and 6 families viz., Chironomidae, Polydora, Penaeoidae, Portunidae, Cypridinidae and Pasiphaeidae.

### *Total Plankton*

### *Density of Total Plankton*

In all the three years of study, total plankton density was minimum in monsoon 2006 with a mean value of 10.6 ± 1.13ml/l and maximum in pre-monsoon 2005 with a mean value of 555.8 ± 66.16/ml. The variation in the density of total plankton across different years was significant ($F= 55.80$; $P<0.001$) as well as the seasonal variation was also significant ($F= 12.71$; $P<0.001$) (Table.1).

### *Richness of Total Plankton*

Total plankton richness was lowest in summer 2004 with a mean value of 1.2 ± 0.28 and highest in pre-monsoon 2005 with a mean value of 13.8 ± 1.45 during the study period. The variation in the richness of total plankton showed significant difference among years ($F= 88.07$; $P<0.001$) and the seasonal variation was also highly significant ($F= 27.46$; $P<0.001$) (Table.1).

### *Diversity of Total Plankton*

Total plankton diversity varied from 0.4 ± 0.09 in summer 2004 to 2.1 ± 0.03 in monsoon 2005 during the study period. The variation in the diversity of total plankton across different years was significant ($F=140.84$; $P<0.001$) as well as the seasonal variation was also significant ($F= 11.25$; $P<0.001$) (Table.1).

***Phyto plankton***

***Density of Phyto plankton***

In all the three years of study, phyto plankton density varied from 9.3 ± 1.46/ml in monsoon 2006 to 300.4 ± 39.79/ml in post-monsoon 2005. The variation in the density of phyto plankton across different years was significant (F= 41.21; P<0.001) as well as the seasonal variation was also significant (F= 12.00; P<0.001) (Table.1).

***Richness of Phyto plankton***

In all the three years of study, phyto plankton diversity was minimum in summer 2004 with a mean value of 0.6 ± 0.14 and maximum in pre-monsoon 2005 with a mean value of 7.7 ± 1.13. The variation in the richness of phyto plankton showed significant difference among years (F= 34.69; P<0.001) and the seasonal variation was also highly significant (F= 18.45; P<0.001) (Table.1).

***Diversity of Phyto plankton***

Phyto plankton diversity was lowest in summer 2004 with a mean value of 0.2 ± 0.04 and highest in pre-monsoon 2004 with a mean value of 0.9 ± 0.35 during the study period. The variation in the diversity of phyto plankton across different years was significant (F= 41.21; P<0.001) as well as the seasonal variation was also significant (F= 12.00; P<0.001) (Table.1).

***Zooplankton***

***Density of Zooplankton***

Zooplankton density ranged between and 1.2 ± 0.31/ml in monsoon 2006 and 255.5 ± 33.59/ml in post-monsoon 2005 during the study period. The variation in the density of zoo plankton across different years was significant (F= 55.30; P<0.001) as well as the seasonal variation was also significant (F= 9.39; P<0.001) (Table.1).

***Richness of Zooplankton***

Zooplankton richness varied from 0.3 ± 0.07 in monsoon 2006 to 6.0 ± 0.37 in pre-monsoon 2005 during the study period. The variation in the richness of zoo plankton showed significant difference among years (F= 100.61; P<0.001) and the seasonal variation was also highly significant (F= 21.66; P<0.001) (Table.1).

***Diversity of Zooplankton***

Zooplankton diversity was lowest in summer 2004 with a mean value of 0.2 ± 0.04 and highest in monsoon 2005 with a mean value of 1.7 ± 0.02 during the study period. The variation in the diversity of zoo plankton across different years was significant (F= 74.16; P<0.001) as well as the seasonal variation was also significant (F= 13.57; P<0.001) (Table.1).

***Benthos***

***Density of Benthos***

In all the three years of study, benthos density was minimum in summer 2004 with a mean value of 24.1 ± 5.63/ml and maximum in post-monsoon 2005 with a mean value of 306.1 ± 20.20/ml. The variation in the density of benthos density across different years was significant (F= 45.79; P<0.001) as well as the seasonal variation was also significant (F= 22.73; P<0.001) (Table.1).

***Richness of Benthos***

Benthos richness ranged between 1.7± 0.13 monsoon 2006 and 3.8 ± 0.11 in post-monsoon 2005 during the study period. The variation in the richness of benthos showed significant difference among years (F= 38.55; P<0.001) and the seasonal variation was also highly significant (F= 8.62; P<0.001) (Table.1).

***Diversity of Benthos***

In all the three years of study, benthos diversity was minimum in monsoon 2006 with a mean value 0.4 ± 0.06 and maximum in pre-monsoon 2004 with a mean value of 1.0 ± 0.15. The variation in the diversity of benthos across different years was significant (F= 23.14; P<0.001) as well as the seasonal variation was also significant (F= 4.72; P<0.001) (Table.1).

**Table .1:** Variations in the density (no./ml), richness (no. of species) and diversity (H′) of plankton and benthos in different seasons[a] of various years at the swamp of Point Calimere Wildlife and Bird Sanctuary, Tamilnadu, Southern India. Values are Mean ± 1 SE.

| Biological parameters | | 2004 | | | | 2005 | | | | 2006 | | | | ANOVA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Season | | Year | |
| | **Variables** | **Mon** | **Post-mon** | **Sum** | **Pre-mon** | **Mon** | **Post-mon** | **Sum** | **Pre-mon** | **Mon** | **Post-mon** | **Sum** | **Pre-mon** | **F** | **P** | **F** | **P** |
| Total plankton | Density | 98.7± 11.01 | 57.3± 8.74 | 24.14± 5.63 | 60.0± 6.24 | 95.12± 7.79 | 555.8± 66.16 | 501.1± 27.15 | 185.1± 21.99 | 10.6± 1.13 | 48.9± 3.11 | 36.0± 3.20 | 88.8± 10.75 | 12.71 | 0.001** | 55.80 | 0.001** |
| | Richness | 4.8± 0.25 | 6.7± 0.26 | 1.2± 0.28 | 7.0± 0.21 | 9.3± 0.47 | 7.6± 0.24 | 9.1± 0.44 | 13.8± 1.45 | 2.6± 0.22 | 7.4± 0.29 | 4.4± 0.31 | 6.0± 0.21 | 27.46 | 0.001** | 88.07 | 0.001** |
| | Diversity | 1.3± 0.05 | 1.7± 0.06 | 0.4± 0.09 | 1.7± 0.05 | 2.1± 0.03 | 1.8± 0.03 | 1.8± 0.07 | 2.2± 0.14 | 0.8± 0.08 | 1.8± 0.03 | 1.4± 0.06 | 1.7± 0.05 | 11.25 | 0.001** | 140.84 | 0.001** |
| Phyto Plankton | Density | 49.9± 5.82 | 76.5± 5.66 | 11.5± 2.67 | 53.0± 2.54 | 31.9± 4.87 | 300.4± 39.79 | 195.3± 17.81 | 82.8± 12.0 | 9.3± 1.46 | 19.9± 2.01 | 17.7± 0.94 | 54.10± 0.60 | 12.00 | 0.001** | 41.21 | 0.001** |
| | Richness | 3.0± 0.12 | 4.7± 0.09 | 0.6± 0.14 | 5.0± 0.25 | 3.4± 0.42 | 5.2± 0.26 | 7.2± 0.26 | 7.7± 1.13 | 2.3± 1.74 | 3.8± 0.22 | 2.4± 0.23 | 3.8± 1.69 | 18.45 | 0.001** | 34.69 | 0.001** |
| | Diversity | 0.7± 0.04 | 0.6± 0.10 | 0.2± 0.04 | 0.9± 0.35 | 0.7± 0.06 | 0.8± 0.08 | 0.6± 0.06 | 0.8± 0.16 | 0.6± 0.07 | 0.8± 0.08 | 0.7± 0.09 | 0.8± 0.02 | 12.00 | 0.001** | 41.21 | 0.001** |
| Zoo Plankton | Density | 48.8± 45.35 | 32.6± 1.73 | 12.7± 2.95 | 7.0 ± 3.21 | 63.2± 26.51 | 255.5± 33.59 | 219.1± 19.3 | 102.3± 11.15 | 1.2± 0.31 | 29.0± 2.21 | 18.4± 2.83 | 6.8± 1.04 | 9.39 | 0.001** | 55.30 | 0.001** |
| | Richness | 1.8± 0.15 | 1.9± 0.06 | 0.6± 0.14 | 2.0± 0.15 | 5.9± 0.10 | 2.4± 0.20 | 3.4± 0.33 | 6.0± 0.37 | 0.3± 0.07 | 3.6± 0.28 | 2.0± 0.21 | 1.1± 0.12 | 21.66 | 0.001** | 100.61 | 0.001** |
| | Diversity | 0.5± 0.04 | 1.0± 0.06 | 0.2± 0.04 | 0.7± 0.01 | 1.7± 0.02 | 0.6± 0.08 | 1.0± 0.08 | 1.5± 0.08 | 0 | 1.1± 0.07 | 0.5± 0.09 | 0.9± 0.07 | 13.57 | 0.001** | 74.16 | 0.001** |
| Benthos | Density | 129.6± 14.77 | 157.1± 9.02 | 24.1± 5.63 | 47.0± 7.12 | 55.7± 5.83 | 306.1± 20.20 | 129.1± 21.47 | 45.8± 4.28 | 24.6± 4.14 | 83.1± 6.25 | 92.7± 5.05 | 53.4± 1.54 | 22.73 | 0.001** | 45.79 | 0.001** |
| | Richness | 3.3± 0.13 | 3.1± 0.07 | 1.9± 0.43 | 3.0± 0.12 | 3.4± 0.21 | 3.8± 0.11 | 3.5± 0.09 | 2.5± 0.15 | 1.7± 0.13 | 3.0± 0.12 | 2.0± 0.11 | 2.3± 0.30 | 8.62 | 0.001** | 38.55 | 0.001** |
| | Diversity | 0.8± 0.03 | 0.9± 0.04 | 0.5± 0.12 | 1.0± 0.15 | 0.8± 0.05 | 0.9± 0.03 | 0.9± 0.04 | 0.5± 0.06 | 0.4± 0.06 | 0.7± 0.06 | 0.6± 0.02 | 0.7± 0.03 | 4.72 | 0.003* | 23.14 | 0.001** |

[a]*Mon = M onsoon ; Post-mon = Post-monsoon; Sum = Summer; Pre-mon = Pre-monsoon. *P < 0.05; ** P<0.01*

## DISCUSSION

Seasonal of annual variations are highly dependent on events like precipitation and general hydrological budget[16]. Seasonal variations in water bird population, abundance and diversity had been reported for other wetlands in different parts of India as well[17]. [18]Temperature may be limiting the development of all major zooplanktonic groups. The water temperature had also been reported to play an important role in controlling the occurrence and abundance of algal diversity[19]. The benthos richness and diversity influenced the population fluctuations of Greater Flamingos in this present study area. Earlier positive relationships between water bird's abundance and invertebrate prey density have also been found in many studies[20]. However, the accessibility and delectability of prey are also important[21]. The rich aquatic biodiversity Since the flamingos are filter feeders, the plankton is one of the major components of their prey, however for Greater Flamingo the benthic fauna is the prime diet. Characteristics of bottom sediments in the wetlands could also influence the faunal and floral community structure in them. So, the water birds which depend on them should also show population fluctuations in response to benthic substrate qualities[22]. Our data collection was coinciding with seasonal variation and species richness and diversity could be associated with benthic organisms.

## CONCLUSION

In conclusion, with the effect of seasonal variation taken into account the phyto and zooplankton and benthos density, richness and diversity varied significantly across the seasons.

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