INTERNATIONAL CONFERENCE

ON

CANCER RESEARCH

Special Issue On

23rd JANUARY 2020

Doi: http://dx.doi.org/10.22376/ijpbs/ijlpr/SP07/Jan/2020.1-87

ORGANIZED BY

LIFE SCIENCE DEPARTMENTS

(BIOCHEMISTRY, BIOTECHNOLOGY & MICROBIOLOGY)

DHANALAKSHMI SRINIVASAN
COLLEGE OF ARTS & SCIENCE FOR WOMEN
(AUTONOMOUS)
(RE-ACCREDITED WITH 'A' GRADE BY NAAC)
PERAMBALUR- 621 212.

In conjunction with

International Journal of Life Science and Pharma Research

ABOUT THIS CONFERENCE

The present conference is organized to concentrate **Cancer Research** in India, with a population close to 1·3 billion, and growing, is epidemiologically interesting and challenging for health-care planners. The conference intends to focus on cancer research, diagnosis and prevention. The conference will be offering a unique gathering in respect to scenario of global challenges. It aims to bring together students, teachers, and researchers working in sciences. The conference is expected to be a platform for the gathering of the ideas for the development of cancer research.

GUEST SPEAKERS

INAUGURAL ADDRESS

Dr.G.Govindaraj ,M.B.B.S.,M.S.,M.Ch., FICS
(Surgical Oncology)
Managing Director
Harshamitra Super Speciality Cancer Centre & Research Institute
Trichy

CHIEF GUEST

Dr. Charles Santhanaraju Vairappan M.Sc., Ph.D
Professor
Institute for Tropical Biology and Conservation (ITBC)
University Malaysia Sabah (UMS)
Natural Product Chemistry/Biotechnology
Kota kinabalu,Sabah,Malaysia.

GUEST OF HONOR

Dr.K.Sabitha M.Sc., Ph.D
Associate Professor
Department of Molecular Oncology
Cancer Institute (WIA)
Adyar, Chennai, Tamilnadu, India

ORGANIZING COMMITTEE

CHIEF PATRON
Thiru. A. SRINIVASAN
Founder & Chairman of
Dhanalakshmi Srinivasan Group of Institutions,
Perambalur.

PATRON
Thiru. S. KATHIRAVAN — Vice Chairman
Thiru.P.NEELRAJ — Secretary
Dhanalakshmi Srinivasan Group of Institutions,
Perambalur.

CONVENOR
Dr. P.SENTHILNATHAN
Principal,
Dhanalakshmi Srinivasan College of Arts
and Science for Women (Autonomous)
Perambalur

CO- CONVENOR
Prof.S.H.AFROZE
Vice-Principal,
Dhanalakshmi Srinivasan College of Arts
and Science for Women, (Autonomous)
Perambalur

ORGANIZING SECRETARIES

Dr. P. Gajalakshmi, M.Sc., M.Phil., Ph.D.,
Assistant Professor & Head
Department of Microbiology
Dhanalakshmi Srinivasan College of Arts & Science for Women (Autonomous),
Perambalur - 621 212.

Dr.G.Sugunadevi, M.Sc., M.Phil., Ph.D.,
Assistant Professor& Head
Department of Biochemistry,
Dhanalakshmi Srinivasan College of Arts & Science for Women, (Autonomous)
Perambalur -621 212.

Dr.C.Sangavai, M.Sc., M.Phil., MBA., PGDBI., PhD.,
Assistant Professor & Head
Department of Biotechnology,
Dhanalakshmi Srinivasan College of Arts & Science for Women (Autonomous)
Perambalur - 621 212.

CONTENT (ORIGINAL RESEARCH ARTICLE)

CONTENT – (ABSTRCT)

SP-01

EXPRESSION OF P^{53} GENE IN CERVICAL CANCER SCREENING

SURIYA PALANIVELU

Assistant Professor& Head, Dept. of Biochemistry, Annai College of Arts and Science, Kumbakonam, Tamilnadu, India.
suriya.biochem@yahoo.com

Abstract
p53 overexpression and its significant role in cervical carcinoma had always been under debate and so far effects on p53 expression and function remain unclear The objective of this preliminary study is to document the biological significance of p53 gene expression in cervical cancer. The expression of p53 gene was assessed in patients diagnosed with HPV positive cervical cancers those underwent myomectomy by RT-PCR analysis and compared with tissues from patients with normal cervix. The study demonstrated that overexpression of p53 gene was significant in all the five cervical cancer cases than the normal tissues thereby concluding that p53 overexpression serves an important biomarker tool in diagnosis for cervical carcinoma.

Key words: cervical cancer, screening, p53 gene expression.

Abbreviations:
DNA: Deoxy ribo nucleic acid, dNTP: Deoxynucleotide, HPV: Human Papilloma Virus, HRHPV: High Risk Human Papilloma Virus, mRNA: messenger Ribonucleic acid, OD: Optical density, PCR: Polymerase Chain reaction.

Introduction

There is a strong association between infection with certain types of genital human papillomaviruses (HPVs) and the subsequent development of cervical cancer. Many epidemiological studies conducted over the last 20 years have established a strong association between the HRHPV (HPV type 16 and 18) and cervical cancer in up to 95% of cases.[1]

In the majority of cervical carcinomas viral DNA sequences are integrated into the host genome resulting in deregulation of the viral oncogenes.[2] It has been established that E6 and E7 oncoproteins of HPVs are bound to tumor suppressor genes p53 and recombinant (Rb) and remove their tumor suppressing properties, inducing malignancies.[3] E6 targets the p53 tumor suppressor for degradation, leading to loss of p53-dependent apoptosis and senescence whereas E7 binds to the pRb tumor suppressor, thereby disrupting G1/S transition control.

Consequently, HRHPV infection may result in malignant alteration and tumor development.The hypothesis whether overexpression of p53 protein is a parameter of more destructive disease in patients with cervical cancer is been in research for long time. However, so far effects on p53 expression and function remain unclear. The first p53 gene mutation in human cancer was described in 1989.[4]It is estimated that p53 mutations are the most frequently identified genetic aberrations in human cancers, accounting for more than 50% of the human cancers.

This preliminary study aims to evaluate the association of p53 gene expression with cervical cancer and its relation to disease prognosis. Henceforth this study on expression of the p53 gene in tissue of the uterine cervix obtained from patients who underwent myomectomy by systematically screening the mRNA expression profiles in between cancerous and normal tissues might play a significant role in the diagnosis of cervical cancer.

Materials and Methods

Sample collection

This is a preliminary case-control study performed in tissue samples collected from 5 subjects each of cases and control. The samples (cases) were obtained from patients presenting with cervical carcinoma and control samples consisted of normal cervical tissues obtained from 5 patients who underwent

myomectomy. The samples were collected from GVN hospital and Doctors Diagnostic Centre in Tiruchirapalli, Tamilnadu, South India.

Ethics

Prior to the study, ethical permission was obtained from Hospital Ethics committee. Demographic/Clinical data was obtained from each subjects regarding name, age, height, weight, history of present illness, personal history and any other clinical history. Consent from the patient was taken for local examination and biopsy.

Processing of Samples

Formalin fixing and paraffin-embedding of tissues (FFPET)

All the tissues obtained from both the cases and the controls were immediately formalin fixed with 10% neutral buffered formalin (4% formaldehyde: 10mM phosphate buffer, pH-7.0) for 48 hrs at room temperature. The tissue is then rinsed in running tap water or I hr. and dehydrated through 79%, 80% and 95% alcohol45 min each followed by 3 changes of 100% alchocol, 1 hr each. The tissue sample is then cleared in 2 changes of xylene 1 hr each and immersed in 3 changes of paraffin 1 hr each and embedded in paraffin. The paraffin tissue block was stored at room temperature.[6]

Deparaffinization

The paraffin-embedded tissue block was sectioned into small pieces on a microtome and placed into small test tubes. Washed with 100% xylene 3 times for 5 minutes each and rehydrated twice with 100% ethanol for 10 minutes each and further with distilled water twice for 5 minutes each.

Extraction of mRNA

RNA isolation from the tissue sample was performed using TRI reagent method.[7] Approximately 50 mg of the tissues were used to isolate the total RNA. The tissue samples were homogenized in 1ml of Tri reagent. The homogenate was incubated for 30 min at room temperature and then centrifuged 12000 g for 10 min at 4˚ c. After centrifugation the supernatant was transferred into a clean 1.5ml tube. 200 µl of chloroform was added and shaken vigorously for 15 sec. The mixture was then incubated at room temperature for 3 min then centrifuged at 12000 g for 30 min at 4°C. The aqueous RNA phase was then transferred to a clean tube and then added 500 µl of Iso propanol / 1 ml of Tri reagent and vortexed for 5 min. The mixture was then centrifuged at 12000g for 8 min at 4°C and then finally 1 ml of 75 % ethanol / 1 ml Tri reagent were added and centrifuged at 7500g for 10 min at 4°C. The above steps were repeated until complete purification of the RNA manifested by the lack of interphase. Air dry the pellet for 5 min then the pellet was resuspended in 10 µl of RNAse-free water. The RNA sample obtained was stored at -20°C till further quantification.

Quantification of RNA

Quality and quality of RNA obtained for both the cases and controls was analysed spectrophotometrically using a calibrated spectrophotometer, Biophotometer 6132. 1 µl of sample was dissolved in 49 µl of RNAse-free water and its optical density (OD) at 260 nm was recorded for calculation of the concentration and the ratios 260/280 was determined for assessing the purity of samples.[8]

P53 expression analysis (PCR)

P53 gene amplification was performed in Eppendorf E331 PCR machine using the following type-specific PCR primers for the p53 gene with Forward sequence: CCTCAGCATCTTATCCGAGTGG,Reverse sequence: TGGATGGTGGTACAGTCAGAGC (product size 128 bp). Amplification was carried out by standard PCR reaction mix containing 1 µl each of forward and reverse primer, premix (dNTPs) 13 µl, template 2 µl and autoclaved double distilled water 3 µl accounting to a total volume of 20 µl. Preliminary denaturation was carried out for 15 min at 62°C followed by 36 cycles of denaturation at 94℃ for 2 min, annealing at 62℃ for 1 min and extension at 72℃ for 7 min.

Electrophoresis

The amplified RNA was examined by electrophoresis in a agarose gel.The digested PCR products were mixed with an appropriate amount of 5x gel loading buffer and with ethidium bromide at a concentration of 0.5µg/ml. 10 µl of the mixture were loaded into the wells using a Hamilton syringe and

electrophoresed at 120 V with a 100-1000bp DNA marker until the marker dyes migrated half the length of the gel. The gel is observed on the UV transilluminator and photographed.

Results

In this present study, five cases of cervical cancer and five normal controls were evaluated for p53 expression. The average purity of the RNA isolated from the cases were 1.258±0.091 and whereas for controls 1.224±0.101 (Table 1). The mean concentration of RNA isolated from the cases were 4.524±3.808 µg/ml and whereas for the controls were 1.54±0.398 µg/ml (Table 2). PCR amplification of the p53 gene and further electrophoration had demonstrated (Fig. 1) that of the 10 samples (n=10) tested 5 samples (cases) from the patients with squamous cell carcinoma and adenocarcinoma of cervix showed a significant expression (367bp). Whereas the 5 control group consisting of normal epithelium of the cervix, the expression of p53 was absent.

Table 1. RNA concentration among cases

S.no.	Age of the cases	Purity of RNA extracted (OD260:OD280 ratio)	RNA concentration (µg/ml)
1	65	1.16	2.3
2	40	1.32	5.95
3	35	1.24	2.04
4	55	1.4	11.36
5	38	1.17	0.97
Average	46.6	1.258	4.524

Table 2. RNA concentration among controls

S.no.	Age of the controls	Purity of RNA extracted (OD260:OD280 ratio)	RNA concentration (µg/ml)
1	37	1.17	1.34
2	50	1.15	0.99
3	50	1.39	1.41
4	45	1.12	1.83
5	46	1.29	2.13
Average	45.6	1.224	1.54

Discussion

Cervical cancer was the 3rd leading cause of death in developing countries, with India accounting for 25% of these deaths in 2012.[9]The pathogenesis of cervical cancer is thought to occur through a multistep process however the exact mechanism of occurrence is yet to be recognized.p53 aberration is the commonest genetic alteration involved in human malignancies.[10] The aim of this study was to correlate the overexpression of p53 gene with cervical cancer thereby demonstrating p53 as a prognosis molecular tool for carcinoma of uterine.

In our study of 5 cases of cervical cancer, the mean ages of the cases recorded were 46.6 years. Similar to our study other works demonstrated the average ages as 48.4 years,[11] 52.1 years,[12] 52 years,[13]51.1 years[14] and 50.3 years[15]. Our study had revealed that all the 5 cervical carcinoma cases demonstrated higher expression of p53 gene than the controls. The results of our study have been supported by Madhumati *et al*.[15] They reported high level of positive p53 gene expression in cervical cancer tissues with low mutation rates.Although p53 mutations are rare in cervical carcinomas,[16] in the present study the expression was greater in all the 5 (100%) cervical cancer cases. Whereas the frequency of p53 expression in previous studies has ranged from 9% to as high as 75% of cervical cancers,[17,18] suggesting p53 may be playing an important role in this cancer. Also earlier reports suggested that expression of p53 protein was much more prevalent in squamous cell carcinoma than in adenocarcinoma similar to previously reported data. [19,20,21] Though it is a base-line study to evaluate p53 expression between the cervical cancer patients and the

normal subjects, similar case control study shall be conducted on a larger population to substantiate the association between p53 expression and prognosis.

References

1. Raju K, Punnayanapalya SS, Mariyappa N. Significance of p53, pRb and Ki-67 markers in cervical intraepithelial lesion and malignancy. *Biomedical Research and Therapy* 2015;2:374-384.
2. Ngan HYS, Tsao SW, Liu SS et al. Abnormal expression and mutation of p53 in cervical cancer-a study at protein, RNA and DNA levels. *Genitourin Med* 1997;73:54-58.
3. Mollamehmetoglu, BV, Erdem H, Keles M. The relationship between p53 expression and human papillomavirus in premalignant and malignant uterine cervical lesions. *Open Journal of Pathology* 2016;6:73-78.
4. Baker SJ, Fearon ER, Nigro JM et al. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989;244:217–221.
5. Feki A, Imringer-Finger I. Mutational spectrum of p53 mutations in primary breast and ovarian tumors. *Oncology Hematology* 2004;52:103-106.
6. Canene-Adams K. Preparation of formalin-fixed paraffin-embedded tissue for immunohistochemistry. *Methods Enzymol* 2013;533:225-233.
7. Cirera S. Highly efficient method for isolation of total RNA from adipose tissue. BMC research notes 2013;6:472.
8. Barbas CF 3rd, Burton DR., Scott JK et al. Quantitation of DNA and RNA. Cold Spring Harb. Protoc. 2007 doi:10.1101/pdb.ip47.
9. Torre LA, Bray F, Siegel RL et al. Global Cancer Statistics, 2012. *CA Cancer J Clin* 2015;65:87-108.
10. Hunt CR, Hale RJ, Buckley CH et al. p53 expression in carcinoma of the cervix. J Clin Pathol 1996;49:971-974.
11. Rajaram S, Gupta G, Agarwal S, et al. High-risk human papillomavirus, tumour suppressor protein p53 and mitomycin-c in invasive squamous cell carcinoma cervix. *Indian J Cancer* 2006;43:156.
12. Tjalma WA, Weyler JJ, Bogers JJ, et al. The importance of biological factors (bcl-2, bax, p53, PCNA, MI, HPV and angiogenesis) in invasive cervical cancer. *Eur J Obstet Gynecol Reprod Biol* 2001;97:223-30.
13. Tan GC, Sharifah NA, Shiran MS, et al. Utility of Ki-67 and p53 in distinguishing cervical intraepithelial neoplasia 3 from squamous cell carcinoma of the cervix. *Asian Pac J Cancer Prev* 2008;9:781-4.
14. Tan GC, Sharifah NA, Salwati S, et al. Immunohistochemical study of p53 expression in premalignant and malignant cervical neoplasms. Med Health 2007;2:125-32.
15. Madhumati G, Kavita S, Anju M, et al. Immunohistochemical expression of cell proliferating nuclear antigen (PCNA) and p53 protein in cervical cancer. *J. Obstet. Gynaecol. India* 2012;62: 557-561.
16. Tommasino M, Accardi R, Caldeira S, et al. The role ofTP53 in Cervical carcinogenesis. *Hum Mutat* 2003;21:307-12.
17. Graflund M, Sorbe B, Karlsson M. Immunohistochemicalexpression of p53, bcl-2, and p21(WAF1/CIP1) in earlycervical carcinoma: correlation with clinical outcome. *Int J Gynecol Cancer*, 2002;12:290-8.
18. Wootipoom V, Lekhyananda N, Phungrassami T, et al .Prognostic significance of Bax, Bcl-2, and p53 expressions in cervical squamous cell carcinoma treated by radiotherapy. *Gynecol Oncol* 2004;94:636-42.
19. Hunt CR, Hale RJ, Buckley CH, et al. p53 expression incarcinoma of the cervix. *J Clin Pathol* 1996;49:971-4.
20. Dimitrakakis C, Kymionis G, Diakomanolis E, et al. Thepossible role of p53 and bcl-2 expression in cervical carcinomas and their premalignant lesions. *Gynecol Oncol* 2000;77: 129-36.
21. Horn LC, Fischer U, Hanel C, et al. p53 in surgically treated and pathologically staged cervical cancer: correlation with local tumor progression, but not with lymphatic spread. *Pathol Res Pract* 2001;197:605-9.

SP-02

ANDROGRAPHOLIDE PREVENTS DRUG AND STRESS INDUCED ULCER IN RATS

P. SARANYA[1] AND A. GEETHA[2]

[1]Research Scholar, Department of Biochemistry, Bharathi womens college, Madras University, chennai
[2]Professor, Department of Biochemistry, Bharathi womens college Madras University, chennai
Email: saranya83_bio@yahoo.co.in

ABSTRACT

Andrographis paniculata (Burm.f.)Wall.ex Nees (Acanthacea) has been used traditionally for the treatment of gastrointestinal complications. Andrographolide, a chief compound present in the leaves has been shown responsible for the therapeutic potential of the plant. The present study investigated the gastroprotective effect of andrographolide (AGL) against aspirin (ASA) and cold restraint stress (CRS) induced experimental ulcer in rats. Male albino Wistar rats were administered orally with AGL (3 mg/kg b.wt.) 30 days prior to ulcer induction by ASA and CRS models. pH, gastric fluid volume, titrable acidity, pepsin, myeloperoxidase and H^+K^+ATPase activity, mucin, thiobarbituric acid reacting substances (TBARS), 4-hydroxy nonenal, GSH/GSSG ratio, antioxidant enzyme activities and prostaglandin E_2 (PGE_2) were investigated. AGL pretreatment significantly decreased the acidity and pepsin concentration in ulcer induced rats. The alterations induced in the activity of myeloperoxidase and H^+K^+ATPase were minimized in AGL pre-administered rats. Mucin, thiol content, PGE_2 and enzymatic antioxidants levels were maintained whereas TBARS (1.48 ± 0.18, 2.25 ± 0.25 nmol/mg protein) level was reduced due to AGL treatment. The results suggest that AGL can prevents drugs such as aspirin and stress induced ulcer. The ulcer preventive effect may probably by maintaining gastric defensive factors such as thiol groups, mucin and PGE_2.

KEYWORDS: *Andrographis paniculata*, Andrographolide, H^+K^+ATPase, Inflammation, Mucin, Myeloperoxidase, Prostaglandin E_2, Thiols, Ulcer score.

INTRODUCTION

Gastric hyperacidity and gastroduodenal ulcer is a common global problem today. Hyperacidity is a pathological condition due to uncontrolled secretion of hydrochloric acid that disturbs the mucosal defense to cause inflammation of mucosa[1]. Decrease in defense mechanism aggravates gastroduodenal ulcer caused by loss of gastroprotection by various factors including prostaglandin E_2, mucin and protective thiol groups. Stress[2], nonsteroidal anti-inflammatory drugs (NSAIDS)[3] and *Helicobacter pylori* infection[4] may lead to hyperacidity and gastric ulceration. Apart from the damaging role of acid, reactive oxygen species (ROS) play a major role in causing oxidative damage to mucosa in almost all types of human ulcer[5].

Many drugs including proton pump inhibitors, prostaglandin analogs and histamine receptor antagonists are currently used for the treatment of gastric ulcer. But most of these drugs produce several adverse reactions on long term use and even ulcer relapse sometimes[6]. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors[7,8] and the search for novel herbal drugs continues due to easy availability, cost effect and low toxicity.

The present study toinvestigate the exploit the efficacy of andrographolide, the diterpenoid of the plant *Andrographis paniculata* (AP) (Burm. f.) Wall.ex Nees (Acanthaceae) as a gastroprotective agent. AP is available abundantly in India, Pakistan and Srilanka, growing in hot and shade places. AP was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations. It was referred as a wonder drug in Siddha and Ayurvedic formulations, used for liver and gastrointestinal ailments[9].

Andrographolide (AGL), is an unsaturated trihydroxy lactone with the molecular formula of $C_{20}H_{30}O_5$ (Fig. 1), whose structure was elucidated by Medforth *et al.*[10] which revealed that the bicyclic diterpene lactone contains three hydroxyl groups and two methyl groups. AGL a chief constituent of the leaves of AP, is a bitter water soluble lactone exhibiting protective effect on CCl_4 induced hepatopathy in rats[11]. This compound has recently shown to work as an anti-inflammatory agent by reducing the generation

of reactive oxygen species in human neutrophils[12]. AGL has aroused the interest of many pharmacologists and numerous experiments have been performed, although much remains to be clarified. A few investigations of the pharmacokinetics have been reported after oral administration of andrographolide[13].

The present study was an attempt to evaluate the effect of AGL against gastric ulcers induced by aspirin and cold restraint stress.

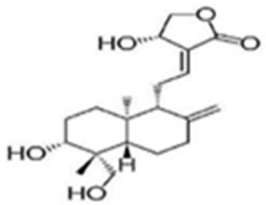


Figure 1
Structure of andrographolide

MATERIALS AND METHODS

Chemicals–Aspirin, alcian blue, diethyl ether, ATP and NADH were purchased from SRL Chemical Company India, and AGL was purchased from Sigma Aldrich, Bangalore, India. All other reagents and chemicals used were of analytical grade.

Eight to ten weeks old Male albino Wistar rats, weighing 120-140 gwere purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, Indiaand were maintained in the animal house, The animals were housed in polypropylene cages and provided with a standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12h light /dark cycle.

Dose response study–An initial dose response study was conducted in rats treated with 1, 3 and 5 mg/kg b.wt of AGL orally for 30 days, to find out the optimal ulcer protective dose against aspirin and cold restraint stress induced gastric ulcer in male albino Wistar rats. A dose of 3 mg/kg b.wt of AGL was then selected on the basis of optimal ulcer protective effect for further studies. Similar dose response study was conducted with 15, 30 and 50 mg/kg b.wt of ranitidine and 30 mg/kg b.wt was found to be the optimum dose[14].

Treatment protocol for antiulcer activity– Rats were divided into 5 groups of 6 animals each.

Group I - Control rats.
Group II - Rats subjected to ulcerogenesis.
Group III - Rats pretreated with 3 mg/kg b. wt of AGL for 30 days and then subjected to ulcer induction
Group IV - Rats pretreated with 30 mg/kg b. wt of standard drug ranitidine for 30 days and then subjected to ulcer induction.
Group V - Rats treated with 3 mg/kg b. wt of AGL for a period of 30 days.

Aspirin (ASA) induced ulcer–ASA was administered as a single dose of 200 mg/kg b.wt to the animals and sacrificed after 4 h[15]. *Cold restraint stress (CRS) induced ulcer*–On day 30, after 30 min of AGL or ranitidine administration, rats were immobilized in a stress cage containing water at 4-6°C then the animals were sacrificed after 2 h[16].*Determination of acid secretory parameters*–The ulcer-induced in rats by ASA and CRS models were considered for the evaluation of *p*H, gastric volume and titrable acidity.

Biochemical analyses

Determination of pepsin activity–Pepsin was assayed according to the method of Shay *et al.*[17] using hemoglobin as substrate. The absorbance of the solution was read at 650 nm. The pepsin content was expressed as μ*M* of tyrosine liberated/ml.

Determination of gastric mucin content–Mucin content was determined by the method of Corne *et al.*[18]. After the collection of gastric juice, the glandular portion was excised and opened down along the lesser curvature. The reverted stomach was soaked for 2 h in 0.1% alcian blue (0.16 *M* sucrose buffered with 0.05 *M* sodium acetate). The uncomplexed dye was removed by 2 successive washes of 15 and 45 min in 0.25 *M* sucrose solution. The dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5

M magnesium chloride for 2 h. The resulting blue solution was shaken briefly with equal volume of diethyl ether and optical density of the aqueous phase was measured at 605 nm. The mucin content of the sample was determined from the standard curve obtained with different concentration of mucin.

Determination of myeloperoxidase activity–Myeloperoxidase (MPO) activity in the gastric tissue was measured according to the method of Bradley *et al*.[19]. Approximately 1.0 g of tissue was homogenized (1:10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide in 50 m*M* potassium phosphate buffer (*p*H 6.0) before sonication in an ice bath for 20 sec. Three freeze/thaw cycles were performed followed by sonication (20 sec in ice bath). The samples were centrifuged at 17000 *g* (5 min, 4°C) and myeloperoxidase in the supernatant was assayed by mixing 0.1 ml of supernatant and 2.9 ml of 50 m*M* potassium phosphate buffer (*p*H 6.0) containing 0.167 g/L *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured for 4min using an UV visible spectrophotometer.

Determination of H^+K^+-ATPase activity–Proton potassium ATPase was prepared from gastric mucosal scrapings[20] and homogenized in 200 m*M* Tris-HCl, *p*H 7.4, centrifuged for 10 min at 5000 X *g*. The resulting supernatant was subsequently centrifuged at 5000 X *g* for 20 min. The protein concentration of the supernatant was determined by using bovine serum albumin as standard[21].

The H^+K^+ ATPase activity in the gastric mucosa was assayed by the method of Reyes- Chilpa *et al*.[22]The assay mixture consisted of an aliquot of enzyme in 20 m*M* Tris-HCl, *p*H 7.4, 2 m*M* $MgCl_2$ and 2 m*M* KCl. The reaction was started with the addition of 2 m*M* ATP and incubated for 30 mins at 30°C and terminated by the addition of 10% trichloroacetic acid followed by centrifugation at 2000 X *g*. The amount of inorganic phosphorous released from ATP was determined spectrophotometrically at 640 nm. The enzyme activity was expressed as n*M* of Pi liberated/min (U)/mg protein.

Determination of prostaglandin E_2 (PGE_2) –Gastric tissues were homogenized with homogenizing buffer (0.05 *M* Tris-HCl at *p*H7.4, 0.1 *M* NaCl, 0.001 *M* $CaCl_2$, 1 mg/ml D-glucose and 28 μ*M* indomethacin to inhibit further PGE_2 formation) for 30 sec. They were then centrifuged at 12,000 rpm for 15 min at 4°C. Supernatants were assayed by using a commercially available PGE_2 enzyme-linked Immunoassay kit – DetectX (Arbor assays: Cat.no K018-H1). The assay procedure was in accordance with the protocol suggested by the manufacturer. Optical density was determined by the Elisa microplate reader at 450 nm. The amount of protein in the sample was determined by the method of Bradford[21]. The mucosal PGE_2 level was expressed as pg per mg of protein and analysis of lipid peroxidation by products and oxidised and reduced glutathione. The homogenates was used for the estimations of TBARS, 4-HNE, GSH, GSSG and antioxidants.

Lipid peroxides in terms of thiobarbituric acid reacting substances (TBARS) was estimated using 1, 1′, 3, 3′- tetra methoxypropane as the standard and expressed as n*M*/mg protein[23] and 4-hydroxy nonenal (4-HNE) was measured by UV spectrophotometric method at 350 nm[24]. Reduced and oxidized glutathione (GSH & GSSG) was determined by the method of Moron *et al*.[25] and Hansen *et al*.[26]. GSH was used as a reference standard and expressed as n*M*/mg protein. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler[27]. The activity of GPx was expressed as n*M* GSH oxidized/min/mg protein.

Superoxide dismutase (SOD) activity was measured according to the method of Kakkar *et al*.[28]. The inhibition of reduction of nitroblue tetrazolium to blue colored formazan in presence of phenazine methosulphate and NADH was measured at 560 nm using *n*- butanol as blank. The enzyme activity was expressed as units/mg protein.

Decomposition of H_2O_2 in the presence of catalase (CAT) was kinetically measured at 240 nm[29]. The enzyme activity was expressed as μ*M* of H_2O_2 consumed/min/mg protein.

STATISTICAL ANALYSIS

Data were analyzed by using a SPSS software.

RESULTS AND DISCUSSION

It is generally accepted that ulcer results from an imbalance between aggressive factors such as acid and pepsin and the defensive factors such as prostaglandins, bicarbonate and mucosal glycoproteins. To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the defense mechanism by increasing mucus production. In our previous study, we reported that the animals which received 3 mg/kg b.wt of AGL showed an optimum reduction of ulcer index in rats subjected to ulcerogenesis by various methods including aspirin and CRS induced models[30].

Effect of AGL on pH, volume of gastric fluid, titrable acidity, MPO and H^+K^+ATPase–Table 1 shows the effect of AGL on *p*H, volume of gastric fluid, titrable acidity, MPO and H^+K^+ATPase activity in ulcer induced rats. The gastric fluid and titrable acidity were found to be decreased significantly in AGL treated animals when compared to that of ulcer control group. The MPO, pepsin and H^+K^+ATPase activity were found to be elevated and were significantly restored in test drugs treated rats.

Pepsin is a proteolytic enzyme present in the gastric secretion of mammals which is derived from an inactive precursor pepsinogen produced principally in the chief cells of the gastric mucosa. Pepsin activity is merely an index of the secretory activity of the pepsinogenic cells of the gastric mucosa. The pepsin activity is significantly elevated in ulcer control rats when compared to that of rats administered with the test drug prior to ulcer induction.

We have previously reported that AP and AGL inhibited the activity of gastric mucosal H^+K^+ATPase, the proton pump *in vitro*, similar to omeprazole[30]. The modest approach to control ulceration may be by reducing H^+K^+ATPase action, the enzyme for acid secretion in the parietal cells of gastric mucosa. Although several antisecretory drugs such as omeprazole, lansoprazole, ranitidine, famotidine and histamine H_2- receptor blockers are being used to control acid secretion, long term use may produce adverse side effects on human health[31]. In recent years, the drugs that reduce the acid secretion and H^+K^+ATPase activity are the preferred choice for ulcer treatment[32]. Mucosal damage, an initial step in ulcer development has been known correlated hypersecretion of HCl through H^+K^+ATPase action along with oxidative stress[33].

MPO activity is an index of neutrophil dependent inflammatory response in a variety of clinical and experimental conditions[19]. MPO mediates lipid peroxidation in the presence of hydrogen peroxide with halide ions to prevent microbial infection to be followed after inflammation[34]. The decrease in MPO activity in test drugs received animals showed that inflammation is significantly controlled by AGL treatment.

Levels of TBARS, 4-hydroxyl nonenal, PGE_2, mucin content, pepsin concentration and GSH/GSSG ratio–The gastric levels of TBARS, 4-hydroxyl nonenal, PGE_2, mucin content, pepsin concentration and GSH/GSSG ratio are presented in Table 2. The lipid peroxidation produts TBARS and 4-HNE were found to be elevated in ulcerogen treated rats and the elevation was controlled in test drug administered rats. Lipid peroxidation is a prominent manifestation of ROS and free radical activity in biological systems. The oxidative modification of lipids and ROS has been implicated in the pathogenesis of many diseases including peptic ulcer[35], including gastroduodenal ulcer[36]. Different methodologies have been developed that measure a variety of lipid peroxidation products used as markers of lipid peroxidation processes. Malondialdehyde (MDA) is cytotoxic and chemically reactive, but 4-HNE is more aggressive than MDA and leads to cell damage at nanomolar concentration[37]. MDA and 4-HNE were significantly higher in aspirin induced ulcer when compared with that of normal rats. Pihan *et al*.[38] reported that radicals such as OH- causes lipid peroxidation and increases gastric lesions induced by necrotizing agents.

The mucin content, GSH/GSSG ratio and PGE_2 were found to be depleted significantly in ulcer induced animals. The test drug pretreated rats maintained the level to near normal. Mucosal barrier is the major factor that provides gastric protection, because it has been proved that more the production of mucus, less is the degree of ulceration. The higher the mucin content, lower is the free acidity and so stimulating mucosal defensive agents is a new dimension in the treatment of gastric diseases[39]. The mucin content was found to be preserved significantly in rats pretreated with the test drug that show the possible role of AGL in enhancing mucosal defense against gastric ulcer.

GSH, a tripeptide containing glycine, glutamic acid and cysteine is the major redox compound of the aqueous phase in cells, scavenging reactive electrophilic substances, mainly ROS. Being the main antioxidant factor, it is also the chief reservoir of cellular SH gropus[40]. The cysteinyl residue of GSH provides nucleophilic thiol which is essential for the detoxification of electrophilic metabolites and metabolically produced oxidizing agents[41]. We could see a significant depletion in the ratio of GSH:GSSG an indicator of thiol redox status in group II rats which were subjected to ulcerogenesis. GSH:GSSG ratio shows the influence of reduced thiols on the inflammatory changes induced by ulcerogen. The levels were found to be restored in drug received rats.

Prostaglandins, particularly PGE_2, is known to have strong antiulcer effects and play key role in gastric defense mechanism by maintaining factors such as mucus, bicarbonate, microcirculation and cell turn over[42,43]. Recent studies have revealed that receptors activated by E-type prostaglandins are divided into 4 subtypes: EP_1, EP_2, EP_3 and EP_4[44]. The EP_4 receptor has also been reported to be abundantly present in gastric mucus producing cells, and PGE_2 appears to stimulate mucus secretion via this receptor[45,46]. We could see a significant depletion of PGE_2 in ulcer control rats when compared to that of rats received the test drug prior to ulcerogenesis.

Aspirin inhibits prostaglandin biosynthesis, irreversibly by acetylating serine residues in the active site of cyclooxygenase (COX). COX-2 is an inducible form, which is activated in response to cytokines at the site of inflammation to produce prostaglandins. PGE_2 is the major prostaglandin (PG) made by the human stomach that is involved in the inhibition of gastric secretion, stimulation of mucus secretion and clearance of acid from the submucosa via local vasodilation. Ferreira *et al.*[47] demonstrated that aspirin like drugs blocked PG release from the perfused, isolated dog spleen. Collier and Flower[48] have also reported that administration of aspirin inhibited human seminal PG production. PGs might be responsible, at least in part, for the genesis of fever or inflammation and that aspirin like drugs might owe their therapeutic activity to their ability to prevent PG biosynthesis. Aspirin selectively acetylates the hydroxyl group of serine residue (Ser 530) located 70 amino acids from the C terminus of the enzyme. Acetylation leads to irreversible COX inhibition.

Levels of enzymatic antioxidants in gastric tissue–Table 3 shows the levels of enzymatic antioxidants in gastric tissue. The activity levels of SOD, CAT and GPx were found to be maintained in the test drug pretreated animals when compared to that of ulcer control rats. The levels were non-significantly elevated in normal rats received AGL. GPx plays a key role in eliminating H_2O_2 and lipid hydroperoxides in gastric mucosal cells. It is also essential for maintaining a constant ratio of reduced glutathione to oxidized glutathione in the cell. [49] GPx inhibition results in H_2O_2 accumulation and subsequent lipid peroxidation which could be related to the gastric damage induced by ulcerogens.[50]

SOD scavenges the superoxide radical O_2^-, one of the reactive oxygen species responsible for lipid peroxidation[51]. This reaction leads to increase in generation of peroxyl radical H_2O_2, which is also capable of producing more oxidative damage[5]. CAT and other peroxidases further reduce H_2O_2. This led to increased generation of H_2O_2 and its accumulation due to decreased CAT level. Inactivation of gastric peroximes during stress[52] may also aggravate the mucosal damage. In the present study we have observed that treatment with the test drugs maintained the levels of SOD and CAT in the rat gastric tissue when compared to that of ulcer control rats.

Rats pretreated with AGL or Ranitidine for 30 days (dose in mg/kg b.wt)	**TBARS (nmol/mg protein)**	**4-Hydroxy nonenal (% of control)**	**GSH/GSSG ratio**	**Mucin (µg Alcian blue/g of glandular tissue)**	PGE_2(pg/mg protein)
None	0.93±0.10	-	15.27±1.63	482.24±53.03	20.5±3.61
ASA	1.48 ± 0.18*	47.3±5.3	6.23±0.67*	233.0 ± 24.46*	10.65±2.6*
AGL (3)+ ASA	0.93 ± 0.07*	21.5±3.9*	13.01±1.47*	468.12 ± 43.45*	17.5±2.1*
Ranitidine (30) +ASA	0.95 ± 0.10*	10.6±1.9*	12.71±1.41*	472.16 ± 47.4*	21.6±3.6*
CRS	2.25 ± 0.25*	48.5 ± 5.7	6.73 ± 0.71*	265.31 ± 26.93*	15.5±2.5$^{\#}$
AGL (3) + CRS	0.89 ± 0.08*	19.93 ± 2.4 *	12.92 ± 1.31*	499.12 ± 49.78*	22.5±3.6*
Ranitidine (30) + CRS	0.85 ± 0.08*	9.56 ± 0.97 *	12.69 ± 1.29*	489.65 ± 48.34*	20.6±3.65*
AGL (3)	0.99±0.13^{NS}	8.9±2.6	14.63±1.77^{NS}	487.09 ± 54.34 ^{NS}	23.6±4.1 ^{NS}
Statistically significant difference is expressed as *P<0.001, $^{\#}$P<0.01 and ns - non significant. Groups are compared as: Control vs ulcer (ASA and CRS), AGL or ranitidine vs respective ulcer, control vs AGL.					

Effect of AGL on enzymatic antioxidants in gastric tissue. [Values are mean ± SD of 6 animals]			
Rats pretreated with AGL or Ranitidine for 30 days (dose in mg/kg b.wt)	**GPx (nmol of GSH oxidized/min/mg protein)**	**SOD (U/mg protein)**	**CAT (µmol of H_2O_2 decomposed/min/mg protein)**
None	211.64±27.12	46.14 ± 5.02	4.13 ± 0.43
ASA	105.0 ± 11.07*	37.32 ±4.39†	2.21± 0.22*
AGL (3)+ ASA	148.11 ± 16.29*	42.11 ± 4.29†	3.89 ± 0.33*
Ranitidine (30) +ASA	196.17 ± 20.0*	43.89 ± 4.56†	3.78 ± 0.40*
CRS	115.27 ± 11.65*	40.03 ± 4.26†	2.18 ± 0.23*
AGL (3) + CRS	250.23 ± 26.32*	45.15 ± 4.57†	3.93 ± 0.39*
Ranitidine (30) + CRS	253.87 ± 27.15*	45.68 ± 4.89†	3.91 ± 0.40*
AGL (3)	215.68 ± 21.97 ^{NS}	45.27 ±4.93 ^{NS}	3.95 ± 0.41 ^{NS}
Statistically significant difference is expressed as *P<0.001, †P<0.05 and ns - non significant. Groups are compared as: Control vs ulcer (ASA and CRS), AGL or ranitidine vs respective ulcer, control vs AGL.			

CONCLUSION

This study concludes that the efficacy of AGL as antiulcer agent when tested with aspirin and cold restraint stress induced ulcer models are comparable to that of ranitidine and the beneficial effect may be probably by reducing the action of offensive factors and also by elevating the levels of defensive factors such as PGE_2, mucin and thiols in gastric tissues.

REFERENCES

1 Sachs G, Maton PN and Wallmark B, Pharmarology of the parietal cell, in: Benjamin SB, ed. *Pharmacology of peptic ulcer disease.* New York: Springer Verlag, (1991) 1.

2 Cook DJ, Witt LG, Cook RJ and Guyatt GH, Stress ulcer prophylaxis in the critically III: A metaanalysis, *Am J Med,* 91 (1991) 519.

3 Langman MJ, Brooks P, Hawkey CJ, Silverstein F and Yeomans N, Non-steroidal anti-inflammatory drug associated ulcer:epidemiology, causation and treatment, *J Gastroenterol Hepatol,* 6 (1991) 442.

4 Konturek PC & Konturek SJ, Role of Helicobacter pylori infection in gastro-duodenal secretion and in pathogenesis of peptic ulcer and gastritis, *J Physiol Pharmacol,* 45 (1994) 333.

5 Das D, Bandyopadhyay D, Bhattacharya M & Banarjee RK, Hydroxyl radical is the major causative factor in stress induced gastric secretion. *Free Radic Biol Med,* 23 (1997) 8.

6 Ariyphisi I, Toshiharu A, Sugimura F, Abe M, Matsuo Y & Honda T, Recurrence during maintenance therapy with histamine H_2 receptor antagonist in cases of gastric ulcer, *Nihon Univ J Med,* 28(1986) 69.

7 Sairam K, Rao CV & Goel RK, Effect of Centella asiatica Linn on physical and chemical factors induced gastric ulceration and secretion in rats. *Indian J Exp Biol,* 39 (2001) 137.

8 Sairam K, Rao CV & Goel RK, Effect of Convolvulus pluricaulis Chois on gastric ulceration and secretion in rats, *Indian J Exp Biol,* 39 (2001) 350.

9 Chopra RN, Nayar SL & Chopra IC, Glossary of Indian Medicinal Plants. NISCOM, CSIR, New Delhi, India, (1956) 18.

10 Medforth CJ, Chang RS, Chen G, Olmstead MM & Smith KM, A conformational study of diterpenoid lactones isolated from the Chinese medicinal herb *Andrographis paniculata*, *J Chem Soc Perkin,* 2 (1990) 1011.

11 Rana AC & Avadhoot Y, Hepatopretective effects of *Andrographis paniculata* against carbon tetrachloride-induced liver damage, *Arch Pharm Sci Res,* 1 (1991) 93.

12 Shen YC, Chen, CF & Chiou WF, Andrographolide prevents oxygen radical production by human neutrophils: possible mechanisnm(s) involved in its anti-inflammatory effect, *Braz J Pharmacol,* 135 (2002) 399.

13 Panossian A, Hovhannisyan A, Mamikonyan G, Abrahamian H, Hambardzumyan E, Gabrielian E, Goukasova G, Wikman G & Wagner H, Pharmacokinetic and oral bioavailability of

andrographolide from *Andrographis paniculata* fixed combination Kan Jang in rats and human, *Phytomedicine,* 7 (2000) 351.

14 Saranya P & Geetha A, A biochemical study on the gastroprotective effect of hydroalcoholic extract of *Andrographis paniculata* in rats, *Indian J Pharmacology,* 43 (2011) 402.

15 Goel RK, Das DG & Sanyal AK, Effect of vegetable banana powder on changes induced by ulcerogenic agents in dissolved mucosubstances in gastric juices, *J Gastroenterol,* 4 (1985) 249.

16 Senay EC & Levine RJ, Synergism between cold and restraint for rapid production of stress ulcer in rats. *Proc Soc Exp Biol Med,* 124 (1967) 1221.

17 Shay H, Komarov SA, Fels SS, Mreanze D, Gruenstein M & Siplet H, A simple method for the uniform production of gastric ulceration in the rat, *Gastroenterol,* 5 (1945) 43.

18 Corne SJ, Morrissey SM & Woods RJ, Proceedings: A method for the quantitative estimation of gastric barrier mucus. *J Physiol,* 242 (1974) 116P.

19 Bradley PP, Priebat DA, Christensen RD & Rothstein G, Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker, *J Invest Dermatol,* 78 (1982) 206.

20 Cheon HG, Lim H & Lee DH, Biochemical properties of a newly synthesized H^+K^+ ATPase inhibitor, 1-(2-methyl-4-methoxyphenyl)-4-[(3-hydroxypropyl) amino]-6-methyl-2, 3-dihydropyrrolo [3,2-c] quinoline. *Eur J Pharmacol,* 411 (2001)181.

21 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem,* 72 (1976) 248.

22 Reyes Chilpa R, Hatsuko Baggiob C, Alavez-Solanoa D, Estrada-Muniza E, Kauffmanc FC, Sanchezc RI & Mesia-Vela S, Inhibition of gastric H+K+ATPase activity by flavonoids, coumarins and xanthones isolated from Mexican medicinal plants, *J Ethnopharmacol,* 105 (1-2) (2006) 167.

23 Draper HH & Hadley M, Malondialdehyde determination as index of lipid peroxidation, *Methods Enzymol,* 186 (1990) 421.

24 Kinter M in: Punchard NA & Keuy GJ, ed. *Free Radicals - A Practical Approach*, Oxford University Press, Oxford, (1996) 136.

25 Moron MS, Depierre JW & Mannervik B, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver, *Biochim Biophys Acta,* 582 (1979) 67.

26 HansenRE, Ostergaard H, Norgaard P & Winther JR, Quantification of protein thiols and dithiols in the picomolar range using sodium borohydride and 4, 4'-dithiodipyridine, *Anal Biochem,*363 (2007) 77.

27 Flohe L & Gunzler WA, Assays of glutathione peroxidase, *Methods Enzymol.* 105 (1984) 114.

28 Kakkar P, Das B & Viswanathan PN, A modified spectrophotometric assay of superoxide dismutase, *Indian J Biochem Biophys,* 21 (1984) 130.

29 Aebi H, Catalase *in vitro,Methods Enzymol,* 105 (1984) 121.

30 Saranya P, Geetha A & Narmadha Selvamathy SMK, The antioxidant and H^+K^+ATPase inhibitory effect of *Andrographis paniculata* and andrographolide-*in vitro and in vivo* studies, *Pharmacologyonline,* 1 (2010) 356.

31 Waldum HL, Gustafsson B, Fossmark R & Qvigstad G, Antiulcer drugs and gastric cancer, *Dig Dis Sci,* 50 (2005) S39.

32 Perlin DS, Ion pumps as targets for therapeutics intervention. Old and new paradigms, *Electron J Biotechnol*, 1 (1988) 55.

33 Phull PS, Green CJ & Jacyna MR, A radical view of the stomach: the role of oxygen derived free radicals and antioxidants in gastroduodenal disease, *Eur J Gastroenterol Hepatol,* 7 (1995) 265.

34 Stelmaszynska T, Kukovetz E, Egger G & Schaur RJ, Possible involvement of myeloperoxidase in lipid peroxidation, *Int J Biochem,* 24 (1992) 121.

35 Mylonas C & Kouretas D, Lipid peroxidation and tissue damage, *In Vivo,* 13 (1999)

36 Repetto GM & Llesuy SF, Antioxidant properties of natural compounds used in popular medicine for gastric ulcers,*Braz J Med Biol Res,* 35 (2002) 523.

37 Schmidt H, Grune T, Muller R, Siems WG & Wauer RR, Increased levels of lipid peroxidation products malondialdehyde and 4-hydroxynonenal after perinatal hypoxia,*Pediatr Res,* 40(1996): 15-40.

38 Pihan G, Regillo C & Szabo S, Free radicals and lipid peroxidation in ethanol-and aspirin-induced gastric mucosal injury, *Dig Dis Sci,* 32 (1987) 1395.

39 Bardhan KD, Omeprazole in the management of refactory duodenal ulcer, *Scand J Gastroenterol Suppl,* 166 (1989) 63.

40 Browne RW & Armstrong D, Reduced glutathione and glutathione disulfide,*Meth Mol Biol,* 108 (1998) 347.
41 Ketterer B, Coles B & Meyer DJ, The role of glutathione in detoxication, *Environ Health Perspect,* 49 (1983) 59.
42 Miller TA, Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms, *Am J Physiol,* 245 (1983) G601.
43 Wilson DE, Role of prostaglandins in gastroduodenal mucosal protection, *J Clin Gastroenterol,* 13 (1991)S65.
44 Coleman RA, Smith WL & Narumiya S, International union of pharmacology classification of prostanoid receptors: properties, distribution and structure of the receptors and their subtypes, *Pharm Rev,* 46 (1994) 205.
45 Ding M, Kinoshita Y, Kishi K, Nakata H, Hassan S, Kawanami C, Sugimoto Y, Katsuyama M, Negishi M, Narumiya S, Ichikawa A & Chiba T, Distribution of prostaglandin E receptors in the rat gastrointestinal tract, *Prostaglandins,* 53 (1997) 199.
46 Narumiya S, Sugimoto Y & Ushikubi F, Prostanoid receptors: structures, properties and functions, *Physiol Rev,* 79 (1999), 1193.
47 Ferreira SH, Moncada S & Vane JR, Indomethacin and aspirin abolish prostaglandin release from spleen, *Nature,* 231 (1971) 235.
48 Collier JC & Flower RJ, Effect of aspirin on human seminal prostaglandins, *Lancet,* ii (1971) 852.
49 Halliwell B & Gutteridge JMC, Biologically relevant metal ion dependent hydroxyl radical generation. An update, *FEBS Letters,* 307 (1992) 108.
50 Naito Y, Yoshikawa T, Yoshida N & Konda M, Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric injury, *Dig Dis Sci,* 43 (1998) 30S.
51 Fridovich I, Biological effects of superoxide radical, *Arch Biochem Biophys,* 247 (1986), 1.
52 Boyd SC, Sasame HA & Boyd MR, Gastric glutathione depletion and acute ulcerogenesis by dimethylmalate given subcutaneously to rats, *Life Sci,* 28 (1981) 2987.

SP-03

IN VITRO ANTIBACTERIAL ACTIVITY OF *ECLIPTA PROSTRATA L* BY USING DISC DIFFUSION METHOD

NALINI DEVI D[1] AND MOHAMED SALEEM GANI A[2]

[1]Research Scholar in Biochemistry (category-B), Bharathiyar University, Coimbatore-46.
[2]Associate Professor in Science & Humanities, M.A.M College of Engineering, Siruganur, Trichy-5.
Email: mail2nalinidevi@gmail.com

ABSTRACT

In the current study attempt toexplore phytochemical components from the various solvents such as ethanol, methanol and aqueous extracts of the whole plant of Eclipta prostrata L by Gas chromatography and Mass spectroscopy (GC-MS). 100gms of the powdered sample was exposed to the sox let extraction monitored by rotary evaporator and investigated using PerkinElmer GC-MS. In which the antibacterial activities were determined by using ethanolic extract of this plant. The GC-MS analysis revealed the presence of various compounds with peak area like 2-cyclopentene-1-tri Dacanoic acid, Octa hydropentalen-1-ol,Tri6,4,0,027)] Dodecane. Dodecanoicacid, tridecanoicacid, Hentriacontane, Heptane 2,2,3,3,5,6,6 Hepta methyl, sulfurous acid Hexy Monyl ester. Extracts and metabolites from this plant have been known to possess pharmacological properties and also exhibit antibacterial activity.

KEY WORDS:

Eclipta prostrata L, GC-MS analysis, ethanolic, 2-cyclopentene-1-tri Dacanoic acid,

1. INTRODUCTION

The herb *Eclipta prostrata L (*Asteraceae,) commonly known as Bhringraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice. It grows commonly in moist places as a weed in warm temperature to tropical areas worldwide. It is widely distributed throughout the India, China, Thailand and Brazil [1]. The plant has been reported for the treatment of liver cirrhosis and infective hepatitis. The plant is known to have some important pharmacological activities such as anti-inflammatory, analgesic, hepatoprotective and also possess antimicrobial activity[1]. *Eclipta prostrata L* has been used in traditional systems of medicine and also by traditional healers especially in south region of India for the treatment of liver diseases since ancient times. The phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, saponins, flavanoids, steroids, phenolic compounds, coumarin, luteolin, wedelolactone, triterpenoids, proteins, amino acids and reducing sugar etc. The present study aimed to investigate the phytochemical constituents present in the ethanol, methanol and aqueous extracts of the whole plant of *Eclipta prostrata L*[1].

2. MATERIALS & METHODS

2.1. Collection and preparation of plant materials *Eclipta Prostrata (L.)* L were collected from Trichy, Tamilnadu, India and confirmed by Dr. S. John Britto, The Rapinat Herbarium, ST. Joseph's college, Tiruchirappalli, (Ref.No: DND 001/2014) The leaves were thoroughly washed and shade dried and coarsely powdered in a grinder. Then it is sieved and stored in airtight container for further activities.

2.2. Soxhlet Extraction:

Eclipta Prostrata (L.) powder consisting phytoconstituents were extracted with 70% Ethanol by soxhlet apparatus. The powdered plant sample was packed in a thimble, sealed properly with cotton. Ethanol in the round bottomed flask is boiled up to its boiling point, the vapours of it passes through the packed powder and collected as condensed extract in the RB flask. After repeated extraction of about15-20 cycles the extract was evaporated to expel solvents, which was then used for further process. Finally, crude extract was obtained. The crude extract was stored at

4°C until further use. GC-MS analysis 20g of powdered sample is soaked with 60ml ethanol overnight and filtered through ash less filter paper with sodium sulphate. The extract is concentrated to 1ml by bubbling nitrogen into the solution. The extract contains both polar and non-polar phyto-componentsThe GC-MS was performed by using column Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30× 0.25 mm × 0.25 µm df, equipment: GC-clarus 500 perkin Elmer carrier gas:1ml per minute, split:10.1 detector: Mass detector Turbo mass gold perkin software: turbomass 5.2. 2µl of ethanolic extract of the whole plant of *Eclipta prostrata L* was employed for GC-MS analysis. The 2µl sample extract injected into the instrument was detected by the Turbo mass software. The GC-MS extraction process was maintained at a temperature of 110 0C with 30minutes.the injector temperature was set at 250 0C [mass analyzer]. The different parameters were involved in the operation of the clarus500MS, were standardized. The helium gas was used as the carrier gas at a constant flow rate of 1.0mL/min.MS program: Library used NIST version year 2005 (Inlet line temperature: 200 0C; source temperature: 200 0C). Mass spectra were taken at 70 eV; a scan interval 0f 0.5s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes[2]Phytochemical screening Phytochemical analysis of ethanol extract of Eclipta Prostrata (L.) L was carried out qualitatively to test for the presence of phenols, alkaloids, proteins, amino acids, tannins, carbohydrates, flavonoids, Phytosterols, saponins etc.

ANTIMICROBIAL ASSAY

The pathogenic bacterial species were collected, and it was determined. Bacterial strains consisted of Shigellaboydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp.and Salmonella paratyphi A. The antifungaleffect of *Eclipta prostrataL*. Was determined against fungal strains such as Aspergiller niger and candida albicans. The strains were Sub cultured bimonthly and the cultured strains were allowed to grow for one week and stored at 5 0C for further analysis. Muller Hinton agar (MHA) was used as the media for culturing of bacterial strains. The stock cultures were maintained in sabouraud dextrose broth and two different strains of fungal pathogens were maintained in sabouraud dextrose broth for 24 hours until used for antifungal activity. The discs were immersed in different concentrations like 50µg to 250µg/ml allowed evaporating. After that the plates were incubated at room temperature (27 ˚C ± 2) for 24hours.After incubation, plates were observed for zones of inhibition and recorded in millimetres [3] Evaluation of Anti-microbial activity The antimicrobial activity of the methanol extracts of various parts and in vitro grown plant of *Eclipta prostrataL.*was evaluated through disc-diffusion method. Minimum inhibitory concentration (MIC) The ethanolic extract of *Eclipta prostrataL.*antimicrobial activity were further tested against all the organisms for the evaluation of its antibacterial and antifungal efficiency at different concentrations (50µg to 250µg/ml) by using filter paper disc diffusion method. The zone of inhibition was calculated in millimeters. Activity index was calculated by comparing the zone of inhibition by plant extract with that of standard drug.[4]

$$\text{Activity index} = \frac{\text{Inhibition zone of test sample (extract)}}{\text{Inhibition zone of standard drug}}$$

Table-1:Represents antimicrobial activity of *Eclipta prostrata L.*

S.NO.	NUMBER OF THE ORGANISMS	ZONE OF INHIBITION(mm)
1.	Pseudomonas Sp.	1.8
2.	Shigella boydii	10.6
3.	Klebsilla pneumonia	7.1
4.	Salmonella paratyphi A	5.1
5.	E.coli	6.5
6.	Aspergiller niger	5.7
7.	candida albicans	4.6

3. RESULTS AND DISCUSSION

The individual fragmentations of the components with molecular structure were illustrated in the antimicrobial activity was determined by measuring the diameter of Zone of inhibition. The whole plant extracts (Leaf stem, flower& root) of *Eclipta prostrata L.* with ethanol as well as aqueous[5]From these results of the various extracts against some pathogenic organisms like Shigella boydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp.and Salmonella paratyphi A Aspergiller niger and candida albicans. Speciesetc.From the qualitative of *Eclipta prostrataL.*analysis having various chemical constituents by using aqueous and ethanol extracts showed some medicinal properties.[6] In this analysis was carried out to understand the presence of various chemical constituents such as alkaloids, Tannins, glycosides, flavonoids, Terpenoids and steroids [7] These compounds are shows the plants having antimicrobial activity and also exhibit the pharmacological activity[8].The different parts of the plants exhibited activity against the pathogenic organisms such as, Shigellaboydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp.and Salmonella paratyphi A Aspergiller niger and candida albicans. Species etc. From the Disc diffusion method ethanolic extract of *Eclipta prostrata L.* shows zone of inhibition in mm represents in Table –I.

4. CONCLUSION:

The various bioactive constituents revealed from the different parts of an *Eclipta prostrata L.* Plant by using ethanol extract suggests that the components having pharmacological activity, antimicrobial activity and also possesses antioxidant properties. This medicinal herb indicates that one of the disease curable medicine like liver cancer, Jaundice, hair stimulator, hepatoprotectant and also skin diseases.

REFERENCES

1. Kirtikar KR, Basu BD. Indian medicinal plants, Deharadun (INDIA): International book distributers, (1999)
2. Saxena AK, Singh B, Anand KK.Hepatoprotective effect of Eclipta Alba on sub cellular levels in rats Ethnopharmacol.1993:40:155-161.
3. Alonso, Paz,C.cedeiras,M.P.Ferndaz,J.Ferreira,F.Moyna,P.Soubes, M.Vaques,A.Vero, S.Zunino,L.,Screening medicinal plants for antimicrobial activity. Journal of Ethnopharmocology, 20:67-69(1995) 6
4. The antimicrobial activity of perfume oils. Journal of American Pharmaceutical Association. 28:471 (1958)
5. Seenivasan Prabhuseenivasan, Manickkam Jayakumar and SavarimuthuIgnacimuthu, In vitro antibacterial activity of some plant essentialoils. BMC Complement Altern Med. 6: 39 (2006).
6. Zaidan, M.R.S. Noor Rain, A. Badrul, A.R. Adlin, A. Norazah, A. and Zakiah, I., In vitro screening of five local medicinal plants for antibacterialactivity using disc diffusion method. Tropical Biomedicine. 22(2): 165-170 (2005).
7. Doughari, J.H. El-Mahmood, A.M. and Tyoyina, I., Antimicrobial activity of leaf extracts of Senna obtusifolia L. African Journal of Pharmacy and Pharmocology. 2(1): 7-13 (2008
8. Murugesan S,Rajeshkannan C,Sumathi R,Manivachakam P,Suresh Babu D.Bioactivity of root hexane extract of coleus forskohlii Briq.labiatae:GC-MS characterization and identification.Eur J Exp boil.2012;2:1469-73.
9. Cowan MM Plant product as antimicrobial agents.Clin Microbiol Rev.1999; 12:564-82.

SP-04

METABOLOMIC AND PHYTONUTRITIVE PROFILING OF *CARICA PAPAYA*.L – AN INVITRO STUDY

S.ALAGENDRAN[1*], FERNANDEZ-SAAVEDRA.G[2], N. PUSHPA[3], S.SAHAYA SATHISH[4], P.SATHISH[1], M. VALARMATHI[1], P.SUDHA[1], V.A.MOHANLAL[1] AND N. VIJAY[1].

1. Dhanalakshmi Srinivasan Agriculture College (Affiliated by TNAU, CBE), Perambalur-12, Tamil Nadu, India
2. Department of Pharmacology, Faculty of Medicine, UNAM, Mexico D.F
3. Department of Microbiology, Cauvery College for Women (Autonomous), Trichy-17
4. Dean, School of Biological Sciences, Department of Botany, St.Joseph College (Autonomous), Trichy-02, Tamilnadu, India.
E.mail: proteinblg@gmail.com

Abstract
The objective of this study was to estimate the biochemical composition in leaves of C. papaya. Carica papaya.L belongs to the family Caricaceae. Dried leaves extract is shows the phytoactive biochemical's Cystatin, Saponins, Carbohydrates, protein, amino acids, steroids, tocopherol, phenols, flavonoids, cyanogenic glycoside, Quercetin, Kaempferol and glucosinolates in multivariate analysis. The concentration of 60 ppm contains more phytoactive molecules are elevated as Cystatin, Quercetin and Kaempferol. Furthermore, Papaya leaves metabolites Cystatin therapeutically valuable to human nutrition and health proved the specificity 99.9%. Carica papaya.L helps to preventing the diseases like flu like dengue, Cancer, diabetes and also acetogenin is the plant metabolite acts as cleanse the blood circulation and reduce the efficacy of platelet aggregation.

Key words:*Papaya leaves, phytoactive metabolites, anticancer and Platelet thrombosis*

Introduction

Plant biochemistry is epitomize the considerable diversity of organic substances that are intricate and accumulated through plants, the chemical composition of these substances, their biosynthesis turn over and metabolism in plants, their innate circulation and their biological task.[1]Medicinal plants are supportive for therapeutic as well as for remedial of human diseases, since of the occurrence of phytochemical constituents.[2] The phytoactive substances are naturally occurring in the alternative & traditional medicinal herbalism of plants, leaves, vegetables and roots that have defense role and protect from various diseases.[1-3]Papaya conveys a wide-ranging continuum of phytoactive substances together with primary and secondary metabolites such as Carbohydrates, polysaccharides, glycosides, enzymes, flavonoids, lectins, saponins, vitamins, steroids. [4-5]These indispensable nutritional specifics with the aim of papaya contains an assay of dietary polyphenols[6], protease inhibitors[7] and flavanoids[8] that may be valuable for gratifying health needs putative nutritional and, therefore, considered as advantageous in support of pervasiveness to health.[8-9]

Phytoactive constituents from plant derived chemicals are the basic source for the establishment of drugs. The phytoactive constituents are more perceive and have the ample biological function in the prophecy of plant based active drugs.[9-10] Cystatin Proteases extracted from papaya revealed a extensive specificity and thermo stability thus make use of in the food production. Cystatin is the metabolites which deploy Papaya proteases are of therapeutic consequence particularly in favor of antiulcer, anti-inflammatory, antitumoral, anthelmintic, degenerative diseases, vitamin deficiency A, C, D & E properties.[11-13] Anti-inflammatory properties of papaya proteases contain cystatin and flavanoids help to reduce pain and suffering from osteoarthritis, edema, and sciatica.[14] The aim of the present work was to quantitate the plant metabolites present in the whole plant of Carica papaya.L (caricaceae)and the leaf parts were used for solvent extraction follow by hexane, petroleum ether, chloroform, methanol and Phenol extracts quantitatively by HPTLC.

MATERIALS AND METHODS

Plant materials

The whole plants of Carica papaya.Land the leaf parts were collected from Field of Dhanalakshmi Srinivasan Agriculture College, Perambalur. The referral of the plant has been deposit, Department of Botany, School of Biological Sciences, St. Joseph College (Autonomous), Trichy was authenticated.

Preparation of extracts

Fresh leaf materials of the whole plant of Carica papaya.Lleaves were collected distinctively in mass, wash down under running tap water to remove stick on dirt trail by rinsing with deionised water. The papaya leaves was followed by air dried and pulverized in a mechanical grinder followed by sieving (sieve no.40) to acquire crude powder. Papaya leaf extraction done by soxhlet method reveals, the liquid extracts were concentrated in vacuum or by lyophilized to yield dry extracts. The concentrated extracts were used for HPTLC analysis. 2 µl of the extracts of Carica papaya.Lwere employed for HPTLC analysis.

High performance thin layer chromatography

According to Wagner and Baldt[15] and Harbone method[16-17], HPTLC fingerprint is determind by the dried leaf extract from papaya has been carried out through solvent extraction methods.[18]The developed plate was air dried and scanned at 254, 366 and 400 nm using Camag scanner 3 with winCATS 1.4.2 software (Camag, Switzerland).

Results and Discussion

The present study exposed to analyze the real-time HPTLC fingerprint profiles of secondary metabolites in methanolic leaf extracts of Carica papaya. It shows the amount of the secondary metabolites, specifically, cystatin, quercetin, flavonoids, glycosides, saponins and kaempferol. A different composition of the mobile phase and spraying reagents used and the colour change is predicted and are presented in Table 1. Further, HPTLC epitomize the UV 254 and 366 nm, the densitogram, and 3 dimensional displays were determined as in Figures 1 and 3. Figure.2 portrayed the variation of standard Cystatin versus methanolic extract of Carica Papaya leaf shows % of inhibition is unpredictably high in concentration of 60mg/g. This is due to formation of antioxidant properties and secondary metabolites acts as radicals scavenging due to formation of phenolicpyrenoids and flavonoids The sample extracts were run along with the standards and it was perceived to validate the presence of metabolomic substrates and compared with phytochemical compounds from chromatogram after derivatization overcome in UV 366 nm the peak range, area and consecutively high in multivariate analysis of papaya leaf supernatant extract revealed cystatin, quercetin and kaempferol are extensively high in their concentration as 99.9%. During HPTLC quantitation at 366nm, the values of cystatin, quercetin and kaempferol are remarkably high in specificity and interassay variation decline as 0.25-0.54 in Carica Papaya. L. Inhibitors of Plant cystatins contain cysteine-proteases of the leaf protein from papaya such as papain C1A and legumain C13 families. Analogous of plant cystatins metabolized novel defense proteins consist of hypervariable; optimistically aromatic amino acid sites apparently propel their biological activity. Cystatin shows that metabolomic profile has the immunosuppressive role which protect antioxidant, gastroinflammation, nephroitic tumor, antihepatic, antidiabetic, anticancer and neuro defense in cell system. The present study revealed that phenolic content and flavonoid of C. papaya leaves encompass the quercetin, kaempferol and their certain glycosides, and imply that kaempferol is an essential flavonoid of C. papaya leaf because of its large quantity and produce antioxidant activity.

Table. 1-Metabolomic Profiling of Cystatin, Quercetin and Kaempferol validating by HPTLC under 366 nm.

Chemical Data	Cystatin (mg/g)	Quercetin (mg/g)	Kaempferol (mg/g)
Recovery (%)	99.76-99.90	97.8-99.82	99.22-99.78
Accuracy – COV			
Interday (n=5)	0.25-0.54	0.12-0.48	0.22-0.51
Intraday (n=5)	0.15-0.39	0.11-0.28	0.12-0.36
Specificity (%)	99.87	99.76	99.58

Fig.1Phytonutrients analysis of Carica Papaya.L using different solvents

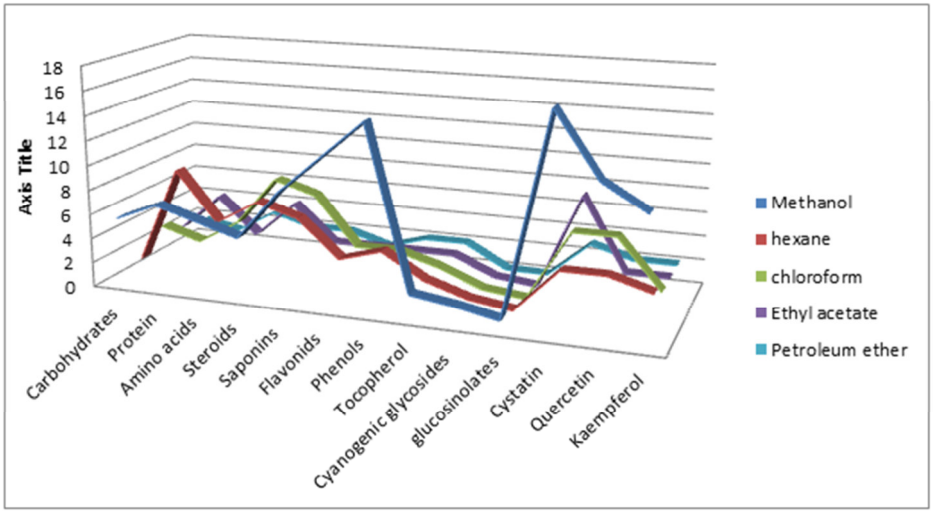


Fig. 2 Cystatin content - Carica Papaya .L

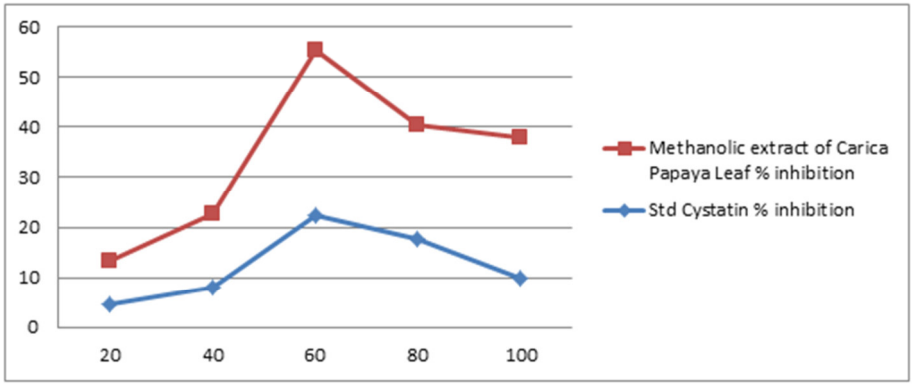
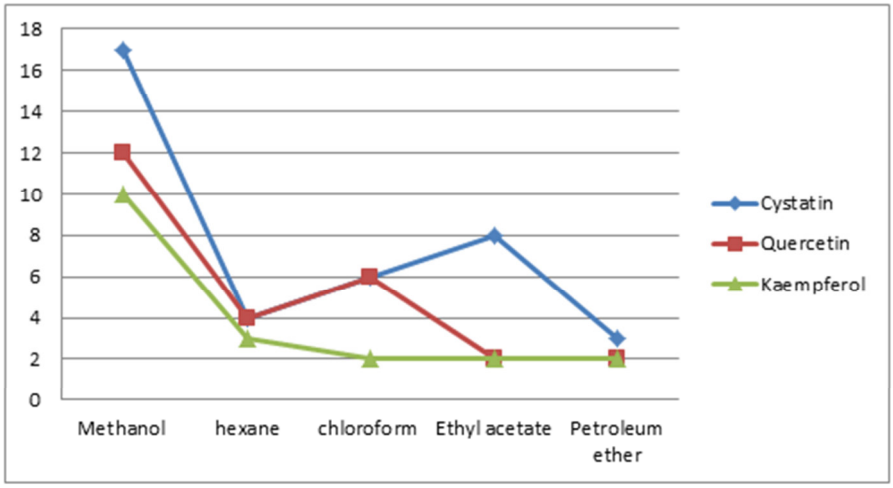


Fig.3 Chemical profiling of Carica Papaya. L extract analyzed by HPTLC



Metabolomic studies shows that HPTLC is a valuable high through put method use for plant secondary derived substances.[20-21] and more efficient in the spot of plant biochemistry and for the detection of plants particularly secondary metabolites.[22] The characterization of metabolomic substances from HPTLC fingerprinting is proving the evidence that validate accurate and clear-cut method for herbal formulations.[20,23] This method is more linear and highly putative for prepared the alternative medicines through herbal and food adulterants using the source of biochemical substrates for drug analysis.[24-25]Therefore, HPTLC can be valuable method used for promote the pharmaceutical drugs through herbal plants. Carica Papaya lead has the defensive role particularly in immune cells and hypersensitive actions also recover by plants to inflame the systematic diseases such as tumorigenesis and antihelminthiasis.[26-27] The qualitative analysis of methanolic leaf extracts of C.papaya analyzed through HPTLC confirmed the presence of many secondary metabolites like Cystatin, Quercetin, Kaempferol, flavonoids, cardiac glycosides, saponins, and Vitamin C.[8-9] The well determined HPTLC profiles also confirming the rate of

these metabolites of medicinal importance which prop up the conventional therapeutic uses of C.papaya leaves.

Cystatin undergoes protein degradation, in which cysteine protease activity response to diverse plant abiotic stresses.[28]Cystatin found in plants which produce biotic and abiotic stress in ROS producing cells of papaya plants leads to cause programmed cell death (PCD).[29] Reactive oxygen species is triggered by enzyme inhibitors like cysteine protease to unregulate the physiological alterations which are controlled by PCD in plants. During inhibition, activities of cysteine proteases possess a cystatin gene which indirectly controls the signaling pathway of apoptosis in plants.[30]Cystatin has rich source of medicinal uses as analgesic, anti-inflammatory, and adaptogenic measures which facilitate to improve pains, host resistance against diseases, and survival against stress and also to cure various illness.[31-33] In the present study, methanolic leaf extracts of C.papaya determined to have three distinct types of plant secondary metabolites with different Retention factor was detected.

The profile of HPTLC determining in C.papaya leaves Out of 32 spots, 9 were identified as flavonoids (3 in leaf extract) in the methanolic extract of papaya leaves. Flavonoids, the most well-known secondary metabolites from phenolic compounds, mainly consist of a complex aromatic ring and a heterocyclic ring of glycoside linkage.[34-35] Alkaloids and Flavonoids are most active against anti-inflammatory, anticancer, hypersensitivity and antioxidant properties.[35] Cystatin, Quercetin, Kaempferol is a glycosides were prominent in methanolic extract of papaya leaves which bear out the therapeutic values of this species in terms of anticancer, high in nutritive value as they would release phenolics on hydrolysis that are toxic to airborne pathogens.[36] Saponins and terpenoids are secondary metabolites which consist of moieties like glycosidic, acetate mevalonate metabolic pathway formed from triglycerides and fatty acid syntheses widely found in plant and are characterized by structures containing terpenes clusters or steroidal aglycones and one or more sugar domains. Even though, their sensibly large structural diversity, saponins give out some distinctive biological properties with the ability to form foam and lyses red blood cells.[37-38] Saponins show signs of a broad range of pharmacological trial which include anti-inflammatory, vasodilations, Immunomodulatory and other central nervous activities. To conclude, a better perceptive of the plant derived metabolites were analyze by two way methods substantiate that Cystatin, Quercetin and Kaempferol has predominantly high in chromatogram which bring out the strong antioxidant and used as the ailments for autoimmune diseases, neurodegenerative and cancer. HPTLC fingerprinting facilitate in functionally confirmed the presence of unlike constituent depends on the polarity of the building block which are exhibited as number of resolved bands. Cystatin, quercetin and kaempferol also exhibits a wide spectrum of cellular activities, including anti-inflammatory and anticarcinogenic properties.

Acknowledgements
The authors wish to thank Dr.G.Gajendran, Principal, DSAC, Perambalur and Dean, School of Biological Sciences, St. Joseph College (Autonomous), Trichy, India for identifying and authenticating the plant materials used in this investigation.

Conflict of Interest
The conflict of interest affirmed none

References
1. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis. Bull World Health Organ 2008; 86: 594-9.
2. Zunjar V, Mammen D, Trivedi BM and Daniel M: Pharmacognostic, Physicochemical and Phytochemical studies on *Carica papaya* leaves: Pharmacognosy Journal 2011; 3(20): 5-8
3. Jurandi, G.O.; Angela Pierre, V. Papaya: Nutritional and pharmacological characterization, and quality loss due to physiological disorders. An overview. Food Research International 2011, 44, 1306–1313.
4. Chan, Y.K.; Paull, R.E. Papaya (Carica papaya L.), Caricaceae. In: Encyclopedia of Fruit and Nuts; Janick, J.; Paull, R.E.; Eds.; CABI: Wallingford, U.K., 2008; 237–247.
5. Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anticancer agents. Fitoterapia 2010; 81:703-14.

6. Jaime, A.; Teixeira, S.; Zinia, R.; Duong, T.N.; Dharini, S.; Abed, G.; Manoel, T.S.; Paula, F.T. Papaya (Carica papaya L.) Biology and Biotechnology. Tree and Forestry Science and Biotechnology 2007, 1, 47–73.
7. Salas, C.E.; Gomes, M.T.R.; Hernandez, M.; Lopes, M.T.P. Plant cysteine proteinases: Evaluation of the pharmacological activity. Phytochemistry 2008, 69, 2263–2269.
8. Miean KH, Mohamed S: Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) Content of Edible Tropical Plants. J Agric Food Chem. 2001, 49: 3106-3112.
9. Esther JA, Nathaniel OW, Mercy B, Selina AS. Phytochemical screening, antimicrobial and antioxidant properties of assorted Carica papaya leaves in Ghana. J Med Plants Stud 2016;4: 193-8.
10. Ogan. A,U, "The basic constituents of the leaves of Carica papaya," Phytochemistry, vol. 10, no. 10, pp. 2544–2547, 1971.
11. Mello, V.J.; Gomes, M.T.R.; Lemos, F.O.; Delfinoa, J.L.; Andradec, S.P.; Lopesa, M.T; Salasb, C.E. The gastric ulcer protective and healing role of cysteine proteinases from Carica candamarcensis. Journal of Phytomedicine 2008, *15*, 237–244.
12. Guzelmeric E, Vovk I, Yesilada E. Development and validation of an HPTLC method for apigenin 7-O-glucoside in chamomile flowers and its application for fingerprint discrimination of chamomile-like materials. J Pharm Biomed Anal 2015;107:108-18.
13. Erlund I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res 2004;24:851-74.
14. Meireles M, Moura E, Vieira-Coelho MA, Santos-Buelga C, Gonzalez-Manzano S, Dueñas M, *et al.* Flavonoids as dopaminergic neuromodulators. MolNutr Food Res 2016;60: 495-501.
15. Wagner.H and Baldt.S Plant Drug Analysis. A Thin Layer Chromatography Atlas, Springer, Berlin, Germany, 2nd edition, 1996.
16. Harborne JB, Phytochemical Methods, Chapman and Hall, London, UK, 3rd edition, 1998.
17. Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nded. London: Chapman and Hall; 1984.
18. Reich E, Schibli A. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. 1st ed. New York: Thieme Medicinal Publisher, Inc.; 2006.
19. Reich E, Schibli A, DeBatt A. Validation of high-performance thin-layer chromatographic methods for the identification of botanicals in a cGMP environment. J AOAC Int 2008;91: 13-20.
20. Gong F, Wang BT, Chau FT, Liang YZ. HPLC data preprocessing for chromatographic fingerprint of herbal medicine with chemometric approaches. Anal Lett 2005;38: 2475-92.
21. Zhao HX, Zhang HS and Yang SF: Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. Food Science and Human Wellness 2014; 3(3-4): 183-90.
22. Ayoola.P.B and Adeyeye.A, "Phytochemical and nutrient evaluation of Carica papaya (Pawpaw) leaves," International Journal of Research and Reviews in Applied Sciences, vol. 5, no. 3, p. 325, 2010.
23. Otsuki, N.; Dang, N.H.; Kumagai, E.; Kondoc, A.; Iwataa, S.; Morimotoad, C. Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. Journal of Ethnopharmacol 2010, *127*, 760–767.
24. Li Y, Zhou S, Li J, Sun Y, Hasimu H, Liu R, *et al*. Quercetin protects human brain microvascular endothelial cells from fibrillar β-amyloid1-40-induced toxicity. Acta Pharm Sin B 2015;5: 47-54.
25. Yushau, M., Onuorah, F. C. and Murtala, Y, "In-vitro sensitivity pattern of some urinary tract isolates to *carica papaya* extracts". Bayero Journal of Pure and Applied Sciences, 2 (2): 75-78. 2009.
26. Xu B, Chang SK. Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. J Agric Food Chem 2009;57: 10718-31.
27. Maisarah AM, Amirah BN, Asmah R, Fauziah O. Antioxidant activity of different parts of Carica papaya. Int Food Res J 2013;20:1043-8
28. Martinez, M., Cambra, I., Carrillo, L., Diaz-Mendoza, M., and Diaz, I. (2009). Characterization of the entire cystatin gene family in barley and their target cathepsin L-like cysteine-proteases, partners in the hordein mobilization during seed germination. *Plant Physiol.* 151, 1531–1545.
29. Pernas, M., Sanchez-Monge, R., and Salcedo, G. (2000). Biotic and abiotic stress can induce cystatin expression in chestnut. *FEBS Lett.* 467, 206–210.

30. Zhang, X., Liu, S., and Takano, T. (2008). Two cysteine proteinase inhibitors from *Arabidopsis thaliana*, AtCYSa and AtCYSb, increasing the salt, drought, oxidation and cold tolerance. *Plant Mol. Biol.* 68, 131–143.
31. Ong ES. Extraction methods and chemical standardization of botanicals and herbal preparations. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812:23-33.
32. Agung N, Hesty H, Jae SC and, Hee-Juhn P: Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity; Asian Pacific Journal of Tropical Biomedicine 2017; 7(3): 208-213.
33. Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G and Colizzi V: Antioxidant and Anti-Inflammatory Activities of Extracts from *Cassia alata*, *Eleusine indica, Eremomastax speciosa, Carica papaya* and *Polyscias fulva* medicinal plants collected in Cameroon; PLOS one 2014; 9(10).
34. Rashed K, Fouche G. Anticancer activity of Carica papaya extracts in vitro and phytochemical analysis. Greener J Pharm Pharmacol 2013;1:1-5.
35. Farooq T. Phytochemical and Pharmacological Investigation of the Leaves of Carica papaya Linn. East West University; 2009. p. 26-37.
36. Davis W, Lamson MS, Matthew S, Brignall ND: Antioxidants and cancer III: quercetin. Altern Med Rev. 2000, 5: 196-208.
37. Dhouib, R.; Laroche-Traineau, J.; Shaha, R.; Lapaillerie, D.; Solier, E.; Rualès, J.; Pina, M.; Villeneuve, P.; Carrière, F.; Bonneu, M.; Arondel, V. Identification of a putative triacylglycerol lipase from papaya latex by functional proteomics. Federation of European Biochemical Societies Journal 2011, 278, 97–110.
38. Pendzhiev, A.M. Proteolytic enzymes of papaya: Medicinal applications. Pharmaceutical Chemistry Journal 2002, 36, 315–317.

SP-05

ANTIHYPERAMMONEMIC EFFECT OF LYCOPENE: A DOSE DEPENDENT STUDY

S. SUBASH

Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600113, Tamilnadu, India
***Corresponding Author mail ID:drsubashselvaraju@gmail.com; ssubrhythm@gmail.com**

ABSTRACT:
Ammonia is a key neurotoxin involved in the neurological complications of liver failure. Elevated ammonia leads to hyperammonemic condition which affects several important central nervous system(CNS) functions.The present work was aimed at evaluate the protective effect of lycopene; a naturally occurring plant component, on ammonium chloride (AC) induced hyperammonemia in male *albino*Wistar rats. Three different doses of lycopene (1, 2, and 4 mg/kg of body weight) were administered to rats treated with AC for 8 weeks. Amelioration of hyperammonemia and its complications of AC induced hyperammonemia by lycopene were measured by assessing the body mass, circulatory levels of ammonia, urea, uric acid, creatinine and bilirubin. AC treatment with lycopene 2 mg/kg body weight dose of lycopene was significantly effective.This could be due to the antioxidant potential of lycopene. Further the administration of lycopene (1, 2, and 4 mg/kg of body weight) to normal rats did not show any noticeable side effects.Thus our results show that lycopene could act as aneffective antihyperammonemic agent without causing any adverse side effects and it could an appropriate natural compound for the treatment of hyperammonemic condition in near future with further extensive study to find its antihyperammonemicpotential.

Keywords: ammonia, urea, creatinine, hyperammonemia, lycopene.

INTRODUCTION

Ammonia is the precursor molecule and essential substrate for biosynthesis of amino acids, proteins and nucleic acids.[1]In mammals, at least 20 metabolic reactions generate ammonia.[2]Hyperammonaemia is defined as an elevated ammonia concentration in the blood, caused by an impairmentof the liver function resulting in inadequate ammonia detoxification. In living organisms, ammonia is an important nitrogen substrate in several reactions, and plays an important role in nitrogen homeostasis of cells. Hyperammonemia is a major contributing factor to neurological abnormalities observed in hepatic encephalopathy and in congenital defects of ammonia detoxication. Ammonia affects both excitatory and inhibitory synaptic transmission in the mammalian brain by a variety of mechanisms. Antiepileptic drugs such as valproate and salicylate cause hyperammonemia in mammalian systems.[3]The most severe effects of high ammonia levels occur in the brain characterized by personalityand behavior changes, confusion and tremors, which may even lead to coma and death.[4,5]

The greatest disadvantage of presently available potent conventional or synthetic antihyperammonemic agents/therapies lies in their toxicity and reappearance of symptoms after discontinuation. These drugs or therapies aresometimes inadequate and can have serious adverse effects.[6] Therefore, the screening and development of drugsfor their antihyperammonemic activity is still obscure, and so there is a worldwide trend to go back to traditionalmedicinal plants and natural products. There is a need for the search of appropriate protective agents against hyperammonemia. This can be achieved by focusing on the active principles of plants because of the leads provided by natural products that may offer better treatment than currently used drugs. The polyphenols/flavonoids occur ubiquitously in foods of plant origin,[7] which have received much attention because of their broad-spectrum pharmacological activities and extensive biological effects.[8]

The antioxidant lycopene is a naturally occurring red carotenoid pigment most commonly found in tomatoes and other red/orange fruits and vegetables, exhibits potent free radical scavenging properties.[9] It is present abundantly in tomatoes, watermelon, pink guavas, pink grapefruit, watermelons and papayas.[10]The structure of lycopene ($C_{40}H_{56}$) is an acyclic isomer of β-carotene with a 40 carbon polyisoprenoid chain and 13 conjugated double bond structure.[11] It is one of the most effective antioxidants in the carotenoid family,

and its activity against biological reactive oxygen species may prevent or ameliorate oxidative damage to cells and tissues both *in vivo* and *in vitro*.[12,13]

This study was designed accordingly to determine the possible protective effect of lycopene on the levels of blood ammonia, plasma urea, uric acid, serum creatinine and bilirubin and on body weight in the AC-induced hyperammonemia in male *albino*Wistar rats.

MATERIALS AND METHODS

Experimental animals

Adult male *albino* rats of Wistar strain (Rattusnorvegicus) (180-200 g) will be used for this study. Animals will be housed in well ventilated rooms (temperature 23 ± 2°C, humidity 65-70% and 12 h light/dark cycle) at Central Animal House, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani Campus and will be fed with standard pellet diet and water ad libitum. The animals used in the present study were approved by the ethical committee (IAEC No: 01/20/2016), University of Madras, India (Reg. No. 205/CPCSEA Dated 01/06/2000).

Chemicals

Lycopene was purchased from Carbosynth Limited, UK. Ammonium chloride and other chemicals used in this study were of analytical grade and obtained from Sigma Chemical Company (St Louis, MO, USA) and HIMEDIA-Mumbai (India).The kit was purchased from Spinreact, Spain and Greiner diagnostic GmbH, Germany.

Experimental Induction of Hyperammonemia

Hyperammonemia will be induced in rats by intraperitoneal injections (i.p.) of ammonium chloride (AC) at a dose of 100 mg/kg body weight thrice a week for 8 weeks.[14]

Preparation of lycopene and treatment schedule

The test drug lycopene will be prepared by dissolving in corn oil[15] given via gastric gavage. Control animals will be given the same amount of vehicle.[14]

Experimental design (Each group 6 rats)

Group I : Normal rats received corn oil (vehicle control) by i.p
Group II : Rats received lycopene (4 mg/kg b.w.)
Group III : Rats received AC (100 mg/kg b.w.) i.p
Group IV : Rats treated with AC (100 mg/kg) + Lycopene (1 mg/kg b.w.)
Group V : Rats treated with AC (100 mg/kg) + Lycopene (2 mg/kg b.w.)
Group VI : Rats treated with AC (100 mg/kg) + Lycopene (4 mg/kg b.w.)

At the end of the experimental period (i.e 8th week), all experimental rats were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected for the biochemical estimations (hyperammonemic markers).

Measurement of body weight

During the experimental period, body weights of experimental rats were measured every week using a digital balance.[16]

Biochemical estimations

Determination of blood ammonia

To determine the levels of ammonia in the blood,[17]triethanolamine(151 mM, 200 mL) buffered substrate (α-ketoglutarate) was added to theblood (20 mL), mixed thoroughly, and the absorbance was measured at 480 nm.

Determination of plasma urea

To determine the levels of urea[18] by adding 0.2mL of plasma & 3.5 mL of distilled water was added. After 0.35 M sulphuric acid (0.4 mL) & 10% sodium tungstate (0.3 mL) were added. After centrifugation, to take supernatant (2.1 mL), diacetylmonoxime (0.5mL), water (2 mL) and sulfuric acidephosphoric acid mixture

(1.6 mL) were added, the mixture was boiled for 30 min's & cooled, and the absorbance was measured at 480 nm.

Estimation of plasma uric acid

Plasma uric acid was determined by adding tungstic acid (5.4 mL) to plasma (0.6 mL). The contents were centrifuged. To 3 mL of supernatant, 20% sodium carbonate (0.6 mL) and1% phosphotungstic acid reagent (0.6 mL) were added, the mixture was incubated at 25˚C for 10 minutes, and the absorbance was read at 700 nm.[19]

Estimation of Serum creatinine

Serum creatinine was determined by adding alkaline picrate (2 mL) to 3 mL of deproteinized supernatant (prepared by centrifugation of 0.2 mL of serum with 4.3 mL of 10% tricarboxylic acid cycle). The mixture was kept at 25˚C for 30 minutes and the absorbance was read at 520nm.[20]

Estimation of serum bilirubin

The level of bilirubin in serum was determined by adding absolute methanol (2.5 mL)1.5% hydrochloric acid (0.5 mL), and a diazo reagent (0.5 mL) to serum (0.2 mL), which were mixed thoroughly and kept at room temperature for 30 minutes, and the absorbance was measured at 540 nm.[21]

Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were expressed as Mean ± Standard Deviation for six rats in each group. p values <0.05 were considered significant.[22]

RESULTS

Table 1 shows the levels of final body weight of normal and experimental rats. Rats induced with AC, showed a significant decrease in the body weight when compared with normal rats.

Treatment with lycopene to AC-induced rats for eight consecutive weeks (thrice a week) nearly normalized body weight when compared with AC induced rats.

Table 2 shows the activities of blood ammonia, plasma urea, uric acid, serum creatinine and bilirubin. Ammonia, uric acid, creatinine, bilirubin activities in blood circulation of rats in AC induced rats were significantly higher and decrease the level of urea than the normal rats. Treatment with lycopene (1 mg/kg, 2mg/kg and 4 mg/kg b.w) to hyperammonemic rats significantly reduced ammonia, uric acid, creatinine, bilirubin and increased urea activities when compared with AC treated rats. Among the three doses, the maximum effect of lycopene was observed at the dose of 2 mg/kg b.w.

Table. 1 Effect of lycopene on body weight changes in control and experimental rats.

Group	Initial (g)	Final (g)
Normal	191 ± 14.55 [a]	352 ± 26.81 [a]
Lycopene (4 mg)	187 ± 14.24 [a]	353 ± 26.88 [a]
AC (100 mg)	192 ± 14.62 [a]	277 ± 21.10 [b]
AC + Lycopene (1 mg)	189 ± 14.39 [a]	320 ± 24.37 [c]
AC + Lycopene (2 mg)	193 ± 14.70 [a]	322 ± 24.52 [c]
AC + Lycopene (4 mg)	190 ± 14.47 [a]	319 ± 24.29 [c]

Values are given as mean ±S.D from six rats in each group.
Values not sharing a common superscripts (a,b,c and d) differ significantly at p<0.05 (DMRT).

Table 2.Effect of lycopene on changes in blood ammonia, plasma urea, uric acid, serum creatinine and bilirubin of normal and experimental rats.

Group	Blood ammonia (μmol/L)	Plasma Urea (mg/dL)	Plasma Uric acid (mg/dL)	Serum Creatinine (mg/dL)	Serum Bilirubin (mg/dL)
Normal	85.15 ± 6.48 [a]	9.54 ± 0.73 [a]	1.69 ± 0.13 [a]	0.83 ± 0.06 [a]	0.94 ± 0.07 [a]
Lycopene (4 mg)	83.24 ± 6.34 [a]	9.61 ± 0.73 [a]	1.65 ± 0.13 [a]	0.81 ± 0.06 [a]	0.92 ± 0.07 [a]
AC (100 mg)	386.53 ± 29.43 [b]	4.13 ± 0.31 [b]	2.92 ± 0.22 [b]	1.78 ± 0.14 [b]	3.43 ± 0.26 [b]
AC + Lycopene (1 mg)	191.39 ± 14.57 [c]	6.45 ± 0.49 [c]	2.17 ± 0.17 [c]	1.15 ± 0.09 [c]	2.16 ± 0.16 [c]
AC + Lycopene (2 mg)	155.16 ± 11.81 [d]	8.05 ± 0.61 [d]	1.89 ± 0.14 [d]	0.98 ± 0.07 [d]	1.72 ± 0.13 [d]
AC + Lycopene (4 mg)	187.20 ± 14.25 [c]	6.23 ± 0.47 [c]	2.09 ± 0.16 [c]	1.12 ± 0.09 [c]	2.11 ± 0.16 [c]

Values are given as mean ±S.D from six rats in each group.
Values not sharing a common superscripts (a,b,c and d) differ significantly at p<0.05 (DMRT).

DISCUSSION

Ammonia is a key factor in the pathogenesis of hepatic encephalopathy, a major complication in acute and chronic liver failure and other hyperammonemic states, such as inborn errors of urea synthesis, during hepatic inadequacy, large quantities of ammonia in portal blood escapes, from the detoxification process and enters systemic circulation. Thus, blood and tissue (brain) ammonia levels are elevated rapidly.[23] Ammonia is a potent inhibitor of α-ketoglutarate dehydrogenase, the rate limiting enzyme in tricarboxylic acid cycle causing the accumulation of α-ketoglutarate, which stimulates glutamate formation in both astrocytes and neurons.[24]Previous studies[14] have reported that hyperammonemic rats showed an increased body weight due to the accumulation of lipid metabolites, proteins, and amino acids in tissuesand blood circulation. In our study, lycopene-treated hyperammonemic rats showed near normalized body mass as compared with hyperammonemic rats. Our results suggest that lycopene supplementation to hyperammonemic rats has managed to have an affirmative effect in opposition to hyperlipidemia.

Liver contains all the detoxification enzymes that are needed for the urea cycle and thus eliminating ammonia. Liver failure and/or liver cirrhosis leads to partial or complete inactivity/ depletion of urea cycle enzymes and can predispose patients to life-threatening hyperammonemia and/ or hepatic encephalopathy.[25] In the present study, increased blood ammonia and decreased urea indicate the hyperammonemic condition in AC-treated rats.[14,26] This may be due to the liver damage caused by the urea cycle disorder and ammonia intoxication.[27] Lycopene administered hyperammonemic rats showed a significantly decreased level of circulatory ammonia and an increase in urea biosynthesis when compared with corresponding AC-treated rats. Numerous investigations have documented that phenolic compounds and flavonoids offer ammonia detoxification by removing excess ammonia, uric acid, and creatinine during various disease conditions such as hyperammonemia, nephrotoxicity and so forth.[14,28-29] The reduction in ammonia and increase in urea synthesis showed antihyperammonemic nature of this compound along with antioxidant[30] and anti-inflammatory[31] potencies of lycopene favouring the normalisation of urea cycle defect and lessens hyperammonemic complications. Further, lycopene has the ability to normalize the levels of urea during renal dysfunction conditions in rats[32] and the results of our experiments corroborate these findings. These observations clearly indicate that lycopene could exert potent antihyperammonemic effect by significant ammonia lowering effect in hyperammonemic rats.

Administration of AC to rats exhibited a significant increase in plasma uric acid and serum creatinine, and a decrease in plasma urea concentration when compared with the control group. Blood urea nitrogen, uric acid, and creatinine levels are useful indicators of renal function. Renal damage can be accompanied by an increase in blood urea nitrogen, uric acid, and creatinine, indicating reduced urea, uric acid, and creatinine clearance.[33] In addition to the hepatic damage, renal damages were also present, as was evident by the elevation in plasma urea levels, which was considered as a significant marker of renal dysfunction.[34] The earliest research[14,35] investigated that the levels of plasma uric acid and serum creatinine

were increased, and the level of plasma urea concentration was decreased after the administration of AC. It might be due to dysfunctional and dystrophic changes in the liver and kidney due to severe renal impairments; as a result, urea excretion decreased and its concentration in plasma increased rapidly. Hyperammonemic rats treated with lycopene showed significantly decreased levels of plasma uric acid and serum creatinine, and an increase in plasma urea concentration when compared with AC - treated rats, indicating the antihyperammonemic effect of lycopene. Furthermore, lycopene has the ability to normalize the levels of creatinine during diabetes conditions in rats,[36] nephrotoxicity conditions in mice[37] and rats.[32,38] Lycopene therapy normalized the uric acid levels in cerebral ischemia-reperfusion injury in rats[39] and gingivitis patients[40] and the results of our experiments corroborate these findings.

Serum bilirubin is used as an index for the assessment of hepatic function, and any abnormal increase in the level of bilirubin in the serum indicates hepatobiliary diseases and severe disturbance of the hepatocellular function.[41]In the present investigation, the rats induced with AC showed a significantly increased level of bilirubin as compared with to normal rats.

CONCLUSION

Lycopene is abundantly present in tomatoes and is the most prevalent antioxidant. It is also present in watermelon, pink grapefruit, and several other red fruits.[42]The lycopene content of fresh tomatoes was 12 mg/100g.[43]In the present study, overall findings suggest that the lycopene has the potential of an excellent antihyperammonemic agent. The AC induced hyperammonemia treated with the lycopene in different concentration (1 mg/kg, 2 mg/kg, and 4 mg/kg b.w.) in the *albino*Wistar rats with consequences of alterations in biochemical parameters components 2 mg/kg b.w. act as an effective dose of hyperammonemia function.

ACKNOWLEDGEMENTS

The financial support by Department of Science and Technology, Government of India, New Delhi, in the form of SERB PI & Young Scientist Scheme (YSS/2015/001958; Dated: 15.07.2016) to Dr. S. Subash is thankfully acknowledged. The author acknowledge the infrastructural support from the Head, Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani campus, Chennai, Tamilnadu, India.

REFERENCES

1 Dimski DS. (1994). Ammonia metabolism and the urea cycle: Function and Clinical Implications. J Vet Int Med, 8:73-78.
2 Cooper AJL, Plum F. (1987).Biochemistry and physiology of brain ammonia.Physiol Rev, 67(2):440-519.
3 Monfort P, Felipo V. (2005). Long-term potentiation in hippocampus involves sequential activation of soluble guanylatecyclase, cGMP-dependent protein kinase and cGMP-degrading phosphodiesterase, alterations in hyperammonemia. BMC Pharmacol, 5(Suppl 1):66.
4 Coltart I, Tranah TH, Shawcross DL. (2013). Inflammation and hepatic encephalopathy. Arch BiochemBiophys, 536:189e196.
5 Scott TR, Kronsten VT, Hughes RD, Shawcross DL. (2013). Pathophysiology of cerebral edema in acute liver failure. World J Gastroenterol, 19:9240-255.
6 Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandan SK, Prakash VR. (2001) Evaluation of anti-inflammatory activity of Pongamiapinnata leaves in rats. J Ethnopharmacol, 78:151-57.
7 Das KD. (1994).Naturally occurring flavonoids: structure, chemistry, and high-performance liquid chromatography methods for separation and characterization. Methods Enzymol, 234:410-20.
8 Holiman PCH, Hertog MGL, Katanc MB. (1996). Analysis and health effects of flavonoids. Food Chem, 57:43-6.
9 Bertram JS, Pung A, Churly M, Kappock TJ, Wilkins SR, Cooney RV.(1991). Diverse carotenoids protect against chemically induced neoplastic transformation. Carcinogenesis, 12:671–78.

10 Chenghao H. Zhu, Erik R. Gertz, YimengCai, Betty J. Burri. (2016). Consumption of canned citrus fruit meals increases human plasma β-cryptoxanthin concentration, whereas lycopene and β-carotene concentrations did not change in healthy adults. Nutrition research, 36:679 – 688.
11 Jun X, Jia L, Shi-Yong Z, Zheng-Hai D, Jing-Ao G, Zi-Xuan H, et al. (2016). Lycopene protects against atrazine-induced hepatotoxicity through modifications of cytochrome P450 enzyme system in microsomes. Experimental and Toxicologic Pathology, 68:223–31.
12 Yonar ME. (2012).The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (Oncorhynchusmykiss, W.). Fish Shellfish Immunol, 32:994–1001.
13 Boeira SP, Filho CB, Del'Fabbro L, Roman SS, Royes LF, Fighera MR, et al. (2014). Lycopene treatment prevents hematological, reproductive and histopathological damage induced by acute zearalenone administration in male Swiss mice. Exp. ToxicolPathol, 66:179–85.
14 Jayakumar M, Subramanian P. (2014).Antihyperammonemic effect of fisetin on hyperammonemic rats: a biochemical study. IJMRR, 2:33-9.
15 Krishnamoorthy G, SelvaKumar K, VenKataraman P, Elumalai P, Arunakaran J. (2013). Lycopene supplementation prevents reactive oxygen species mediated apoptosis in Sertoli cells of adult albino rats exposed to polychlorinated biphenyls. InterdiscipToxicol, 6(2):83-92.
16 Subash S, Subramanian P. (2009). Morin a flavonoid exerts antioxidant potential in chronic hyperammonemic rats: a biochemical and histopathological study. Mol Cell Biochem, 327:153-61.
17 Wolheim DF.(1984).Preanalytical increase of ammonia in blood specimens from healthy subjects.ClinChem, 30:906-08.
18 Natelson S. Microtechniques of Clinical Chemistry for the Routine Laboratory. Springfield, IL: C.C. Thomas;1957.
19 Brown H. (1945).The determination of uric acid in human blood. J BiolChem, 158:601-08.
20 Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffee reaction. J BiolChem 1945;158:581-91.
21 MalloY HT, Evelyn KA. (1937).The determination of bilirubin with the photometric colorimeter. J BiolChem, 119:481-90.
22 Duncan BD. (1957). Multiple range tests for correlated and heteroscedastic means. Biometrics, 13:359e364.
23 Reddy GR, Devi BC, Chetty CS. (2004). Developmental lead neurotoxicity: alterations in brain cholinergic by ST. Neurotoxicology, 23:402-07.
24 Lai JCK, Cooper AJL. (1986). Brain a-ketoglutarate dehydrogenase complex: kinetic properties, regional distribution and effects of inhibitors. J Neurochem, 47:1376-386.
25 Enns GM. (2008). Neurologic damage and neurocognitive dysfunction in urea cycle disorders. SeminPediatrNeurol, 15:132-39.
26 Subash S, Subramanian P. (2012).Chronotherapeutic effect of morin in experimental chronic hyperammonemic rats.Int J NutrPharmacolNeurol Dis, 2:266-71.
27 Rama Rao KV, Jayakumar AR, Norenberg MD.(2005). Differential response of glutamine in cultured neurons and astrocytes. J Neurosci Res, 79:193-99.
28 Subash S, Subramanian P. (2010). Morin improves the expression of urea cycle enzymes in hyperammonemic rats. Journal of Pharmacy Research, 3(10):2557-560.
29 Subash S, Subramanian P. (2011). Impact of morin (a bioflavonoid) on ammonium chloride-mediated oxidative damage in rat kidney.Int J NutrPharmacolNeurol Dis, 1:174-78.
30 Jiang W, Guo MH, Hai X. (2016).Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat. World J Gastroenterol, 22(46):10180-188.
31 Cha JH, Kim WK, Ha AW, Kim MH, Chang MJ.(2017). Anti-inflammatory effect of lycopene in SW480 human colorectal cancer cells.Nutr Res Pract, 11(2):90-6.
32 Ateşşahin A, Ceribaşi AO, Yilmaz S. (2007). Lycopene, a carotenoid, attenuates cyclosporine-induced renal dysfunction and oxidative stressin rats. Basic ClinPharmacolToxicol, 100(6):372-76.
33 Palabiyik SS, Erkekoglu P, Zeybek ND, Kizilgun M, Baydar DE, Sahin G, et al. (2013). Protective effect of lycopene against ochratoxin A induced renal oxidative stress and apoptosisin rats. ExpToxicolPathol, 65(6):853-61.
34 Kumar G, Murugesan AG, Rajasekara M. (2006). Effects of Helicteresisora bark extract on blood glucose and hepatic enzymes in experimental diabetes. Pharmazie, 61:353-55.

35 Fan SH, Rao YK, Tseng YM. (2009). Anti-oxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from. Phyllanthusurmaria. J Ethnopharmacol, 116:333-40.
36 Li W, Wang G, Lu X, Jiang Y, Xu L, Zhao X. (2014). Lycopene ameliorates renal function in rats with streptozotocin-induced diabetes. Int J ClinExpPathol, 7(8):5008-**0**15.
37 Dai C, Tang S, Deng S, Zhang S, Zhou Y, Velkov T, et al. (2015). Lycopene attenuates colistin-induced nephrotoxicity in mice via activation of the Nrf2/HO-1pathway. Antimicrob Agents Chemother, 59(1):579-85.
38 Bayomy NA, Elbakary RH, Ibrahim MAA, Abdelaziz EZ. (2017). Effect of Lycopene and Rosmarinic Acid on Gentamicin Induced Renal Cortical Oxidative Stres,Apoptosis, and Autophagy in Adult Male *Albino* Rat. Anat Rec (Hoboken), 300(6):1137-149.
39 Wei Y1, Shen X, Shen H, Mai J, Wu M, Yao G.(2010). Effects of lycopene on cerebral ischemia-reperfusion injury in rats.Wei Sheng Yan Jiu, 39(2):201-04.
40 Tripathi P, Blaggana V, Upadhyay P, Jindal M, Gupta S, Nishat S. (2019). Antioxidant therapy (lycopene and green tea extract) in periodontal disease: A promising paradigm. J Indian SocPeriodontol, 23:25-30.
41 Martin P, Friedman LS. Assessment of liver function and diagnostic studies. (1992). In: Friedmann LS, Keeffe EB, eds. Handbook of Liver Disease. Philadelphia: Churchill Livingstone, 1-14.
42 Story EN, Kopec RE, Schwartz SJ, Harris GK. (2010).An update on the health effects of tomato lycopene. Annu Rev Food SciTechnol, 1:189-210.
43 Alda LM, Gogoaşă I, Bordean DM, Gergen I, Alda S, Moldovan C, et al. (2009). Lycopene content of tomatoes and tomato products. Journal of Agroalimentary Processes and Technologies, 15 (4):540-42.

SP-06

IN VITRO ANTIOXIDANT PROPERTIES AND FREE RADICAL SCAVENEING ACTIVITY OF ETHANOLIC EXTRACT OF *COSTUS SPICATUS (JACQ*-1788)

AZHAGU MADHAVAN S[1*], VINOTHA P[2], UMA V[2]

1.PG & Research Department of Zoology & Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous) Poondi, Thanjavur – 613503, India.
2. Department of Biochemistry, School of Life Science Bharathidasan University, Tiruchirappalli -620 024, Tamil Nadu, India.
Email: mathavan062@gmail.com

ABSTRACT

Anti oxidant is reactive free radicals In the assay, DPPH radical is converted to the corresponding hydrazine, and the color of the solution is changed from violet to yellow indicates the scavenging behavior of the plant sample due to bioactive compounds such as phenolic compounds, flavonoids, tannins, and their derivatives. From the ethanolic extract *Costus spicatus*, showed a percentage inhibition of 64.61±0.227 while ascorbic acid showed a percentage inhibition of 85.31±0.29 at a concentration of 80µg/mL. The IC^{50}46.66, 37.64 and 27.29µg/mL for EECS and ascorbic acid respectivelyhigher antioxidant activity than EECS extract possessed a good radical scavenging capacity. No single inhibitor will combat the results of each atom. Just as free radicals have different effects in several areas of the body, every antioxidant behaves differently due to its chemical properties. The results obtained for the scavenging activity against hydrogen peroxide is presented in and the graphical representation. The antioxidants free radical production of the enhancement body defense system.

KEY WORDS: Anti-oxidant, Reducing power assay, Superoxide radical activity, DPPH radical scavenging activity.

INTRODUCTION

Antioxidants and free radicals

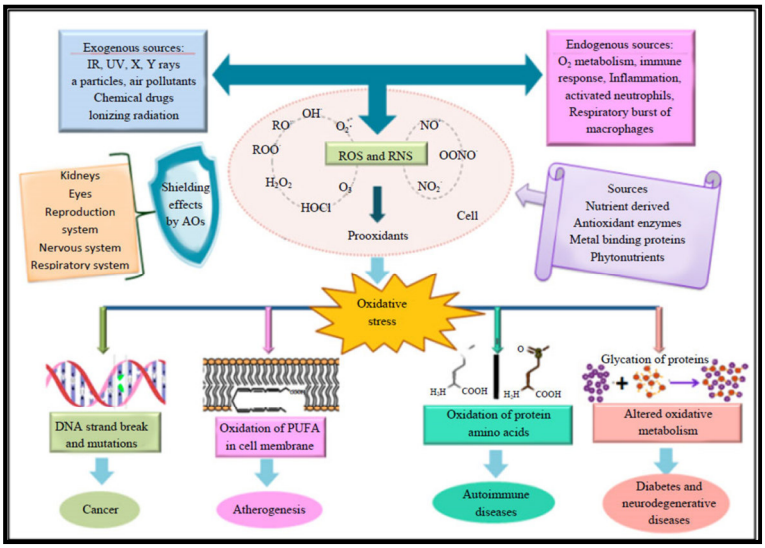
Without seeing at least one ad that claims to fight with antioxidants to age, it is difficult to watch television. Antioxidants square measure molecules that forestall the reaction of alternative molecules. Antioxidants are chemicals that reduce or stop the consequences of free radicals. They present lepton to free radicals, thereby reducing their reactivity. Antioxidants distinctive are that they'll present a lepton while not turning into reactive free radicals themselves. No single inhibitor will combat the results of each atomJust as free radicals in several areas of the body have different characteristics, each antioxidant acts differently because of its chemical properties.

Nevertheless, certain antioxidants can become prooxidants in certain situations, which absorb electrons from other molecules, causing chemical instability that induces oxidative stress. Antioxidants square measure molecules that might safely move with free radicals and terminate the chain reaction before important molecules square measure broken. Although there square measure many catalyst systems inside the body that disarm free radicals, the principle antioxidants are vitamin E, beta-carotene, vitamin C and selenium. When these Antioxidants neutralize free radicals by donating a lepton particle they're left with a tiny low downside. The antioxidants square measure currently missing a lepton and become free radicals themselves. The body cannot manufacture these antioxidants so that they should be provided in your diet.

Diabetes is a disease caused by an imbalance between blood sugar absorption and insulin secretion. Postprandial symptom plays a very important role within the development of the polygenic disorder. Regulating plasma glucose level is vital for delaying or preventing diabetes. One big strategy among the various antidiabetic therapeutic strategies is to limit gastrointestinal glucose absorption by inhibiting carboh ydrate metabolizing enzymes alpha-amylase and alpha-glucosidase.[8]lpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable

molecules. On the other hand, mammalian α-glucosidase in the mucosal brush border of the small intestine catalyzes the end step digestion of starch and disaccharides that are abundant in the human diet. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates within the intestine and diminish the postprandial blood sugar excursion. An effective means of lowering the levels of postprandial hyperglycemia has been offered by α-amylase and α- glucosidase inhibitors [2]. The treatment goal of diabetic patients is to maintain near-normal levels of glycemic control, in both fasting and post-prandial conditions. Many natural sources are investigated regarding the suppression of aldohexose production from the carbohydrates within the gut or aldohexose absorption from the viscus [3].

Impressive progress of research interest in the field polymeric nanoparticle-based drug delivery systems was reported earlier; however, only a few products are available in the market presently. Major limitations in evaluating the above-said system include longer time consumption and difficulty in studying the drug release kinetics and mechanism of interaction of the polymer with the drug molecules. In vitro drug release is a measure of the release of the active pharmaceutical ingredient or drug from the drug delivery system in a controlled laboratory environment. It is a key evaluation method in drug development and quality control. In silico approaches are defined as the use of information in the creation of computational models or simulations that can be used to make predictions suggest hypotheses, and ultimately provide discoveries or advances in medicine and therapeutics.



(https://Free+radical+formation+oxidative+stress+and+pathogenesis)

Free radical growth, oxidative stress and chronic disease pathogenesis

Dietary and endogenous antioxidants react by eliminating them with oxidizing free radicals and preventing cell injury. The involvement of therapeutic agents in cancer treatment could cause the generation of free radicals inflicting cellular injury and gangrene of malignant cells. So, a priority arises on whether or not exogenous inhibitor compounds taken at the same time throughout therapy might scale back the useful impact of therapy on malignant cells. In addition to pure antioxidants or foods rich in antioxidants, oxidative damage to nucleic acids can be reduced. An inhibitor is outlined as: "any substance that once gift in low concentrations compared to it of associate degree oxidizable substrate considerably delays or inhibits the reaction of that substrate". As this concept indicates, the physiological function of antioxidants is to stop damage to cellular components caused by chemical reactions involving free radicals.

Ethanolic extract of the Macrotyloma uniflorum leaves produced momentous antioxidant activity when compared with the standard ascorbic acid demonstrating that the extract exhibited a potential free radical scavenging ability. In the current study, M. uniflorum confirmed α- amylase and α-glucosidase inhibitory potential which may serve as a lead for the isolation and identification of compounds responsible for it. However, the active ideology responsible for the inhibitory action of α-amylase and α-glucosidase needs to be acknowledged and characterized for the improvement of indigenous botanical possessions for the development of a novel hypoglycemic drug. Due to the possession of secondary metabolites, this study justifies that the plant may exhibit hypoglycaemic activity which may be used in the management of diabetes. Intense study in this plant will also help to identify the active principle and this can be used as a

clue for developing new drugs that may be used in the pharmaceutical industries for modern drug discoveries.

Antioxidant activities are affected by buckwheat processing based on the changes in chemical composition. Some report investigates major anti-oxidative constituents from the seeds of buckwheat by detecting the components of the mixture of extracts and DPPH suggesting that rutin and quercetin were the major anti-oxidative constituents that are responsible for the quality control of buckwheat. The sources of buckwheat production had some necessary influence on the DPPH radical scavenging activity. They may be inhibited the activity or maintain the blood glucose values close to normal. Some of the drugs targeted to insulin secretion (sulphonylureas, glinides) while others are targeting insulin resistance (metformin, thiazolidinediones), [5]. One therapeutic approach for treating in the early stage diabetes is to decrease postprandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase, and β-glucosidase, in the digestive tract. Consequently, inhibitors of those enzymes determine a discount within the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise.

MATERIALS METHODS

Collection, Identification and Authentication of plant species

The plant, Costus spicatus were collected from the Saliyamangalam, Thanjavur district, Tamilnadu, India. It was taxonomically known and genuine by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamilnadu, India. The voucher specimens are deposited at the Rapinat herbarium and the voucher number is SAM 001. We analysed DPPH radical scavenging activity, Hydrogen peroxide scavenging activity[7], 1989Superoxide radical scavenging activity. [4]

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Statistical analysis

All the biochemical assays were analysed in triplicate and repeated at least three times. The results square measure as mean ± variance. All the experimental data were compared using the Student's t-test. P.

RESULT AND DISCUSSION

Several studies have shown the link between the traditional drug formulations rich in anti-oxidants and the incidence certain diseases such as cancer, diabetes, heart disease and other diseases related to aging. Antioxidants have a dual role of prevent food oxidation, especially lipid oxidation, and at an equivalent time, increase the antioxidant intake from diet. In organism, these exogenous antioxidants can manifest a good sort of actions, including inhibition of oxidizing enzymes, chelation of transition metals, transfer of hydrogen or one electron to radicals, singlet oxygen deactivation, or enzymatic detoxification of reactive oxygen species,cellular membrane stability and involves inhibition of radical production alongside enhancement of the body defense system .

DPPH radical scavenging assay

DPPH may be a common abbreviation for associate degree organic matter one, 1-diphenyl-2-picryl group. Scavenging of DPPH radical is that the basis of a standard inhibitor assay. In the assay, DPPH radical is converted to the corresponding hydrazine, and the color of the solution is changed from violet to yellow indicates the scavenging behavior of the plant sample due to bioactive compounds such as phenolic compounds, flavonoids, tannins, and their derivatives. The DPPH scavenging activity of the extracts was used as a model for anti-oxidant capacity. Polyphenols, flavonoids, terpenes and tannins compounds present in plant and plant products have been reported to have the anti-oxidative capacity.

Table 1: Percentage inhibition of ethanolic extract *costus spicatus*& standard ascorbic acid against DPPH at 517nm

S.NO	Conc.in µg/mL	Percentage inhibition by *Costus spicatus*	Percentage inhibition by ascorbic acid
1	10	37.87 ± 0.60	35.31±0.115
2	20	48.91 ± 0.60	44.62±0.115
3	40	67.86 ± 0.27	48.64±0.394
4	60	79.49 ± 0.30	60.95±0.167
5	80	85.36 ± 0.29	64.61±0.227
	IC50	**27.29 µg/ml**	**46.66µg/mL**

***mean ± SEM (n=3)**

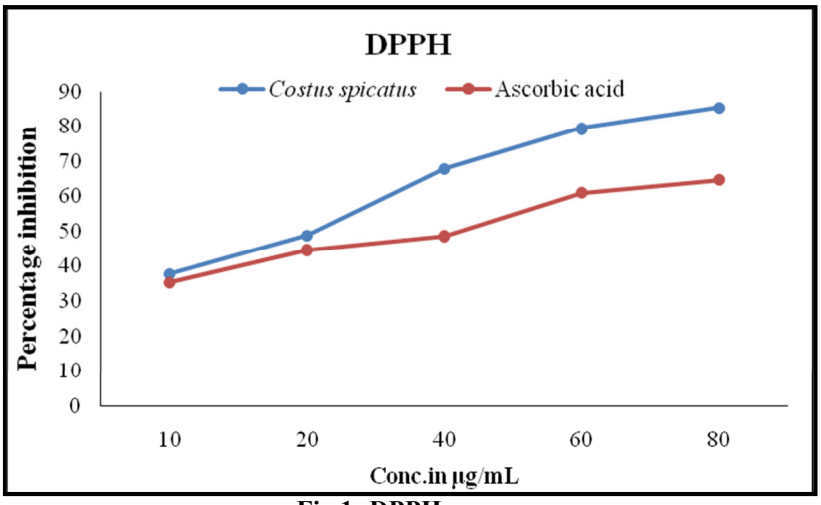


Fig 1: DPPH

The ethanolic extract of *Costus spicatus* showed considerable DPPH radical scavenging activity, which was concentration-dependent. From the it can be seen that theEthanolic Extract *Costus Spicatus*, showed a percentage inhibition of 64.61±0.227 while ascorbic acid showed a percentage inhibition of 85.31±0.29 at a concentration of 80µg/mL. The IC^{50} value calculated using the linear regression analysis was found to be 46.66, 37.64 and 27.29µg/mL for EECS and ascorbic acid respectively**.** From this, EECS showed higher antioxidant activity than EECS. The extract possessed a good radical scavenging capacity.

Hydrogen peroxide

The results obtained for the scavenging activity against hydrogen peroxide is presented in and the graphical representation.

Table 2: Determination of scavenging activity against hydrogen peroxide

S.NO	Conc.in µg/mL	Percentage inhibition by ascorbic acid	Percentage inhibition by *Costus spicatus*
1	80	32.44 ± 0.81	23.61±0.35
2	160	35.47 ± 0.91	32.9±0.46
3	240	60.73 ± 0.51	43.45±0.28
4	320	67.32 ± 0.53	64.36±0.33
5	400	79.99 ± 0.51	74.07±0.29
	IC50	**221.21 µg/mL**	**287.14 µg/mL**

***mean ± SEM (n=3)**

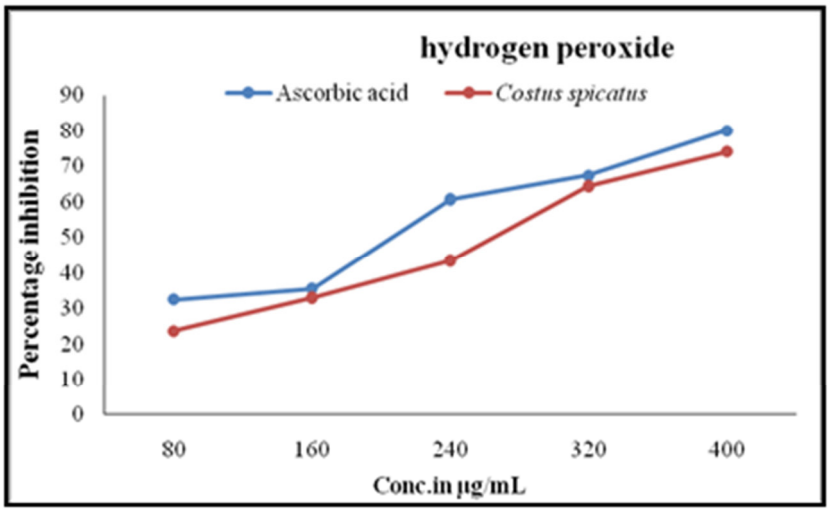


Fig : 2 hydrogen peroxide

From the table, it can be seen that the Ethanolic Extract *Costus Spicatus* showed a percentage inhibition of 74.07 ± 0.29 while ascorbic acid showed a percentage inhibition of 79.99 ± 0.51 at a concentration of 400µg/mL. The IC_{50} value calculated using the linear regression analysis was found to be, 287.14, 251.82 and 221.21µg/mLfor EECS and ascorbic acid respectively. From this, EECS showed higher antioxidant activity than EECS.

Superoxide Radical Activity

The results obtained for the Superoxide Radical Activity are presented inthe graphical representations of the superoxide radical activity of the EECSand ascorbic acid are presented.

Table 3: Determination of Superoxide Radical Activity

S.NO	Conc.in µg/mL	Percentage inhibition by ascorbic acid	Percentage inhibition by *Costus spicatus*
1	12.5	0.457 ± 0.001	0.36±0.0006
2	25	0.576 ± 0.004	0.44±0.0017
3	50	0.667 ± 0.003	0.57±0.0017
4	75	0.821 ± 0.001	0.72±0.0014
5	100	**0.936 ± 0.002**	**0.79±0.0011**

***mean ± SEM (n=3)**

From the table, it can be seen that the Ethanolic Extract *Costus Spicatus* showed an absorbance of 0.79 ± 0.0011 concentration of 100µg/mL while ascorbic acid showed an absorbance of 0.936 ± 0.002 at a concentration of 100µg/mL. The extract and the *Costus spicatus* rhizme extract showed a dose dependent superoxide radical activity. That the same Concentration showed much better activity than the extract alone.

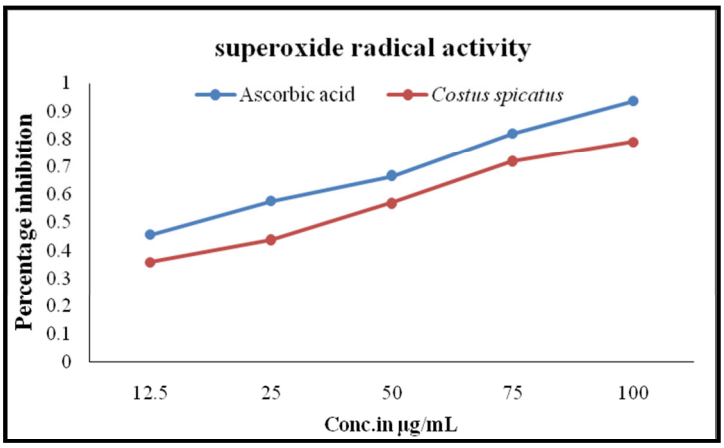


Fig 3: Superoxide Radical Activity

Determination of Reducing Power Assay

The results obtained for the Reducing Power assay of Ethanolic Extract *Costus Spicatus* and standard ascorbic acid are presented in the graphical representation is presented.

Table 4: Determination of Reducing Power Assay

S.NO	Conc.in µg/mL	Reducing Power by ascorbic acid	Percentage inhibition by *Costus spicatus*
1	20	0.745 ± 0.012	0.340±0.002
2	40	0.820 ± 0.003	0.414±0.004
3	60	0.930 ± 0.002	0.641±0.002
4	80	0.958 ± 0.059	0.689±0.003
5	100	**1.052 ± 0.007**	**0.780±0.007**

***mean ± SEM (n=3)**

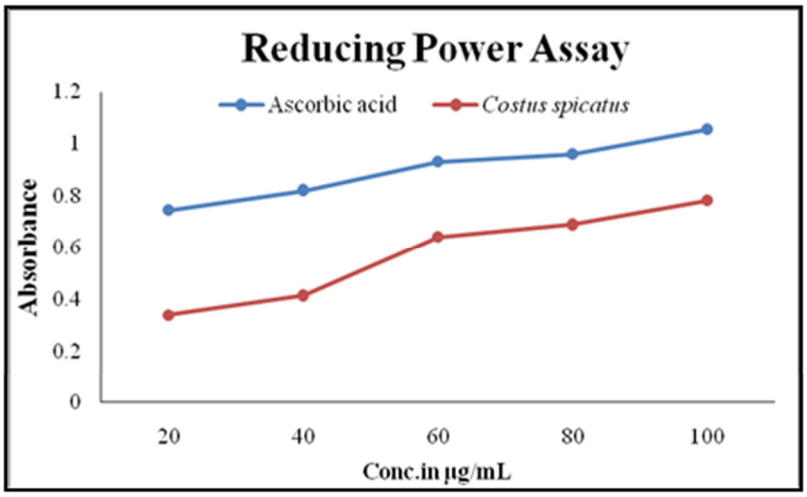


Fig 4:Reducing Power Assay

From the Ethanolic Extract *Costus Spicatus* and ascorbic acid showed the absorbance of 0.780±0.007 and 1.052±0.007 at a concentration of 100µg/mL. The EECS showed higher antioxidant activity.

CONCLUSION

The present study *in vitro anti oxidant* from ethanolic rhizome extract of *C. spicatus* possessed the potential antibacterial activity, antioxidant and anticancer activity and it could be used as natural source for treating humandiseases.Free ROS radicals are generated primarily from various sources in the biological system that cause a cascade of oxidation events leading to cell membrane disruption and attacking other major cell organ els.Normally an antioxidant works by stopping this oxidation process triggered by these unstable entities . SOD and CAT are the two scavenging enzymes that remove the toxic free radicals. In the enzymatic antioxidant defence system, SOD is one of the most important enzymes and scavenges $O^{2}-$ anion (which is the first product of O2 radicals) to form H_2O^2 and hence diminishes the toxic effects of to this radical or other free radicals derived from secondary reactions.

Acknowledgment

This research is supported by PG & Research Department of Zoology & Biotechnology, A.V.V.M. Sri Pushpam College (Autonomous) Poondi, Thanjavur – 613503, India.

REFERENCES

1. Blois, M.S., (1958). Antioxidant determinations by the use of stable free radical. Nature, 1: 1199-2000.
2. Kazeem M.I., J.O. Adamson and I.A. Ogunwande, (2013).Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of Morinda lucida Benth leaf.BioMed research international.
3. Kumar, V., A.K. Abbas.and J.C. Aster.(2017). Robbins Basic Pathology E-Book.Elsevier Health Sciences.
4. Liu F., V.E.C. Ooi and S.T. Chang, (1997). Free radical scavenging activity of mushroom polysaccarides extract. Life Sci., 60: 763-771.
5. Meena H., H.K. Pandey, P. Pandey, M.C. Arya and Z. Ahmed, (2012).Evaluation of antioxidant activity of two important memory enhancing medicinal plants Baccopa monnieri and Centella asiatica. Indian journal of pharmacology, 44(1), p.114
6. Oyaizu, M., (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-315.
7. Ruch R.J., S.J. Cheng and J.E. Klaunig, (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea.Carcinogenesis., 10:1003-1008.
8. Santhiya, N., Priyanga, S., Hemmalakshmi, S and Devaki, K., (2016).Phytochemical analysis, Anti inflammatory activity, in vitro antidiabetic activity and GC-MS profile of *Erythrina variegata* L. bark**.**J. Appl. Pharm Sci, 6(07),147-155**.**

SP-07

DIAGNOSING BREAST CANCER WITH MACHINE LEARNING ALGORITHMS

S. THIYAGARAJAN[1],Dr. T. CHAKRAVARTHY[2],Dr. P.V. ARIVOLI[3]

[1]Research Scholar, Department Of Computer Science, A.V.V.M Sri Pushpam College (Autonomous), (Affiliated to Bharathidasan University, Trichy), Poondi, Thanjavur – 613 503.strprc@gmail.com
[2]Associate Professor, Department Of Computer Science, A.V.V.M Sri Pushpam College (Autonomous), (Affiliated to Bharathidasan University, Trichy) Poondi, Thanjavur – 613 503.tcvarthy@gmail.com
[3]Assistant Professor, Department Of Computer Science, Government Arts and Science College, (Affiliated to Bharathidasan University, Trichy) Kudavasal, Thiruvarur – 612 601.pvatvr@gmail.com

Abstract: Breast cancer is a type of cancer found in the breast. Cancer starts when cells begin to grow out of control. Breast cancer cells usually form as a tumor felt as a lump. Breast cancer occurs in women. It is important to find that most breast lumps are benign (good cells) and not malignant (cancerous cells). Periodic breast cancer check-ups give the disease to be diagnosed and treated prior to it causing noticeable symptoms. Machine learning automates the identification of cancerous cells and provides considerable benefits to the health care systems. Automated process provides physicians to spend less time in diagnosing and more time for diseases treatment, thus it improves the efficiency of the detection process. This research work investigate the application of machine learning methods for detecting breast cancer by using measurements of biopsied cells from women with abnormal breast masses. It uses the Wisconsin Breast Cancer Diagnostic dataset from the UCI Machine Learning Repository. Various performance estimation methods are used to validate the methods used in the work.

Keywords: Breast cancer detection, Classification, Machine learning, WD, UCI Machine,

I INTRODUCTION

Breast cancer is a kind of cancer found in the breast of women. It starts when cells begin to grow out of control. Non-cancerous breast tumors are abnormal growth, but they do not spread outside of the breast. They are not harmful, but breast lumps can raise the risk of getting breast cancer. Any breast lump or change requires to be checked by a physician to identify if it is benign or malignant (cancer). Common breast cancer includes Ductal Carcinoma in Situ (DCIS) and invasive carcinoma, besides there are many different types of breast cancer. Once a biopsy is done, breast cancer cells are identified with various characteristics. If the cancer cells get into the blood or lymph system and are carried to other parts of the body, then breast cancer can spread.

The rates of breast cancer have increased slightly by 0.3 percent per year in recent times. In women, breast cancer is the second prominent cause of cancer death. The possibility of a woman will die from breast cancer is around 1 in 38 (2.6%). From 1989 to 2016, the rates of death from female breast cancer slashed around 40%. These shrinkages are accepted to be the result of discovery of breast cancer prior through diagnosis and elevated awareness, as well as more appropriate treatments. It is essential for us to be familiar of changes in breasts and to identify the signs and symptoms of breast cancer. A new lump of mass is the simplest symptom of breast cancer. Hard mass without pain that has uneven edges is more possibly to be cancer. To find breast cancer initially before any symptoms to be appear, screening test can be helpful. Early finding of breast cancer will give us a good chance of fruitful medication.

Early detection steps contain examining the tissue of breast for abnormal lumps or masses. A fine-needle aspiration biopsy is performed if a lump is found. It extracts a small sample of cells from the mass using a hollow needle. A physician then examines the cells under a microscope to find whether the mass is likely to be malignant or benign.

To find good ways to inhibit, identify and medicate breast cancer and to improve the condition of life of patients and survivors scientists around the world are working. Several research works invent that style of

living and factors, rooted genes affect breast cancer hazard. Scientists go on to look for medicines that may help reduce breast cancer hazard, especially women who are at more chance.

Computers can assist an oncologist in the task of breast cancer detection. Machine learning is a computational strategy which can be used to determine optimal solutions to a given problem without explicitly being programmed into a computer program by a programmer. Over the last two decades, the use of machine learning algorithms has spread to several fields including medical data analysis [3]. Classification is a supervised machine learning task for classifying data accurately and efficiently. Classification can be used effectively in diagnosing breast cancer data. Machine learning supports various algorithms for classification task. Some of the well-known algorithms are K-nearest neighbor, Decision trees, Neural Networks, Naïve Bayes classifier, Support vector machine, Statistical models and Deep Learning model, Decision rules, Regression methods and so on. Each method or algorithm will be used to construct a single classifier model using training dataset. Based on testing and performance measure the single classifier model will be used for classifying unknown instances. Recently, DNN (Deep Neural Networks) have provided good results in many areas like natural language processing, pattern recognition, audio recognition and image classification. DNN is also called Deep learning model, which is used effectively in the analysis of bioinformatics signals.

An automated diagnosing system removes the inherently subjective human component from the process and provides greater detection accuracy. The main objective of this research work is to investigate the application of various machine learning algorithms in breast cancer detection.

There are several research works carried out by the researchers earlier. Some of them are summarized here. Habib Dhahri et al provide an empirical study on breast cancer diagnosis using machine learning and soft computing techniques. The work is based on genetic programming and machine learning algorithms. Sensitivity, specificity, precision, accuracy and ROC curves are used to measure the performance. Wisconsin Breast Cancer dataset from UCI machine learning repository is used. WEKA, an open source machine learning software is used to conduct experimental work [1]

Meriem Amrane et al gave another work on breast cancer detection using machine learning algorithms viz. Naïve Bayes and K nearest neighbor. In their work, KNN is proved in achieving higher accuracy rate. UCI – Wisconsin breast cancer dataset was used [2]. Burak Akbugday used KNN, Naïve Bayes and SVM for classifying breast cancer data. He used WEKA software and Breast Cancer Wisconsin (original) dataset for his experimental work. He suggested that KNN and SVM were the best in classification of breast cancer data [3].

Ahmet Saygili adopted SVM, KNN, Naïve Bayes, Decision trees, Random forest and Multilayer perceptron methods for classifying breast cancer data. He used Wisconsin diagnostic breast cancer dataset. He used 10-fold cross validation and achieved high degree of accuracy [4]. Ch. Shravya et al used Logistic regression, SVM, and KNN in predicting breast cancer. They used Python – Spyder and UCI WDBC dataset for empirical analysis. They proved SVM was more accurate in predicting breast cancer [5]. David A. Omondiagbe et al used SVM with radial basis kernel, ANN and Naïve Bayes. They used WDBC dataset for experimental setups and achieved more accuracy by using a hybrid method of combining SVM and LDA [6]. Siyabend Turgut used microarray breast cancer data, eight machine learning methods, and two feature selection methods and finally suggested that SVM is best. He used Python and K-fold cross validation for empirical study.

The rest of the paper is organised as follows. Section II gives Materials and methods, section III provides results and discussions, and finally, section IV concludes the work.

II MATERIALS AND METHODS

2.1 Materials

The work used Wisconsin Breast Cancer Diagnostic dataset from the UCI (University of California, Irvine). The dataset is from the University Hospital of California, created by Wolberg and contributed by other researchers of the University of Wisconsin. It contains the measurements from digitized images of fine-needle aspirate of a breast mass. The features represent the properties like shape and size of the cell

nuclei present in the digital image. The breast cancer dataset includes 569 instances of cancer biopsies, each with 32 feature-values. They are numeric measurements of mean, standard error and worst (largest) values of cell nuclei's radius, texture, perimeter, area, smoothness, compactness, concavity, concave points, symmetry and fractal dimension. The instances of entire dataset are divided into two partitions with two third of the instances are used for train the model and one third are used to test the model.

2.2 Methods

The important machine learning task, classification can be done using KNN, Naïve-Bayes, Decision trees and Rules based classifier, neural networks and Support Vector Machines.

A. KNN

The term KNN means K nearest neighbor, a popularly used simple classification machine learning algorithm. It uses information about a tuple's K nearest neighbors to classify unlabeled tuples. Value of K can be any number. After deciding K, the algorithm requires a training dataset to be classified into classes as labelled by a target class variable. Then, for each unlabeled tuple in the test dataset, K-nn identifies K nearest neighbors and assigns to the class that is most nearest

B. Naïve-Bayes

Classifier based on Naïve Bayes method uses probabilistic learning. It uses training data to calculate probability of each target class based on the evidence provided by feature values. It uses probabilities to find the most likely target class for the features of unlabeled tuples when they are applied to the classifier for classification.

C. Decision trees and Rules

The two machine learning methods make complex decisions from sets of simple choices. Decision tree classifier is a powerful, which uses a tree structure to construct a model using training dataset. Later, the testing and unclassified tuples will be used to classify using the tree model. Choosing best split among the tuples of training dataset in constructing the decision tree model is an important event. Also one can prune unwanted nodes of a decision tree to improve the performance. A classification rule represented in the form of logical if-else statement assigns a target class to unlabeled tuples.

The condition part of the rule contains feature values, while the action portion gives the target class value to assign when the rule's condition is met. There are a set of rules to be used in classifying several testing and unlabeled tuples, while rules are constructed using training dataset tuples.

D. Neural Networks

A Neural network, also called Artificial Neural Network (ANN) similar to the biological brain that responds to stimuli from sensory organs models the relationship between a set of input values (feature vectors) and a set output values (target features). Although there are various neural networks models, each can be constructed using various activation functions, network topologies and training algorithms. After data collection, exploration and preparation, an ANN model will be trained using training data sets. During the time, weights, threshold function values are get calculated and also adjusted for results with or without feedbacks. Later, testing dataset and unclassified tuples are applied for classification.

E. Support Vector Machine

A Support Vector Machine (SVM) is a hyper plane that creates boundaries between points of data plotted which represent tuples and their feature values. The SVM learner combines the aspects of both the K-nn model of learning and the linear regression model. The combination is highly robust, making SVM to model highly complex problems.

The steps in machine learning process includes: 1. Collecting data, 2. Exploring and preparing the data (Transformation – normalizing numeric data, data preparation – creating training and test datasets), 3. Training the model on the dataset, 4.Evaluating model performance and 5.Improving model performance (using various transformations and testing alternative parameter values of the models). These steps are clearly illustrated in the figure 1.

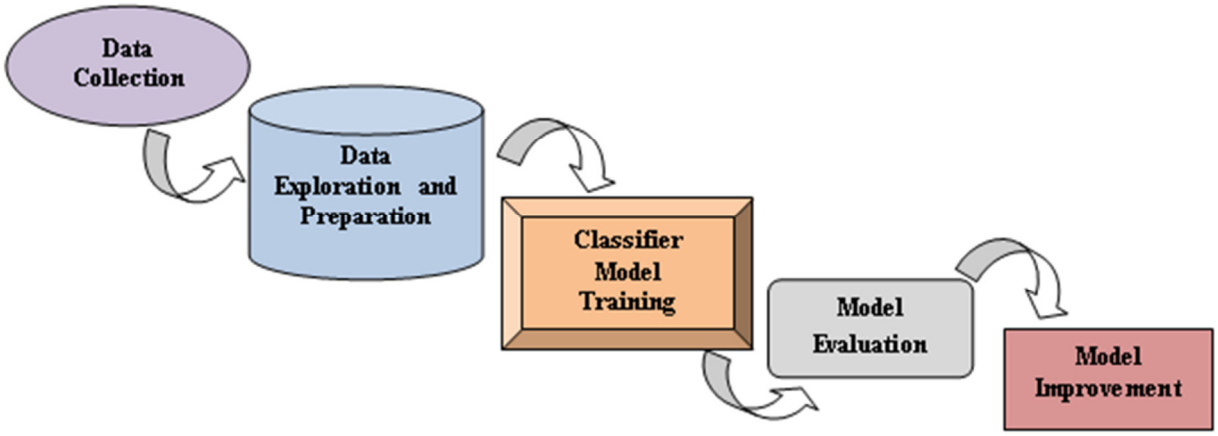


Figure 1: Architecture of the classification system[4]

III Results and Discussions

There are various evaluation metrics for the predictive accuracy of a classifier model. They are accuracy, error rate, sensitivity, specificity, precision, and recall (See table I). These measures are calculated with the help of confusion matrix and its value entries. Figure 2 represents a confusion matrix of class prediction made by KNN algorithm compared with class labels in the dataset. Table II represents the performance of various machine learning algorithms when applied with breast cancer data.

S. No.	Name of the performance measure	Meaning
1	Confusion Matrix	TP (True Positive), TN (True Negative), FP (False Positive) and FN (False Negative)
2	Accuracy	(TP+TN)/N
3	Error rate	1- Accuracy
4	Sensitivity	TP/(TP+FN)
5	Specificity	TN/(TN+FP)
6	Precision	TP/(TP+FP)
7	Recall	TP/(TP+FN)
8	ROC curves	Receiver Operating Characteristic Curve

Table I: Various Performance measures

Predictions By classifier model		**Labels**	
		Benign	**Malignant**
	Benign	**61** ***TN***	**0** ***FP***
	Malignant	**5** ***FN***	**34** ***TP***

Figure 2: Confusion matrix of class prediction made by KNN

Machine Learning Method	Accuracy	Error Rate
KNN	95%	5%
Decision tree	94.27%	5.73%
ANN	96.2%	3.8%
SVM	92.54%	7.46%

Table II: Performance of various machine learning methods

IV CONCLUSION

This work attempts to solve the problem of diagnosing breast cancer using various machine learning algorithms. Different experiments were conducted using the Wisconsin breast cancer dataset. It proved that

the machine learning algorithms can achieve more performance, effective and accurate. All experiments were performed using the R library.

REFERENCES

[1] Dhahri, Habib, et al. "Automated Breast Cancer Diagnosis Based on Machine Learning Algorithms." *Journal of Healthcare Engineering* 2019 (2019).

[2] Amrane, Meriem, et al. "Breast cancer classification using machine learning." *2018 Electric Electronics, Computer Science, Biomedical Engineerings' Meeting (EBBT)*.IEEE, 2018.

[3] Akbugday, Burak. "Classification of Breast Cancer Data Using Machine Learning Algorithms." *2019 Medical Technologies Congress (TIPTEKNO)*.IEEE, 2019.

[4] Saygılı, Ahmet. "Classification and Diagnostic Prediction of Breast Cancers via Different Classifiers." *International Scientific and Vocational Studies Journal* 2.2: 48-56.

[5] Israni, Priyanka. "Breast Cancer Diagnosis (BCD) Model Using Machine Learning." *cancer cells* 1: 10.

[6] Omondiagbe, David A., Shanmugam Veeramani, and Amandeep S. Sidhu. "Machine Learning Classification Techniques for Breast Cancer Diagnosis." *IOP Conference Series: Materials Science and Engineering*. Vol. 495.No. 1.IOP Publishing, 2019.

[7] Turgut, Siyabend, Mustafa Dağtekin, and Tolga Ensari. "Microarray breast cancer data classification using machine learning methods." *2018 Electric Electronics, Computer Science, Biomedical Engineerings' Meeting (EBBT)*.IEEE, 2018.

[8] Thiyagarajan, S., and T. Chakravarthy. "A Survey on ECG Data Classification Using Machine Learning Approaches." (2019): 401-408 ADALYA JOURNAL

[9] Thiyagarajan, S., and T. Chakravarthy. "Classification of ECG Data with Homogeneous Ensemble Deep Neural Networks."JAC : A Journal Of Composition Theory

SP-08

ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF *PIPER BETLE* LEAVES, (NATTU VETRILAI VARIETY) AGAINST CERTAIN BACTERIAL PATHOGENS

C.UMA*, B.ASWINI, S.ARULJOTHI, K. MUTHULAKSHMI

Division of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India
***umasaravanan1@gmail.com**

Abstract
Piper betle L., belonging to family Piperaceae is an evergreen creeper which is known to possess numerous medicinal values. The betel vine is cultivated for its deep green heart-shaped leaf for chewing purposes. The leaves are known to possess antioxidant, anti-inflammatory, anti-apoptotic, anti-cancer and anti-microbial properties. Different extracts (methanol, ethanol, acetone) prepared from Nattuvetrilai leaf variety grown in parts of Tamil Nadu and Kerala were used against five pathogenic bacteria. Methanol extracts from the Nattuvetrilai variety of *Piper betle* exerted good antibacterial activity against test organisms which were collected from parts of Tamil Nadu. The pathogens *viz*. *Salmonella typhi*, *Streptococcus mutans*, *Escherichia coli* and *Staphylococcus aureus* were exhibited greater susceptibility towards methanol extract of betel leaves. The results also revealed that there was a marked difference among the same variety of *P. betle*cultivated in two different regions in their antimicrobial nature.

Key Words: Piper betle, Antibacterial activity

Introduction

The use of medicinal plants as a source for relief from illness can be traced back to over five millennia. The potential of higher plants as a source for a new drug is still largely unexplored [1]Nowadays, multidrug antibiotic resistance has been observed in pathogens due to its widespread use of antibiotics. So, inventions of new medicines have become more important because of these resistant pathogens [2]. Nowadays, traditional medicinal plants play a major role nowadays due to the negative impact displayed by synthetic drugs, such as antibiotic resistance, side effects, high cost and loss of public reliance [3].

The antiseptic property of the betel plant has been known since 600 BC[4]. Ancient Indian culture witnessed the vital role played by *Piper betle*. The traditional books of Ayurveda, CharakaSamhitas, and KashyapaBhojanakalpa which has enlisted the practice of chewing *P. betle* leaf after meals [5]. The *P. betle* (Tamil: Vetrilai) is a perennial dioeciousglabrous climbing vine belonging to the family. It is an evergreen creeper which is known to possess numerous medicinal values. The betel vine is cultivated for its deep green heart-shaped leaf for betel leaf. The leaves are not only used directly for chewing purposes but also possess antioxidant, anti-inflammatory, anti-apoptotic, anti-cancer and anti-microbial properties. Leaves are simple alternate and yellow to bright green in color[6].

The plant grows well in warm, humid climates[8]. The comparative study is essential because the phytochemical concentration of the plant may tend to differ according to habitation, soil, climate, and agronomic practices followed. Even though numerous researches have been done on various medicinal uses of *P. betle* leaves, the comparative study between leaves varieties grow under different climate is limited. Therefore, the objective of this study is to compare the antimicrobial properties of betel leaf (NattuVetrilai variety) collected from Tamil Nadu and Kerala state against selected bacterial pathogens.

MATERIALS AND METHODS

Collection of plants

The *P. betle* Nattuvetrilai variety leaves grown in Tamil Nadu and Kerala state were selected for this study. Healthy and well-grown, young greenish leaves were selected and then collected in sterile polythene bags directly from the cultivated fields and transported safely to the laboratory for further studies. The leaves were washed alternately with tap water and distilled water, then surface sterilization was done with 10% concentrated sodium hypochlorite solution, rinsed again with sterile distilled water and kept at room temperature for shade drying.

Preparation of plant extracts

Using mixer grinder dried leaves were homogenized into a fine powder. Three different solvents *viz*. methanol, ethanol and acetone were chosen for the extraction of bioactive compounds from the sample. About 50g of powdered samples were loaded in the Soxhlet apparatus with 250ml of respective solvent separately and extracted for about 72 h. The extraction was continued until the extractive become colorless. Then after the extraction all the successive extracts were evaporated in a rotary vacuum evaporator at 40°C for the removal of water content. The crude extract thus obtained was transferred into glass vials and stored at 4°C until it is required.

Test organisms used

The bacterial pathogens used in this study were procured from the Institute of Microbial Technology (IMTech), Chandigarh. The lyophilized cultures were revived by inoculating the strains into the nutrient broth. The stock culture was maintained on Nutrient Agar slants and stored at 4°C in a refrigerator.

Preparation of inoculum

The active young cultures for the study were prepared by subculturing a loop full of cells to the Nutrient broth and incubated for 24 h at 37°C. The 24-h-old cultures were suspended into the sterile Nutrient broth to match the turbidity of 0.5 McFarlandstandard, which is approximately 1.0 x 10^6 CFU/ml.

***In vitro* antibacterial activity of the extracts**

Antibacterial activity of the *P. betle* leaf extract was assessed by the disc diffusion method (Bauer *et al*., 1966). About 15–20ml of sterilized Mueller-Hinton (MH) agar medium was transferred to sterile Petri plates and allowed to solidify. After solidification, the test bacterial cell suspension (0.1%) was uniformly spread over the agar surface using sterile cotton swabs. The sterile discs impregnated with 300µg/ml of the extracts were placed at equal distance and then incubated at 37°C for 24 h. The standard antibiotic ciprofloxacin (5µg/disc) was also placed to compare the inhibition results contributed by the extracts. At the end of incubation, the zone of growth inhibition around the disc was measured in mm unit.

Minimum inhibitory concentration

For the determination of minimum inhibitory concentration (MIC), a twofold serial dilution method was followed with Mueller Hinton Broth. The plant extracts were made up to the concentration ranging from 3.9 to 1000µg/ml. The uninoculated MH broth served as a negative control whereas broth tube inoculated with respective culture was served as a positive control. The tubes were incubated for 24 h at 37°C, and the results were noted. The MIC was the lowest concentration of the extract in which there was no visible growth on the inoculated tubes[11].

Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined by subculturing the above (MIC) serial dilution after 24h in MH agar plates and incubating at 37°C for 24h. MBC is defined as the lowest concentration that inhibits the growth of any bacterial colony on solid media[10].

RESULTS AND DISCUSSION

The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties. The plant is the cheaper and safer alternative sources of antimicrobials[13].

The present study was aimed to compare the antimicrobial properties of NattuVetrilai grown in two different regions i.e., Tamil Nadu and Kerala. In the present study, 3 different solvents were used *viz.,* methanol, ethanol and acetone. Of all, the methanolic extracts of all the plant materials showed antimicrobial activities than other solvents. The solvent methanol can be able to extract more antimicrobial compounds including polyphenols, tannins, terpenoids, saponins, xanthoxylines, lactones, flavones and phenol, etc[14]. Most of the studies reported that methanol is a better solvent for the extraction of different compounds from plants and this has been confirmed in our present study. The inhibitory action was increased with an increase in the concentration of extract used. Ethanolic extract from *P. betle* leaves also recorded good result after methanolic extract[11].

The antimicrobial activity of P. betle leaf (NattuVetrilai) grown under two different climatic conditions was compared in this study. The comparative study is essential because of the phytochemical

concentration of the plant may tend to differ according to habitation, soil, climate and agronomic practice followed. Even though numerous researches have been done on various medicinal uses of P. betle leaves, the comparative study between leaves varieties grow under different climate is limited. The data obtained from this study would be the first record where the differences in the antimicrobial nature among the same variety of betle leaves were reported.

Table-1: List of bacterial pathogens used in this study

S. No	Bacterial strains
1.	*Salmonella typhi* MTCC1168
2.	*Klebsiellapneumoniae* MTCC39
3.	*Streptococcusmutans*MTCC121
4.	*Staphylococcus aureus* MTCC90
5.	*Escherichia coli* MTCC40

Table-2: Antibacterial activity of leaf extract of PBN1 variety cultivated in Dindigul (District), Tamil Nadu against bacterial pathogens

Zone of inhibition(mm)/ Organism used						
S.No.	**Extracts used µg/ml**	***S. typhi* (A)**	***K. pneumonia* (B)**	***S. mutans* (C)**	***Staph.aureus* (D)**	***E.coli* (E)**
1.	**Methanol**					
	400	14.8 ± 0.5	11.9 ± 0.3	16.4 ± 0.5	12.8 ± 0.6	14.7 ± 0.5
	MIC	125	125	125	125	125
	MBC	250	250	250	250	250
Standard Ciprofloxacin (5 µg/disc)		27.5 ± 0.3	27.3 ± 0.5	29.3 ± 0.7	25.6 ± 0.4	29.4 ± 0.2
2.	**Ethanol**					
	400	13.7 ± 0.3	10.7 ± 0.5	15.8 ± 0.5	12.8 ± 0.5	11.8 ± 0.6
	MIC	125	250	125	250	250
	MBC	250	500	250	500	500
Standard Ciprofloxacin (5 µg/disc)		27.6 ± 0.2	27.7 ± 0.4	29.3 ± 0.6	25.2 ± 0.4	29.5 ± 0.2
3.	**Acetone**					
	400	12.2 ± 0.6	10.6 ± 0.4	13.7 ± 0.8	10.8 ± 0.8	12.7 ± 0.6
	MIC	250	250	125	250	250
	MBC	500	500	250	500	500
Standard Ciprofloxacin (5 µg/disc)		27.6 ± 0.2	27.7 ± 0.4	29.5 ± 0.2	25.1 ± 0.5	29.7 ± 0.5

Table-3: Antibacterial activity of leaf extract of PBN2 variety cultivated in Palakkad (District), Kerala against bacterial pathogens

Zone of inhibition(mm)/ Organism used						
S. No	**Extracts used µg/ml**	***S. typhi* (A)**	***K. pneumonia* (B)**	***S. mutans* (C)**	***Staph.aureus* (D)**	***E.coli* (E)**
1.	**Methanol**					
	400	14.5 ± 0.5	11.6 ± 0.4	15.7 ± 0.4	13.5 ± 0.7	14.6 ± 0.6
	MIC	125	250	125	250	125
	MBC	250	500	250	500	250
Standard Ciprofloxacin (5 µg/disc)		29.2 ± 0.4	28.2 ± 0.5	28.8 ± 0.5	28.6 ± 0.4	29.6 ± 0.3
2.	**Ethanol**					
	400	13.7 ± 0.3	11.7 ± 0.4	14.9 ± 0.4	12.8 ± 0.8	13.8 ± 0.5
	MIC	250	250	125	250	250
	MBC	500	500	500	500	500
Standard Ciprofloxacin (5 µg/disc)		27.6 ± 0.2	28.2 ± 0.5	28.8 ± 0.5	25.2 ± 0.4	29.6 ± 0.3

3.	**Acetone**					
	400	12.9 ± 0.5	10.7 ± 0.7	13.8 ± 0.3	11.5± 0.4	12.5 ± 0.5
	MIC	250	250	125	250	250
	MBC	500	500	250	500	500
Standard Ciprofloxacin (5 µg/disc)		27.3 ± 0.5	27.5 ± 0.3	29.1 ± 0.5	25.3 ± 0.2	29.7 ± 0.4

A-Salmonella typhi, B- Klebsiellapneumoniae, C- Streptococcus mutants, D- Staphylococcus aureus, E- Escherichia coli

Conclusion

The present study revealed that the methanolic extract from NattuVetrilai variety ofbetel leaves are potential source of certain bioactive compounds which should be investigated further to develop drugs against pathogens.

Reference

1. Mahesh, B. and Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences 4 (S); 839-843.
2. Kaveti, B. and Tan, L. (2011). Antibacterial activity of *Piper betle* leaves. *International Journal of Pharmacy Teaching & Practices*. 2(3):129–132.
3. Livermore, D. M. (2004). The need for new antibiotics.*ClinMicrobiol Infect*. 4: 1–9.
4. Sudewo, B. (2010). Red betel. Jakarta. *PT AgromediaPustaka*. 37–47.
5. Kumar, N., Misra, P., Dube, A., Bhattacharya, S., Dikshit, M. andRanade, S. (2010). *Piper betle* Linn. amaligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. *Curr Sci*. 99: 922–932.
6. Sharma, S., Khan, I. A., Ali, I., *et al*. (2009). Evaluation of the antimicrobial, antioxidant and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. *Antimicrobial Agents and Chemotherapy*. 53: 216–222.
7. Jayaweera, D. M. A. (1982). Medicinal plants (Indigenous and Exotic) used in Ceylon. *National Science Council of Sri Lanka*. Part IV.
8. Nanayakkara, B. S., Abayasekara, C. L., Panagoda, G. J., Kanatiwela, H. M. D. K. and Senanayake, M. R. D. M. (2014). The anti-candidal activity of *Piper betle* (L.), *Vitexnegundo* (L.) and *Jasminumgrandiflorum* (L.). *African Journal of Microbiology Researc*h. 8(23): 2307–2231.
9. Bauer, A. W., Kirby, W. M. M., Sherris, J. C and Turck, M. (1966). Antibiotic susceptibility testing by a standard single disc method. *Am J ClinPathol*. 36: 493–496.
10. NCCLS (1997). Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.
11. Aruljothi, S., Uma.C, Sivagurunathan P. (2016). Comparative Evaluation on the Antibacterial Activity of Karpoori Variety Piper betle Leaves against Certain Bacterial Pathogens. 3 (3): 35-45.
12. Ericsson, H.M and Sherris, J.C.1971.Antibacterial sensitivity testing. Report of an international collaborative study. *Acta.Pathol.etMicrobiol.Scand.Section B.Suppl.No.*217.
13. L. Doughari, J. H., Elmahmood, A. M. and Manzara., (2007). Studies on the antibacterial activity of root extracts of Carica papaya *S.African Journal of Microbiology Research,*. 037-041
14. Cowan,M.M.,(1999)Plant Products as Antimicrobial Agents.*Clin Microbiol Rev*. 12(4): 564–582

SP-09

ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *ADHATODA VASICA* AND *SOLANUM TRILOBATUM* AGAINST RESPIRATORY INFECTION CAUSING PATHOGENS OF CLINICAL ORIGIN

SUBHASHREE JENA, *D. SANGEETHA, P. RAMYA, LIPUN KUMAR PRADHAN

Department of Microbiology, Faculty of Science, Annamalai University,
Annamalai nagar - 608002, Tamilnadu, India.
E-Mail: sangeethadau@gmail.com

Abstract
Plants are used for medicinal purposes from the prehistoric period. Traditional medicine continues to be broadly practised on several reasons. Rising population, scarcity of drugs, cost of treatments, side effects of synthetic drugs and development of resistance to currently using drugs have led to increased demand on the use of plant materials as a source of medicines for a wide variety of human ailments.The microbial pathogens enters into the human body through respiratory tract as the main portal. World Health Organization reports that respiratory tract infections like tuberculosis, influenza, and pneumonia caused more than 4 million deaths worldwide yearly. On account of such problems in the present study, various extracts of two medicinal plants namely *Adhatoda vasica* and *Solanum trilobatum* were chosen to evaluate the antibacterial activity against respiratory infection causing pathogens of clinical origin. Both of the plantsshowed antimicrobial activity against the respiratory infection causing microorganisms. The methanolic extract of *Adhatoda vasica* showed more activity when compared with the plant extract of *Solanum trilobatum.*

Keywords: *Adhatoda vasica, Solanum trilobatum,*antibacterial activity

1. Introduction

In recent years, the infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant ability of some microorganisms. However, synthetic antimicrobial agents provide broad spectrum characteristics, but often associated with the adverse effects on the host, including immune suppression, hypersensitivity and several allergic responses[1, 2, 3].

Herbal medicine is the foundation for about 75–80% of the world population, mainly targeting primary health care in the developing countries because of better cultural acceptability, compatibility with human body and lesser side effects. However, there is a drastic increase in the usage of herbal medicine was found in last few years from the developed countries [4].

Respiratory diseases, including allergies, asthma and chronic obstructive pulmonary disease (COPD) are a major public health burden worldwide. Each year, 250,000 people die of asthma. The prevalence of these diseases is increasing and there is a continued need for new and improved therapies. Some respiratory diseases are caused by bacteria. For instance, *Staphylococcus aureus* causes pneumonia [5], *Escherichia coli* causes community- acquired pneumonia[6].

Adhatoda vasica, native to Asia is a well-known plant in Siddha, Ayurveda and Unani systems of medicine. Various parts of this plant have been used to treat of several ailments as herbal remedy such as, cold, cough, whooping cough, chronic bronchitis, fever, jaundice asthma as sedative expectorant, diarrhoea and dysentery and rheumatic painful inflammatory swellings. This is one of the most potent anti-tuberculosis drugs [7].

Solanum trilobatum Linn (Family: Solanaceae), a thorny creeper with bluish white flower and grows as a climbing under shrub. It is one of the important medicinal plants, more commonly available in Southern India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma and tuberculosis[8].

2. Materials and methods

2.1. Selection of medicinal plants

In the present work, *Adhatoda vasica* and *Solanum trilobatum* plants were screened for potential antibacterial activity.

2.2. Collection, Identification and Processing of Leaves of *Adhatoda vasica* and *Solanum trilobatum*

Fresh plants were collected from Chidambaram, Cuddalore Dist, Tamilnadu. The taxonomic identities of these plants were determined. The leaves were washed with 70% alcohol and then rinsed with sterilized distilled water, shade dried for 10-12 days and powdered using mechanical grinder and then stored in air tight containers for further use.

2.3. Preparation of crude leaf extracts

The powdered materials used for the preparation of methanol, ethanol and acetone extracts.

2.3.1. Preparation of methanol extract

10 grams of leaf powder of each *Adhatoda vasica* and *Solanum trilobatum* were soaked in 100ml of methanol in conical flask separately, plugged with cotton and kept at room temperature for 3days and filtered through Whatman No:1 filter paper. The filtrate was evaporated in petri dish at room temperature for 2-3 days till the volume was reduced to one- fourth of the original volume of the solvent used and stored at4℃ in airtight bottles [9].

2.3.2. Preparation of ethanol extract

10 grams of leaf powder of each *Adhatoda vasica* and *Solanum trilobatum* were soaked in 100ml of ethanol in conical flask separately, plugged with cotton and kept at room temperature for 3 days and filtered through Whatman No:1 filter paper. The filtrate was evaporated in petri dish at room temperature for 2-3 days till the volume was reduced to one- fourth of the original volume of the solvent used and stored at 4℃ in airtight bottles [9].

2.3.3. Preparation of acetone extract

10 grams of leaf powder of each *Adhatoda vasica* and *Solanum trilobatum* were soaked in 100ml of acetone in conical flask separately, plugged with cotton and kept at room temperature for 3 days and filtered through Whatman No:1 filter paper. The filtrate was evaporated in petri dish at room temperature for 2-3 days till the volume was reduced to one- fourth of the original volume of the solvent used and stored at 4℃ in airtight bottles [9]. 3ml of DMSO solvent was added to each bottle to dissolve the extracts.

2.4. Bacterial strain used for assay

The bacterial cultures used in the study were procured from the Department of Microbiology, RMMCH, Annamalai University, Tamilnadu. The organisms used were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Streptococcus pyogenes, Klebsiella pneumoniae, Haemophilus influenzae.*

The strains were maintained on nutrient agar slant at 4℃ and sub cultured on nutrient agar slant and incubated at 37℃ for 24 hours before doing antimicrobial susceptibility test.

2.5. Agar disc diffusion method

The inoculation of microbes was prepared from bacterial culture. About 15-20 ml of Muller-Hinton agar medium was poured in sterilized petridishes and allowed to solidify. One drop of bacterial strain was spread over the medium by swab plate method.Sterile discs were impregnated with the different concentration of solvent extracts of *Adhatoda vasica* like 25µl, 40µl and 50µl and of *Solanum trilobatum* like 25µl, 40µl and 50µl. The discs were allowed to dry and placed on the agar surface. The plates were incubated at 37℃ for 24 hours. The antibacterial activities were evaluated by measuring inhibition zone diameters at the end of the incubation period.

2.6. Phytochemical analysis

The phytochemical analysis of the methanolic plant extract was carried out to find alkaloids, steroids, flavonoids, tannins, saponins and glycosides.

3. Results

The bacterial cultures *viz*., *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Streptococcus pyogenes, Klebsiella pneumoniae, Haemophilus influenzae* procured from the Department of Microbiology, RMMCH, Annamalai University were tested against the ethanol, methanol and acetone extracts of *A.vasica* and *Solanum trilobatum* at different concentrations like 25µl,40µl,50µl concentrations.

3.1. Antibacterial Activity of *Adhatoda vasica* Against Clinical Pathogens:

Methanolic extract of *Adhatoda vasica* showed maximum antibacterial activity against all the pathogens *viz*., *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Streptococcuspyogenes, Klebsiella pneumoniae, Haemophilus influenzae* than the ethanol and acetone extracts except *Staphylococcus aureus*. Among all the organisms, maximum antibacterial activity was exhibited against the organisms in the order *Proteus vulgaris, Staphylococcus aureus* and *Klebsiella pneumoniae*. However, ethanol extract of *Adhatoda vasica* showed maximum antibacterial activity against *Staphylococcus aureus* than the other pathogens.

Table-1: Antibacterial Activity of *Adhatoda vasica* Against Clinical Pathogens:

SL. No.	PATHOGENS	ZONE OF INHIBITION (mm)								
		Methanol			Ethanol			Acetone		
		25µl	40µl	50µl	25µl	40µl	50µl	25µl	40µl	50µl
1.	*Staphylococcus Aureus*	8±0.3	11±0.2	13±0.2	8±0.1	11±0.2	13±0.3	7±0.1	9±0.3	10±0.2
2.	*Pseudomonas Aeruginosa*	7±0.1	9±0.3	10±0.2	NZ	6±0.2	8±0.3	NZ	6±0.1	8±0.2
3.	*Escherichia coli*	6±0.1	7±0.2	9±0.2	NZ	6±0.1	7±0.2	NZ	6±0.1	7±0.2
4.	*Proteus vulgaris*	11±0.2	13±0.3	14±0.1	7±0.1	10±0.2	11±0.1	9±0.1	11±0.2	12±0.3
5.	*Streptococcus Pyogenes*	6±0.1	7±0.2	8±0.2	NZ	6±0.1	8±0.2	NZ	6±0.1	7±0.2
6.	*Klebsiella pneumoniae*	8±0.2	11±0.3	11±0.1	8±0.1	10±0.2	11±0.2	8±0.1	9±0.1	11±0.2
7.	*Haemophilus influenzae*	7±0.1	8±0.1	8±0.2	6±0.1	7±0.1	8±0.2	NZ	6±0.1	8±0.2

NZ= No Zone, ±SD

3.2. Antibacterial Activity of *Solanum trilobatum* against Clinical Pathogens

Methanol extract of *Solanum trilobatum* showed maximum antibacterial activity against all the pathogens. The methanolic extract of *Solanum trilobatum* showed maximum zone of inhibition (12±0.3mm) against *Proteus vulgaris* followed by *Klebsiella pneumoniae* (11±0.3mm) in the concentration of 50 µl. The ethanolic and acetone extract showed highest inhibition against *Staphylococcus aureus* followed by *Proteus vulgaris*.

Table-2: Antibacterial Activity of *Solanum trilobatum* against Clinical Pathogens

Sl. No.	**Pathogens**	**zone of inhibition (mm)**								
		Methanol			**Ethanol**			**Acetone**		
		25µl	**40µl**	**50µl**	**25µl**	**40µl**	**50µl**	**25µl**	**40µl**	**50µl**
1.	*Staphylococcus Aureus*	8±0.2	10±0.3	11±0.2	8±0.1	10±0.2	12±0.3	8±0.1	9±0.2	11±0.3
2.	*Pseudomonas aeruginosa*	6±0.1	8±0.2	9±0.2	NZ	7±0.1	9±0.2	NZ	6±0.1	8±0.2
3.	*Escherichia*	NZ	6±0.2	8±0.2	NZ	6±0.2	7±0.1	NZ	6±0.1	7±0.2

	Coli									
4.	*Proteus Vulgaris*	8±0.2	10±0.2	12±0.3	9±0.1	11±0.3	11±0.3	8±0.1	10±0.2	11±0.2
5.	*Streptococcus pyogenes*	NZ	6±0.1	8±0.2	NZ	6±0.1	8±0.2	NZ	6±0.1	7±0.1
6.	*Klebsiella pneumoniae*	8±0.2	9±0.1	11±0.3	8±0.1	10±0.2	11±0.2	8±0.1	9±0.2	10±0.3
7.	*Haemophilus influenzae*	6±0.1	8±0.3	8±0.2	6±0.2	7±0.3	8±0.1	NZ	6±0.2	7±0.2

NZ= No Zone, ± SD

3.4. Phytochemical analysis of Methanolic Extractof *Adhatoda vasica* leaves

The methanolic leaves extract of *A. vasica* has been analyzed for the presence of phytochemicals. From the Table-3 it can be observed that the methanolic extract of *A. vasica* leaves contain phytochemicals such as alkaloids, flavonoids, tannins and saponins.

Table-3: Phytochemical analysis of Methanolic Extract of *Adhatoda vasica* Leaves

S. No	Phytochemicals	Methanol Extract of A. vasica
1.	Alkaloids a. Dragendorff's test b. Mayer's test	 + +
2.	Steroids	-
3.	Flavonoids	+
4.	Tannis	+
5.	Saponins	+
6.	Glycosides a.Borntrager's test	 -

4. Discussion

The bacterial strains used in the present study were *Staphylococcu saureus, Proteus vulgaris, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa, Haemophilus influenzae* and *Klebsiella pneumoniae* for both the plants were taken from Department of Microbiology, RMMCH, Annamalai University and the sub-cultures were maintained at 4°C in nutrient agar slant. Zabta Khan Shinwari *et al*., 2009 [10] had used Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis,* Gram-negative bacteria, *Pseudomonas aeruginosa*, *Escherichia coli* and *S. typhimurium* against *Adhatoda vasica* and the cultures were obtained from culture library of Microbiology Laboratory of QRI. They also maintained the sub-culture at 4°C but in agar broth.

Sankar Kumar *et al*., [11] revealed that *Adhatoda vasica* showed the antimicrobial activity *against Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumoniae* which also exhibited the highest activity in methanol extract than the aqueous extract. This is an agreement with the current study (i.e.) the methanol extract of *Adhatoda vasica* exhibited high activity against the tested organisms rather than ethanol and acetone extract of *Adhatoda vasica*. In the present study, the methanolic extracts of leaves of *Adhatoda vasica* showed highest antibacterial activity against the clinical pathogens than the ethanol and acetone extracts.

Swapna Latha and Kannabiran, [12]revealed that Aqueous extract from of *S. trilobatum* leaves showed antibacterial activity against tested bacterial strains in the order of *Klebsiella* (10 mm; MIC-0.63

mg/ml), *B. subtilis* (9 mm; MIC-0.5 mg/ml), and *S. aureus* (8 mm;MIC0.5mg/ml). Methanol extracts of flowers showed inhibition over *E.coli* (11mm) and *S. aureus* (9 mm) whereas stem extract showed inhibition only against *S. aureus* (11 mm)**.** In the present study, Methanol extract of *Solanum trilobatum* showed maximum antibacterial activity against all the bacterial strains.

Mohmed Yusuf and Shafat Ahmad Khan, [13]had analyzed the leaves extracts (ethanol, acetone, ethyl acetate and petroleum ether) of *A. vasica* for the presence of phytochemicals. It can be observed that the extracts of *A.vasica* leaves, contain phytochemicals such as alkaloids, flavonoids, terpenoids, tannins and saponins. Therefore, tannins and saponins were observed in ethanol and petroleum ether extracts only. In present study, the methanolic leaves extract of *A. vasica* has been analyzed for the presence of phytochemicals. It can be observed that the methanolic extract of *A.vasica* leaves, contain phytochemicals such as, alkaloids, flavonoids, tannins and saponins.

5. Conclusion

In the present study, both *Adhatoda vasica* and *Solanum trilobatum* showed antimicrobial activity against the respiratory infection causing microorganisms. Hence there could be probability to use these extracts for the treatment of bacterial infectious diseases, as these are eco-safe and biodegradable.

Reference

1. Selvakumar, S. Preliminary phytochemical analysis of aerial parts of *Crossandra infundibuliformis,* J. Chem. Pharmaceut. Res., 2015, 7(12):784-787.
2. Ahmad, Z. Mehmood, F. Mohammad. J. Screening of some Indian medicinal plants for their antimicrobial properties.Ethnopharmacol., 1998, 183-193.
3. Yusuf, A. Ahmad, M. Shahid, M.I. Khan, S.A. Khan, N. Manzoor, F. Mohammad. Assessment of colorimetric, antibacterial and antifungal properties of woollen yarn dyed with the extract of the leaves of henna (Lawsonia inermis), J. Clean. Prod., 2012, 42-50.
4. Vethanarayanan P, Unnikannan P, Baskaran L, Sundaramoorthy P. Asi.J.Biochem. *Solanum trilobatum* (Solanaceae) - an overview, Pharma. Res., 2011, 351–361.
5. Rubinstein E, Kollef M H, Nathwani D., Pneumonia caused by methicillin resistant *Staphylococcus aureus*. Clin. Infect Dis., 2008, 378-385.
6. Tillotson JR, Lerner AM., Characteristics of pneumonias caused by *Escherichiacoli*. N. Engl. J. Med., 1967, 115-122.
7. Chopra. Indigenous Drugs of India. Academic Publishers, Kolkata, India, 1982.
8. Ramakrishnan Akshara, Chandrasekaran Elakiya, Peraman Muthukumaran and Nachimuthu Saraswathy., Comparative evaluation of antibacterial, antifungal activity and phytochemical screening of leaf and bark extract of *Solanum trilobatum* L., Journal of Chemical and Pharmaceutical Research, 2016, 198-202.
9. Harbone JB., Phytochemical Methods. London: Chapman and Hill, 1973, 17.
10. Zabta Khan Shinwari, Irfan Khan, Shamma Naz and Altaf Hussain., Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases, African Journal of Biotechnology., 2009, 7082-7086.
11. Josephin Sheeba.B and Selva Mohan. T., Antimicrobial activity of *Adhatoda vasica* against clinical pathogens, Asian Journal of Plant Science and Research, 2012, 83-88.
12. Inderjit Kaur, P.K. Chauhan, Munish Jaryal, Shubham Saxena and Kanishak., Antioxidant and Antimicrobial activity of leaf extract of *Adhatoda vasica* against the bacteria isolated from the sputum samples of Asthmatic patients, Int. J. Drug Res. Tech., 2012,273-278.
13. Swapna Latha P and Kannabiran K., Antimicrobial activity and phytochemicals of *Solanum trilobatum* Linn., African Journal of Biotechnology., 2006, 2402-2404.

SP-10

ANTICANCER ACTIVITY OF ETHANOLIC EXTRACT OF *VITIS VINIFERA* L. USING MCF7 CELL LINES

S.DHASARADHA BAI[1], A.SILPHA TENISH[2], P.G.GEEGI[3], and P.ANITHA[4]

[1] II M.Sc. Biochemistry, St. Joseph's College, Trichy
[2] M.Sc., Biochemistry, St. Joseph's College, Trichy
[3]Assistant Professor, Department of Biochemistry, St. Joseph's College, Trichy
[4]Head, Department of Biochemistry, Srimathi Indira Gandhi College, Trichy

ABSTRACT:

In spite of good advancements fordiagnosis and treatment, cancer is still abig threat to our society. In the lastfew decades, there has been anexponential growth in the field ofherbal medicine. The aim of thepresent study is to evaluate theanticancer activity of Vitis viniferaagainst MCF 7 breast cell line.100gof Vitis vinifera pulp extract wasmixed with 300 mL of ethanol. Pasteform of the extract obtained by filtration was subjected to pre-clinical screening. The in vitro cytotoxicand antitumor potentials of GPE wasassessed by tryphan blue dye exclusionmethod and MTT assay method. Thecytotoxicity was estimated by LDHassay. From the result, it wasobserved that Grape Pulp Extract – GPE expressed goodcytotoxic and antiproliferatingactivities against MCF-7 cell lines. Therefore, it would of significanceto investigate in future about GPEwhich have a wide implication incancer chemo preventions.

KEYWORDS-Vitis vinifera, MCF 7 breast cell line,MTT assay,LDH assay,GPE

INTRODUCTION:

In spite of good advancements for diagnosis and treatment, cancer is still a big threat to our society[48]. This is the second most common disease after cardiovascular disorders for maximum deaths in the world[44]. It accounts for about 23 and 7% deaths in USA and India, respectively. The world's population is expected to be 7.5 billion by 2020 and approximations predict that about 15.0 million new cancer cases will be diagnosed. The prevalence of cancer in India is estimated to be around 2.5 million with about 8,00,000 new cases and 50,000 deaths per annum[49].During the last one decade, about 70% cancer cases have been diagnosed and treated with survival of a few patients[49] It is believed that in the near future the number of cancer patients will increase in the developing and under developed countries, which may rise up to 70% which is a serious issue for all of us. Most frequently observed cancers in Indian population are of lungs, breast, colon, rectum, stomach and liver[49]. Nowadays, India is growing with a good progress rate and probably will become a developed country within a few decades. In view of these facts, attempts have been made to study the status of cancers in India including its causes, preventive measures, effect on Indian economy and comparison with global scenario. The chemical, biological and other environmental identities are responsible for uncontrolled and unorganized proliferation of cells (carcinogens). The causes for cancers can be both either internal factors like inherited mutations, hormones, and immune conditions or environmental factors such as tobacco, diet, radiation, and other infectious agents. A significant variation of cancer has been reported due to life styles and food habits[39]. Cancer cells differ from their normal cells in that they have abnormal regulation. As normal cells progress to a neoplastic state, they acquire the hallmark capabilities. Sustaining, proliferation, evading, growth suppressors, resisting cell death (apoptosis),enabling replicative immortality, sustained angiogenesis, activating, invasion and metastasis are the stages of cancer.

ANTIOXIDANT EFFECTS

Grape seed extract has antioxidant and free radical scavenging activity. The sparing/recycling effect of procyanidins from *V. vinifera* seeds on alpha-tocopherol was established in phosphatidyl choline liposomes and red blood cells. Procyanidines in addition to scavenging free radicals, strongly and non-competitively inhibit xanthine oxidase activity.

CELL LINES

Cell lines seem to be a key element for the molecular diagnosis in breast cancer as they can be widely used in many aspects of laboratory research and particularly, as *in vitro* models in cancer research[50]. As for breast cancer, MCF-7 cells represent a very important candidate as they are used ubiquitously in research for estrogen receptor (ER)-positive breast cancer cell experiments and many sub-clones which have been established.

MATERIALS AND METHODS:

- COLLECTION OF PLANTS: Plant source selected for the present study was *Vitis vinifera.* The pulp of the selected plant was collected from Trichy fruit market.
- PREPARATION OF PLANT EXTRACT: The pulp part of *Vitis vinifera* was coarsely grinded with mortar and pestle. 100 g of *Vitis vinifera* pulp was mixed with 300 ml of ethanol. Then it was incubated for two days, until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre- clinical screening.
- PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS: Preliminary phytochemical screenings of ethanolic extract of *Vitis vinifera* pulp were carried out as per the standard textual procedure outlined in Evans and Harborne.
- Test for Alkaloids- A quantity (0.2g) of the sample was boiled with 5ml of 2% HCl on a steam bath.
- Test for Flavonoids - A quantity (0.2g) each of the extracts was heated with 10ml of ethylacetate in boiling water for 3 minutes.
- Test for Glycosides- Dilute sulphuric acid (5ml) was added to 0.1g each, of the extracts in a test tube and boiled for 15 minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxide solution. A mixture, 10ml of equal parts of Fehling's solution A and B was added and boiled for 5 minutes. A more dense red precipitate indicates the presence of glycoside.
- Test for Saponins - A quantity (0.1g) each of the extracts (aqueous and n-hexane) was boiled with 5ml of distilled water for 5 minutes.
- Test for Proteins - A quantity (5ml) of distilled water was added to 0.1g each, of the extracts. This was left to stand for 3 hours and then filtered. To 2ml portion of the filtrate was added 0.1ml Millon's Test for Fats and Oil - A quantity of 0.1g each of the extracts was pressed between filter paper and the paper observed. A control was also prepared by placing 2 drops of olive oil on filter paper. Translucency of the filter paper indicates the presence of fats and oil.
- Test for Phenols - To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added. Formation of blue color indicates the presence of phenols in the sample extract.
- Test for Carbohydrates - Take 1 ml of extract and heated and then add few drops of Benedict's reagent. Formation of orange red color indicates the presence of carbohydrates in the sample extract.

IN-VITRO CYTOTOXICITY ASSAY--TRYPAN BLUE

Short- term *in-vitro* cytotoxicity was assessed using MCF7 cell lines by incubating different concentrations of the ethanolic extracts of plant materialat 37°C for 3 hours. The cells underwent trypsinization and then washed in PBS and the cell number was determined using a haemocytometer and adjusted to $1x10^6$cells/ml. different concentrations of the extracts (100-1000 µg/ml) used for the cytotoxicityassay and the final volume was adjusted. Control tubes were maintained with the DMSO and tumor cells but without the plant extract. All the tubes were incubated at 37^0c for 3 hours. After incubation 0.1ml of 0.2% tryphan blue dye in isotonic saline was added to a watch glass along with 0.1ml of test sample and the number of viable (unstained) and non-viable (stained) cells were counted using haemocytometer.

MTT ASSAY

The MTT Cell Proliferation and Viability assay is a safe, sensitive, in vitro assay for the measurement of cell proliferation. When metabolic events lead to apoptosis or necrosis, there is a reduction in the cell viability. Cells were cultured in flat-bottomed, 96-wellplates. The tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) is added to the wells and the cells are incubated. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals. Detergent is then added to the wells, solubilizing the crystals so the absorbance can be read using a spectrophotometer. The optimal wavelength for absorbance is 570 nm. The rate of tetrazolium reduction is proportional to the rate of cell proliferation. Increasing concentrations of ethanolic extracts of plant material were added to the cells

and incubated at 37ºC for 24hrs in CO_2 incubator with 5% CO_2. The samples were drawn every 2hrs and observed the reading at 570nm. Each experiment was conducted in triplicate form. The average was calculated, and compared with the control test samples. The percentage growth inhibition was calculated.

ESTMATION OF LACTATE DEHYDROGENASE RELEASE ASSAY

Lactate dehydrogenase catalyses the conversion of pyruvate to lactate; NADH is oxidized to NAD in the process. The rate of decrease in NADH is directly proportional to the LDH activity and is determined photometrically.Lactic dehydrogenase activity is spectrophotometrically measured in the culture medium and the cellular lysates at 340 nm by analyzing NADH reduction during the pyruvate lactate transformation. Cells are lysed with 50 mM 0.1ml Tris-HCl buffer, pH 7.4 + 0.1ml 20 mM EDTA + 0.1 ml of 0.5 % Sodium Dodecyl Sulfate (SDS), and centrifuged at 13,000 X g for 15 min. The precipitate obtained was added with 33 µl of the ethanolic extracts of Plant material(50-250 µg /ml in 48 mM PBS), 0.2ml of 1 Mm pyruvate and 0.2ml of 0.2 mM NADH. After 15 min incubation, reduction in NADH was observed at 340 nm in UV **spectrophotometer.**

RESULT AND DISCUSSION:

Preliminary phytochemical indicates the presence of Tannin, Terpene, Coumarin, Alkaloid and Sugar.

TEST	INFERENCE	INTENSITY
ALKALOIDS **1) Dragendorff's reagent**	**Alkaloids present**	
2)Mayer's reagent		
3)Picric acid solution 1%		
FLAVANOIDS **1) Ammonium test**	**Flavanoids present**	
GLYCOSIDES	**Glycosides**	
CARBOHYDRATES	**Carbohydrates present**	+
SAPONIN	**Saponin present**	++
TANNINS	**Tannins present**	++
PROTEIN	**Protein**	
OILS		
PHENOLS		

CYTOTOXIC POTENTIAL OF PLANT EXTRACT AGAINST MCF-7 CELL LINES - TRYPAN BLUE DYE EXCLUSION METHOD

Concentration (µg/ml)	Death cells (%)
Control	-
100	8.1
250	12.7
500	25.7
750	34.1
1000	42

CYTOTOXIC POTENTIAL OF GRAPE PULP EXTRACT AGAINST MCF – 7 CELL LINES – MTT ASSAY METHOD

Concentration (µg/ml)	% of Cytotoxicity
15.62	9.2
31.25	17.5
62.5	31.9
125	38.1
250	52.2

CYTOTOXIC POTENTIAL OF GPE AGAINST MCF – 7 CELL LINES – LDH ASSAY EXCLUTION METHOD:

Concentration (µg/ml)	% of Cytotoxicity
15.62	47.43
31.25	48.32
62.5	61.24
125	79.06
250	83.74

SUMMARY AND CONCLUSION:

Grape is one of the finest fruits and the healthiest food, have the anticancer activity. Grapes are a rich source of vitamins and minerals that can contribute to a balanced healthy life and it is a good source for minerals like calcium and iron and vitamins A, C and B. The flavonoids present in grapes act as antioxidants and reduce the damage caused by full radicals. Due to its medicinal properties, grape juice is popularly known as 'nectar of the gods'. In this study the in *vitro* cytotoxic and antitumor potentials of GPE was assessed by tryphan blue dye exclusion method and MTT Method. The cytotoxicity was estimated by LDH release assay. Tryphan blue method showed the progressive reduction in all cell viability in all the MCF - 7cell lines with increasing concentration of ethanolic extract of grape pulp. In MTT assay a formation of formazone leads to viability of cancer cell is decreased with increasing concentration GPE. MTT reduction assessing the functional metabolic activity of mitochondria based on the enzymatic reduction of a tetrazolium salt by mitochondrial dehydrogenases of viable cells, while tryphan blue is based on cell membrane integrity. LDH has been widely used to evaluate the presence of damage and toxicity of tissue and cells. This enzyme-release cytotoxicity assay is convenient, inexpensive, and precise and should be applicable to the study of other cytotoxicity reactions. From this result, it was observed that GPE expressed good cytotoxic and antiproliferating activities against MCF - 7cell lines. In conclusion, the pharmaceutical industry develops the drugs with the consideration of important characteristics such as solubility, membrane permeability, metabolic stability and systemic pharmacokinetics and pharmacodynamics.

ACKNOWLEDGEMENTS:

I place a record of sincere thanks to our guide Mr. P.G. GEEGI, M,Sc., M.Phil, PGDBI, SET.,Asst. Prof of the Department of Biochemistry, St. Joseph's College, for providing valuable insights and inspiring guidance.

REFERENCE:

1) Adriana Dalpicolli Rodrigues a, Thamiris Becker Scheffel, Neuroprotective and anticonvulsant effects of organic and conventional purple grape juices on seizures in Wistar rats induced by pentylenetetrazoleNeurochemistry International, 2012; 60: 799–805
2) Adriana D. Rodrigues, Thamiris B. Scheffel, Purple grape juices prevent pentylenetetrazol-induced oxidative damage in the liver and serum of Wistar rats Nutrition research, 2013; 33:120-125
3) Ana Bucic´-Kojic, Mirela Planinic, Srec´ko Tomas, Lidija Jakobek & Marijan S, Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. International Journal of Food Science and Technology 2009; 44:2394–2401
4) Anand P, Ajaikumar BK, Sundaram C, Kuzhuvelil BH, Sheeja TT, Oiki SL, Sung B, Bharat BA Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharm Res. 2008; 25: 2097-2116
5) Baan R, Straif K, Grosse Y (2007) Carcinogenicity of alcoholic beverages. Lancet Oncol 8, 292 3.
6) Baguley BC and Leung E: Heterogeneity of Phenotype in Breast Cancer Cell Lines. *In*: Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways (Gunduz M, Gunduz E (eds.). Rijeka, InTech, pp. 245-256, 2011.
7) Brayand F, Moller B. Predicting the future burden of cancer. Nat Rev Cancer. 2006; 6: 63–74.

8) Brenes. B, A. Viveros, I. Goni, *Effect of grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens,*Spanish Journal of Agricultural Research 2010; 8(2): 326-333.
9) Brisken C, O'Malley B: Hormone Action in the Mammary Gland. Cold Spring Harb Perspect Biol 2010.
10) Burdall S, Hanby A, Lansdown MR and Speirs V: Breast cancer cell lines: friend or foe? Breast Cancer Res *5*: 89-95, 2003.
11) Caroline Dani, Lívia S. Oliboni Antioxidant Activity and Phenolic and Mineral Content of Rose Grape Juice, Journal of medicianal food, 2009; 12 (1): 188–192
12) Cheng. M, H.Q.Gao, L.Xu, B.Y.Li, H.Zhang, X.H.Li; Cardioprotective effects of grape seed proanthocyanidins extracts in streptozocin induced diabetic rats.J.CardiovascPharmacol, 50, 503-509, (2007).
13) Cheng G, Li Y, Omoto Y et al.: Differential regulation of estrogen receptor (ER) alpha and ERbeta in primate mammary gland. J Clin Endocrinol Metab 2005; 90: 435-444.
14) Clarke RB, Howell A, Potten CS, Anderson E: Dissociation between steroid receptor expression and cell proliferation in the human breast. Cancer Research, 1997; 57: 4987-4991.
15) Claudiu-Ioan Bunea, Nastasia Pop, Anca Cristina Babes*, Carotenoids, total polyphenols and antioxidant activity of grapes (Vitis vinifera) cultivated in organic and conventional systems*, Chemistry Central Journal 2012; 6 :66
16) Cooper AP: *On the anatomy of the breast*. Jefferson; 1840.
17) Cristiane L. Antioxidant effect of organic purple grape juice on exhaustive exercise, Applied Physiology of Nutrition and metabolism, 2013; 38: 1–8
18) Dani. C, L.S. Oliboni, D. Pra, D. Bonatto**1**, C.E.I. Santos**,** Mineral content is related to antioxidant and antimutagenic properties of grape juice, Genetics and Molecular Research. 2012; 211 (3): 3154-3163
19) Despina Sahpazidou, George D. Geromichalos, Dimitrios Stagos, Anna Apostolou, Anticarcinogenic activity of polyphenolic extracts from grape stems against breast, colon, renal and thyroid cancer cells, Toxicology Letters, 2014; 230: 218–224
20) Deugnier MA, Teuliere J, Faraldo MM, Thiery JP, Glukhova MA: The importance of being a myoepithelial cell. Breast Cancer Res 2002; 4: 224-230.
21) Done SJ: Preface. In Breast Cancer - Recent Advances in Biology, Imaging and Therapeutics. Rijeka, InTech, p IX, 2011.
22) Doyle, D., Hanks, G., Churney, N., Calman, K., and (2005) Oxford Textbook of Palliative Medicine 3rd ed., New York: Oxford University Press.
23) Doyle, C. Lawrence, H.K., Byers, T., Courneya, K.S., Demark-Wahnefried, W., Grant, B., McTiernan, A., Rock, C.L. (2006) "Nutrition and Physical Activity During and After Cancer Treatment: An American Cancer Society Guide for Informed Choices", American Cancer Society, 56(6): 323-353.
24) Durando M, Kass L, Piva J, Sonnenschein, Soto AM, Luque EH, Munoz-de-Toro M (2007) Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environ Health Perspect 115, 80-6.
25) En-Qin Xia, Gui-Fang Deng, Ya-Jun Guo and Hua-Bin Li, *Biological Activities of Polyphenols from Grapes,*International jounal of molecular science*,* 2010; *11*: 622-646.
26) Enrique G. Yanes, Samuel R. Gratz and Apryll M. Stalcup Tetraethylammonium tetrafluoroborate: a novel electrolyte with a unique role in the capillary electrophoretic separation of polyphenols found in grape seed extracts *Analyst*, 2000, 125, 1919–1923
27) Evans, W. C. (2002). Trease and Evans Pharmacognosy (15th edn), Elsevier Science limited, New York, pp 156-200.
28) Facino M.R, M.Carini, G.Aldini, E.Bombardelli, P.Morazzoni, R.Morelli; Free radicals scavenging action and antienzyme activities of procyanidines from*Vitisvinifera*Amechanism for their capillary protective action,Arzneimittelforschung, 44, 592- 601 (1994).
29) Gest C, Joimel U, Huang L, Pritchard LL, Petit A, Dulong C, Buquet C, Hu CQ, Mirshahi P, Lauren M, Fauvel-Lafeve F, Cazin L, Vannier JP, Lu H, Soria J, Li H, Varin R and Soria C: Rac3 induces a molecular pathway triggering breast cancer cell aggressiveness: differences in MDA-MB-231 and MCF-7 breast cancer cell lines. BMC Cancer *13*: 63, 2013.
30) Gompel A, Chaouat M, Hugol D, Forgez P: Steroidal hormones and proliferation, differentiation and apoptosis in breast cells. Maturitas 2004; 49: 16-24.

31) Gupta AK and Tandon N. Reviews on Indian Medicinal Plants. Vol 2. New Delhi: Indian Council of Medical Research; 2004.
32) Hadjiiski L, Sahiner B, Helvie M, Chan H, Roubidoux M, Paramagul C (2006) Breast masses: Computer: Aided diagnosis with serial mammograms. Radiology 240, 343- 56.
33) Hala EL-Adawi,Inhibitory effect of grape seed extract (GSE) on cariogenic bacteria Journal of Medicinal Plants Research, 2012; 6(34): 4883-4891.
34) Halkett, G., Kristjanson, L., Lobb, E., Little, J., Shaw, T., Taylor, M., Spry, N., (2012) "Information needs and preferences of women as they proceed through radiotherapy for breast cancer', *Patient Education & Counselling*" 86(3): 396-404
35) Harborne, J. B. (1973). Phytochemical Methods – A Guide to Modern Technique of Plant Analysis. Chapman and Hall
36) Hayes, S.C., Johansson, K., Stout, N.L., Prosnitz, R., Armer, J.M., Gabram, S., Schmitz, K.H. (2012), "Upper-body morbidity after breast cancer", *Cancer*, 118(8): 2237–2249Hammond EC, Horn D (1958) Smoking and death rates: Report on 44 months of follow-up of 187,783 men. II. Death rates by cause. JAMA 166, 1294-04.
37) Haslam SZ, Woodward TL: Host microenvironment in breast cancer development: epithelial-cell-stromal-cell interactions and steroid hormone action in normal and cancerous mammary gland. Breast Cancer Res 2003; 5: 208-215.
38) Hazem M.M. HassanHepatoprotective Effect of Red Grape Seed Extracts against Ethanol-Induced Cytotoxicity Global Journal of Biotechnology & Biochemistry 2012; 7(2): 30-37
39) Helbock HJ, Beckman KB, Shigenaga MK, Walter PB, Woodall AA, Yeo HC, Ames BN. DNA oxidation matters: The HPLC- electrochemical detection assay of 8- oxodeoxyguanosine and 8-oxo-guanine. Proc Nat Acad Sci USA. 1998; 95: 288-293
40) Ho SM, Tang WY, Belmonte de Frausto J, Prins GS (2006) Developmental exposure to estradiol and bisphenol increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66, 5624-32.
41) Irede Dalmolin a, Marcio A. Mazutti b, Eduardo A.C. Batista, *Chemical characterization and phase behaviour of grape seed oil in compressed carbon dioxide and ethanol as co-solven,t* Jounal for Chemical and Thermodynamics, 2010; 42: 797–801
42) Irish Cancer Society (2011) "Symptoms and Diagnosis of Breast Cancer", [online] available: http://www.cancer.ie/cancer-information/breast-cancer/symptoms-and-diagnosis [accessed 5 January 2013]
43) .Jayaprakasha G.K,T.Selvi, K.K.Sakariah;Antibacterial and antioxidant activities of grape (*Vitisvinifera*) seed extracts, Food Res.Int., 36, 117-122 (2003).
44) Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, Cancer J Clin. 2007; 57:43-66.
45) Juliana M. Prado a, Irede Dalmolin a, Natalia D.D. Supercritical fluid extraction of grape seed: Process scale-up; extract chemical composition and economic evaluation Journal of Food Engineering 2012; 109: 249–257
46) Kawakib J. sadd, Phytochemical investigation of fruits and seeds (Vitis vinifera) grown in Iraq,International Journal of pharmacutical science research and review, 2017; 42(2): 65-66
47) Keith W. Singletary and Brandon Meline*, Effect of Grape Seed Proanthocyanidins on Colon Aberrant Crypts and Breast Tumors in a Rat Dual-Organ Tumor Model*, nutrition and cancer, *39*(2): 252–258
48) Kotnis A, Sarin R, Mulherkar R. Genotype, phenotype and cancer: Role of low penetrance genes and environment in tumor susceptibility. Journal of biological science. 2005; 30, 93-102.
49) Nandakumar A. Consolidated report of the population based cancer registries. National Cancer Registry Programme. Indian Council of Medical Research, 1990-96; New Delhi, India.
50) Marjan Nassiri-Asl1 and Hossein Hosseinzadeh Review of the Pharmacological Effects of Vitis vinifera (Grape) and its Bioactive Constituents an Update Phytotherapy research, 2016; 30: 1392–1403

SP-11

ASSESSMENT OF GENETIC DIVERSITY OF *STEVIA REBAUDIANA* BERTONI BY DNA FINGERPRINTING

R.VELMURUGAN AND S.R.SIVAKUMAR*

Department of Botany, Bharathidasan University, Tiruchirappalli-620 024, Tamilnadu, India
***Corresponding author: drsrsivakumar@gmail.com**

Abstract
The Present study was investigated to the analysis of RAPD-PCR *Stevia rebaudiana Bertoni*genomic DNA polymorphism variation. A total of a primer were finally selected to generated RAPD fingerprints that revealed III bands, which 88 bands (80%) were found to be polymorphic bands. Five accessions were clustered in to two major groups in the dendrogram. The present results suggested that there was strong correlation between the variables in DNA polymorphism data. The present study concluded that the results of the genetic diversity could be used to select best accessions for planting and conservation of the *Stevia rebaudiana* pharmaceutical applications.

Key words: *Stevia rebaudiana,*RAPD-PCR,polymorphism.

Introduction

Genetic and environmental factors and their interactions affect the pharmaceutically important secondary metabolites in medicinal plants. A variety of environmental factors, such as season ,altitude, radiation, and soil nutrients, have been proven to significantly influence the secondary metabolite profile [5]. Successful management and conservation of natural population depend on accurate assessment of genetic diversity to address all questions regarding genetic relationships among individuals as well as levels and structure of genetic variation[1].DNA markers are used to assess genetic diversity at various levels of taxon –species, inter and intro population and progeny. At the species level, the know of genetic diversity helps understand the features which make it unique and distinct from other species diversity studies at this level help in ex situ conservation programs. Different types of molecular markers have been used to ascertain DNA polymorphisms. One of the most efficient molecular methods in terms of ability to produce abundant polymorphic DNA (RAPD) technique it is a PCR based technology, a simple and cost-effective tool for analysis of plant genome it is technically least demanding and offer a fast method for analysis of plant genome it is technically least demanding and offer a fast method for providing information from a large number of loci, particularly in species where no study has previously been undertaken. RAPD is being widely used in various areas of plant research and it has proved to be a valuable tool in studying inter and intra-specific genetic variation patterns of gene expression, and identification of specific gene [6]. The first linkage map of *steiva* has been constructed based on RAPD makers by Yao *et al*., (1999) [12] Influence of genetic variation on morphological diversity in ten accessions of s rebaudiana in malaysia was studied by Osman and abdullateef (2011)[1]. Recently the genetic and metabolic variability in s rebaudiana among accessions of different geographical regions of india, using random amplified polymorphic DNA (RAPD) markers has been reported by chester *et al*., (2013)[3]. The combination of two methods will be highly useful for understanding the level of secondary metabolite biosynthesis and genetic variability exist within a particular medicinal plant species accessions. Therefore, it is a urgent need to investigate the variation among the different populations to relate the genetic diversity of this important antidiabetic medicinal plant. Genetic diversity of various medicinal plants was positively correlated with the level of bioactive molecules in the recent past [10, 2]. Hence it is hypothesized that an increased level of genetic diversity in medicinal plants may have influence on level of bioactive component biosynthesis. The major goal of this study was to correlate the genetic variability with the level of phytochemical content among *Stevia* accessions for commercial applications.

Material and Methods

Plant materials

A total of five accessions (Table-1) of *Stevia rebaudiana* Bertoni were collected from various locations in Tamil Nadu, India. The leaves was extracted with ethanol .Young leaves were used for DNA extraction, while excess leaf materials were stored in -80˚C for future use.

DNA extraction

Total genomicDNA was extracted from leaves, using a modified CTAB method based on the protocol of Doyle and Doyle (1990). Quality of total DNA was verified by (0.8%) agarose gel electrophoresis, visualized under UV light and image was recorded using gel documentation system (Alpha image, USA).

DNA concentration

For each sample, a series of assay were carried out to estimate the optimum DNA concentration (12-20ng/µl) for PCR amplifications. The DNA diluted with sterile TE buffer 10-15ng/µL and used for PCR amplification and stainedwith ethidium bromide.

PCR amplifications

RAPD-PCR was carried out according to willams *et al*.,(1990) and the random oligonucleotide primers were obtained from Operon Ino., CA, USA. The reaction was carried out in a volume of 20µl consisted of 2µl, 10 x PCR buffer 10 Mm Tris HCl (Ph 8.3), 50Mm KCl, 1.5Mm $MgCl_2$), 2 µl, 1,5 mM dNTPs (dATP, dGTP, dCTP, and dTTP) 250 nM random decamer primer (1.0 µl), 0.5 units of Taq DNA polymerase, 2 µl of genomic DNA (15 ng) and finally added 13 µl of sterile water. Amplifications were performed in a PCR thermal cycler under the PCR amplification profile with initial denaturation at 94˚C for 4min, followed by 40 cycles at 94˚C for 1min/37˚C for 1,30 seconds 72˚C for 2 min with a final extension step 72˚C for 7 min. After PCR cycles, loading dye was added to the amplified products. The RAPD products were separated on 1.5% (w/v) agarose gel electrophoresis in 1.5% (w/v) containing 0.5µg/ml ethidium bromide in 1x TAB buffer. Electrophoresis was performed at 60V for about 2h, until the bromophenol blue dye front had migrated to the bottom of the gel. The molecular standard used was the lambda DNA double digested by EcoRI/Hind III. The gel were visualized under UV light and photographed. Among primers, those which exhibited clear banding pattern after PCR amplification were selected for further RAPD analysis [11].

Fingerprinting data scoring and analysis

RAPD banding patterns were analyzed by biostatic type scoring. The occurrence of a specific band of amplified DNA was scored as (+) and absence as (-) for all prominent bands with in a finger print profile. Therefore, a sequence of 'p' and 'o' was generated for each primer species to form a data matrix. DNA fragments sizes were estimated by agarose gel and comparison with molecular marker. Cluster analysis dendrogram was generated by using unweighted paired group method with arithmetic average (UPGMA) based on jaccard's similarity co-efficient. The fit of dendrograms obtaind were checked by bootstrapping.

Phylogenetic tree analysis

To investigate the genetic relationship between different location of *Gymnema* plant species, DNA bands from seven accessions produced by different random primers were scored and used for construction of phylogenetic tree. The lambda DNA double digested by EcoR I/Hind III was used to determine the size of each amplified DNA fragment. RAPD fragments were assigned a DNA length and recorded as a binary matrix for each individual as presence (1) or absence (0) of a given band. Phylogenetic relationship of *S. rebaudiana* plant species was generated, using Hierarchical clustering of DARwin 5.0 software based on UPGMA method.

Results and Discussion

Genomic DNA polymorphism of S. *rebaudiana* Bertoni accessions was accessed by RAPD-PCR analysis. In a preliminary screening hundred oligonuclotide primers were used to ampilify the genomic DNA isolated from three accessions of *S. rebaudiana* plant species (L1-L5). Out of hundred primers screened, only nine RAPD primers (OPA10, OPA13, OPA16, OPB01, OPB07, OPC09, OPC15, OPC16, and OPC19), produced clear and reproducible DNA fragments among the five accessions of *S. rebaudiana* plant species. The selected primers produced 108 bands and 88 bands were showed polymorphiams. The highest number of RAPD bands was detecte

d in OPC 16 primers (16 bands) and followed by OPA 10 (13 bands) while the lowest number of DNA bands was observed with OPA 13 (4 bands) (Figure -1). The DNA finger printing profile for five accessions of of *S.rebaudiana* is showed in Table -2In OPA10 primer produced RAPD profiles have a specific 1100 bp DNA fragment in S1 accessions which was absent in S2, S3, S4 and S5 accessions. In case of OPC16 primer, S1 acccessions produced a specific DNA fragment with approximate size 1772bp, but it was not amplified in S2, S3, S4 and S5 accessions (figure -1).

Phylogenetic analysis

To investigate the genetic relationship between different locations of *S. rebaudiana*, DNA bands from five accessions produced by random primers were scored and used for construction of phylogenic tree. RAPD fragments were assigned a DNA length and recorded as a binary matrix for each individual as presence (1) or absence (0) of a given band. Phylogenetic relationship of *S. rebaudiana* was constructed using Hierarchical clustering of DARwin 5.0software based on UPGMA method (Figure -2).

A dendrogram of genetic similarities among the five populations was constructed, using the genetic distance coefficients. Based on the clustering analysis, we classified the populations into two major clusters: Group I (Accessions S1) and Group II (Accessions S2, S3, S4 and S5). The present results showed that the variation exist among the different accessions of *S. rebaudiana* which can be utilized for the production of quality herbal formulations. DNA molecular markers are useful genetic marker and hence may have diverse applications [8]. Their applications in the study of medicinal plant species would enhance the accuracy of selecting better accessions and accelerate both their commercial cultivation and industrial applications.[8]

The present study revealed that genetic relationship among five populations, using DNA polymorphism data, classified the populations into two major clusters, and found correlations among genetic makeup and place of origin of the populations. These findings may be highly beneficial for protecting genetic resources and promoting sustainable use of *S. rebaudiana* in pharmaceutical industry.

Table-1Collection locations with altitude, latitude and longitude

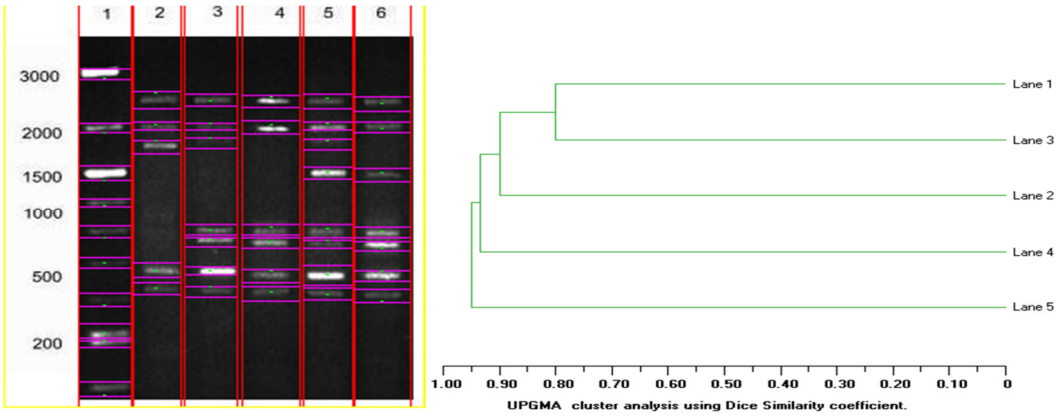
Accessions ID	Areas of the study District in Tamil Nadu	Altitude	Latitude	Longitude
S1	Horticulture Research Station (HRS), TNAU Kodikanal, Dindigul	2300 m	10˚20' N	77˚50'E
S2	Centre for siddha medicinal garden, Meetur, Salem, Dt, TN	49 m	11˚47' N	77˚48'E
S3	Horticulture Research Station (HRS), TNAU Yercurd, Salem, Dt, TN	1515 m	11˚77' N	78˚20' E
S4	Government Botanical Garden, Ooty, TN	2500m	11.4189'N	76.7114'E
S5	Kollimalai hill, Namakkal, Dt, TN	1400m	11.2485'N	78.3387'E

Table 2: RAPD primer, products, their sequences generated by random amplified polymorphic DNA (RAPD) primers in *Stevia rebaudiana* Bertoni collected from different location of Tamil Nadu

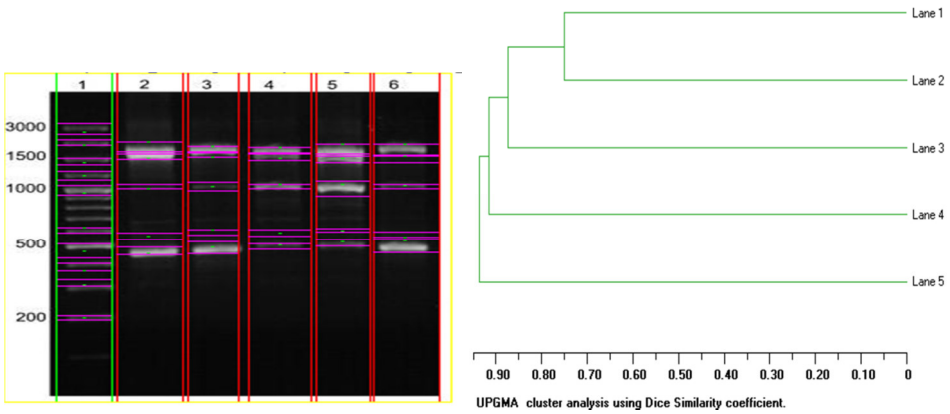
Primers	Nucleotide sequence 5 'to 3'	Total No. of bands	No. of polymorphic bands	Polymorphism	Banding range (base pair)
OPA10	GTGATCGCAG	16	13	81	2620-250
OPA13	CAGCACCCAC	9	4	44	1171-300
OPA16	AGCCAGCGAA	7	7	100	1096-250
OPB01	GTTTCGCTCC	9	7	77	2911-564
OPB07	GGTGACGCAG	12	10	83	1801-300
OPC09	CTCACCGTCC	14	12	85	2227-300
OPC15	GACGGATCAG	12	11	91	2728-250
OPC16	CACACTCCAG	20	16	80	2163-250
OPC19	GTTGCCAGCC	12	10	83	2778-250
	Total	111	90	80.44	

Figure-1: RAPD pattern generated using various selected oligonucleotide primers.

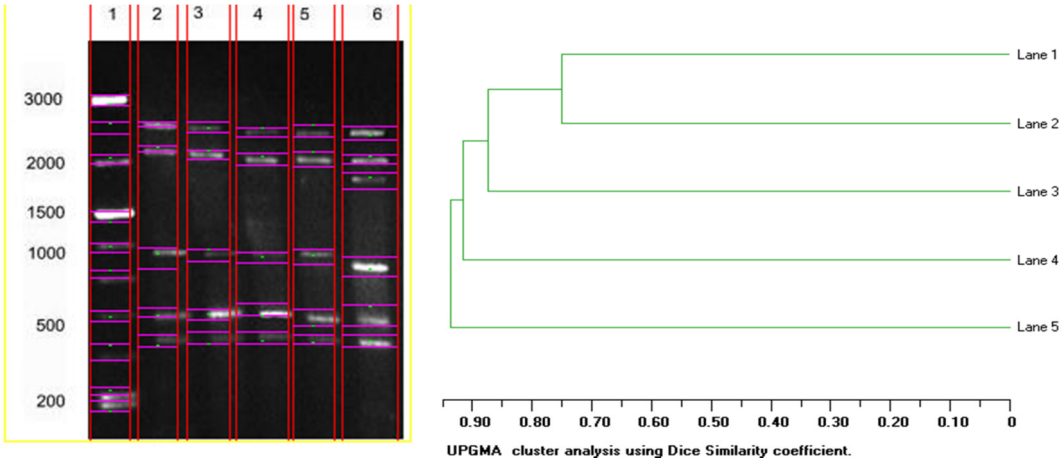
S 1-OPA16



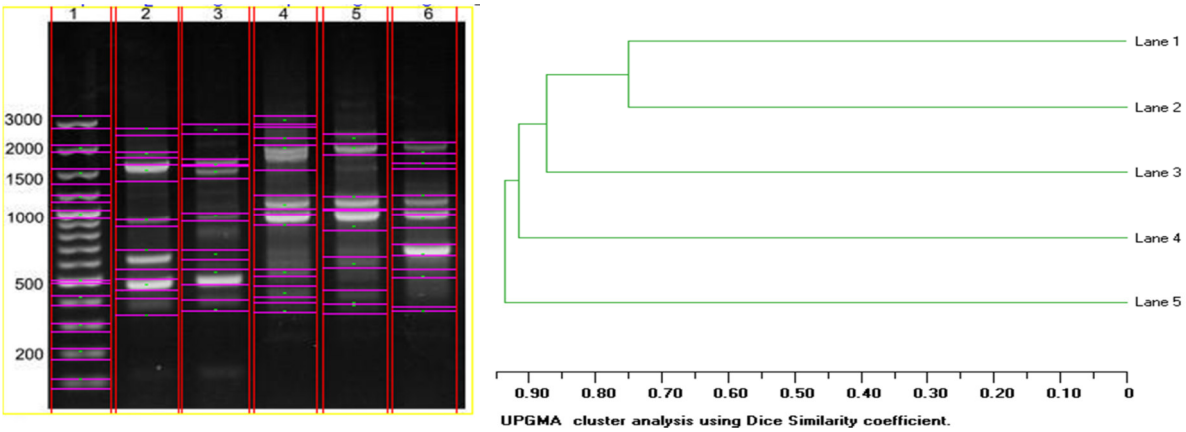
S 2-OPA10



S 3 –OPA13



Sample 4-OPC16



S 5-OPC15

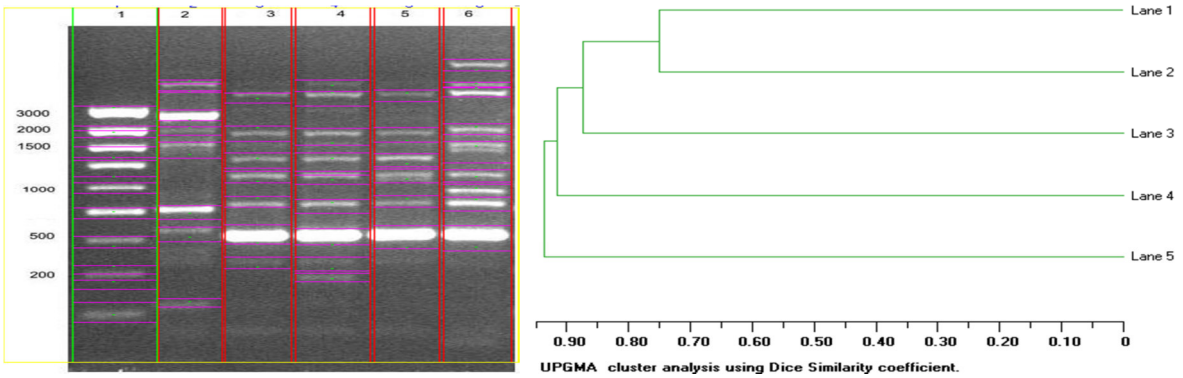
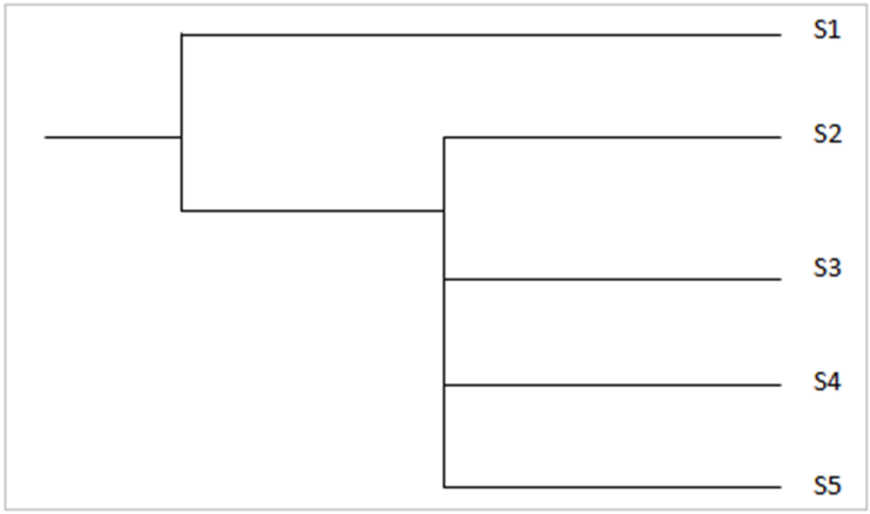


Figure 2 : Unweighted pair group method with arithmetic average (UPGMA) five showing the genetic relationship between five accessions of *S. rebaudiana* Bertoni as determined by RAPD markers



Conclusion

The present study was concluded that, genetic diversity linearly correlated to the concentration of the bioactive constituents of (stevioside) may be present in the plant sample mong the *S. rebaudiana* accessions tested, S1Kodaikanal accessions may be more suitable for cultivation and production of high yield of bioactive compound content followed by S3 Yercaud accessions. The correlation between RAPD markers indicates the potential to use RAPD analysis as a reliable method for identification and authentication of high yield accessions of pharmaceutically important medicinal plants including *S. rebaudiana* for future industrial applications.

Acknowledgement

Authors are thankful to the Dr.S.R.Sivakumar, Assistant professor in botany Bharathidasan university, Tiruchirappalli for providing the necessary facilities to carry out the research work.

References

1. Abdel-Mawgood, A.L,; Assaeed, A.M. and Al-Abdallatif, T. I. (2006) Application of RAPD technique for the conservation of an isolated population of capparis deciduas, Alex. J. Agric. Res., 51: 171-177.
2. Ali Azizi, Javad Hadian, Mansour Gholami, Wolfgang Friedt and Bernd Honermeier (2012).Correlations between genetic, morphological and chemical diversities in a germplasm collection of the medicinal plant Origanum vulgare, Chem. Biodiver., 9:2784-2801.
3. Chester, K: Tamboli, E.T,: Parveen, R, and Ahmad, S. (2013). Genetic and metabolic diversity in Stevia rebaudiana using RAPD and HPLC analysis. Pharm. Biol., 51: 771-777.
4. Doyle, J.J. and Doyle, J.L. (1990).A rapid total DNA preparation procedure for fresh plant tissue. Focus, 12:13-15.
5. Hartmann, T. (2007).From waste products to eco-chemicals: fifty years research of plant secondary metabolism. Phytochem., 68:2831-2846.
6. Kuddus, R.H,: Kuddus, N.N. and Dvorchik, I. (2002). DNA polymorphismin the living fossil Ginkgon biloba from the eastern united states. Genome, 45:8-12
7. Li, X.B. and Tu, P.F (2003).Fingerprint system of Chinese medicinal materials.Chinese Trad. Herbal Drugs, 34:385-387.
8. Liang, M.S.; Zeng, Y,; Zhou, X.; Hou, L.J. and Li, X. (2001). Genetic marker and their applications in identifiying crop cultivars. Chinese Bulletin Bot., 18:257-265.
9. Mohamad Osman and Raji Akintunde Abdullateef (2011).Influence of genetic variation on morphological diversity in accessions of *Stevia rebaudiana* Bertoni. Inter. J. Biol., 3:66-72.
10. Tihg Han, Yuan Hu, Shan-Yong Zhou, Hui-Liang Li, Qiao-Yan Zhang, Hong Zhang, Bao-Kang Huang, Khalid Rahman, Han-Chen Zheng and Lu-Ping Qin (2008). Correlation between the genetic diversity and variation of total phenolic acid contents in Fructus xanthii from different populations in china.Biomed.Chromatogr., 22:478-486.
11. Venkatachalam, P,; Lakshmi, R,; Nair Geetha, N. and Thulaseedharan, A. (2008). Genetic relationship analysis in rubber tree (*Hevea brasilliensis* Muell.Arg) by DNA fingerprinting.Plant Cell Biotech.Mole. Biol., 9:101-108.
12. Yao, Y,; Ban, M. and Brandle, J. (1999).A genetic linkage map for Stevia rebaudiana. Genome, 42:657-661.

AB-01

AN INNOVATORY AND SUSTAINABLE APPROACH FOR GREEN SYNTHESIS OF BIOPLASTIC FROM SILK INDUSTRY WASTE – A PROMISING VIABLE ECOPLASTIC ALTERNATIVE

[*]SANJAY A V, [1*]N. VIJAYAKUMAR, [2]G. RUBALAKSHMI

[*&1*] Department of Biochemistry&Biotechnology, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India.
[2] GRD Bioclinical Research, Rasipuram, Namakkal, Tamilnadu, India.
[1]*E-mail: nvkbiochem@yahoo.co.in

ABSTRACT

Plastic pollution is one of this century's major issues impacting the environmental health of our planet. The major environment concerns behind extensive synthetic plastics usage are its biodegradability, and production of toxins whiles its degradation. Bioplastics have attracted widespread attention, as eco-friendly and eco-tolerable alternative.To address these issues, the proposed work aims at the synthesis of completely biodegradable materials, by using sericin synthesized from cocoon waste respectively with nano reinforcement of montmorillonite/cellulose crystals. The product as such can find its place as a strong replacement of packaging, holding and single use plastic goods. Besides waste reduction in terms of use of cocoon waste as a source of sericin also gains significance in terms of wellbeing of the environment. Sericin a promising natural protein that could be extracted from cocoon waste is an abundant, hidden biopolymer to be exploited. Sericin is abundant non food protein featuring excellent biocompatible and biodegradable properties.The produced bio material is expected to revoltionize the bioplastic market with its versatile biodegradable plastic properties. The outcome of the proposed project is expected to provide an alternative ecofriendly biomaterial to the existing non degradable plastics.

Key words:Bioplastic, sericin, industrial waste, *Arabidopsis thaliana*.

AB-02

A MOLECULAR TRAPPING STRATEGY FOR PRODUCTION OF SAPONIN FROM *DECALEPIS HAMILTONII* AND ITS PHARMACOLOGICAL PROPERTIES - A NOVEL APPROACH

[*]D.S.DINESH RAM, [1*]N.VIJAYAKUMAR AND [2] G. RUBALAKSHMI

[* & 1*]Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India.
[2] GRD Bioclinical Research, Rasipuram, Namakkal, Tamilnadu, India.
[1*]E-mail: nvkbiochem@yahoo.co.in

ABSTRACT

The world is crammed with rich sources of medicinal plants. Medicinal plants are now getting more attention because they have potential of myriad benefits to the society. The last 50 years have witnessed a vast search of kingdom for secondary metabolites with anti-cancer, antimicrobial, antipyretic, antiulcer, antidiabetic, antioxidant, anti-inflammatory, chemoprotective, cytoprotective, insecticidal, neuroprotective and hepatoprotective activities. One such a plant is *Decalepis hamiltonii*belonging to the family *Apocynaceae,* in Ayurvedic system of medicine in India and is used in curing various diseases like stomach disorders, gastric ulcers and to stimulate appetite. Saponins are a diverse group of compounds widely distributed in the plant kingdom. Consumer demand for natural products coupled with their physicochemical properties and mounting evidence on their biological activity such as anticancer and anticholesterol activity has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors. The present study has been formulated to understand the phytochemical screening, *in vitro* antioxidant and anticancer properties elicited by saponin from roots of *Decalepis hamiltonii.*The cytotoxic properties were evaluated, on cancer cells of Breast cancer(MCF-7), lung cancer (A-549) cell lines and compare with normal cell line L-6 (Rat, Normal muscle) using MTT colorimetric assay for 24, 48 and 72 hrs.There is no systematic work that has been undertaken on this plant and this is the first report of the phytochemical screening, antioxidant and anticancer activities elicited by saponin from *Decalepis hamiltonii.*

Keywords:Saponin, Anticancer, Antioxidant, *Decalepis hamiltonii,* Medicinal plant.

AB-03

ANTIDEPRESSANT ACTIVITY OF MORIN, A FLAVONOID ON RATS INDUCED BY CHRONIC UNPREDICTABLE MILD STRESS

KIRUTHIKA R AND JUSTIN THENMOZHI A

Department of Biochemistry & Biotechnology, Annamalai University, Chidambaram-608 002, Tamilnadu, India

ABSTRACT

This study evaluates the antidepressant effect of morin against unpredicted chronic mild stress (UCMS) in rats. Depression is the leading causes of disability, affecting up to 120 million people worldwide. Chronic exposure to stressful events is an established risk factor for the development of depression in humans. Morin a natural flavanoid found in wide array of plants including white mulberry, almond, sweet chestnut, etc displays potential anti-amyloid, antianxiety, anti-tardive dyskinesic, antiparkinsonian, antioxidant, anti-hyperlipidemic, anti-diabetic, hepatoprotective, cardioprotective, nephroprotective, anticancer, antihypertensive, antiosteoarthritic and antibacterial activities. However, the anti-depressive role of morin was not investigated so far. Therefore, the present study will be carried out to examine the neuroprotective effect of morin by analyzing the neurochemical indices, oxidative stress variables, behavioral deficits and expressions of inflammatory and apoptotic markers in UCMS induced rat model of depression. At the end of the experimental period, behavioural tests will be carried out. Rats will be fasted overnight and will be sacrificed by cervical dislocation to procure hippocampus and cortex tissues. Various neurochemical, biochemical and molecular markers in the control and experimental animals will be evaluated to assess the neuroprotective effect of morin.

AB-04

A GREEN WAY GENESIS OF SILVER NANOPARTICLES FROM *INDIGOFERA TINCTORIA* AND ITS PHARMACOLOGICAL PROPERTIES - "A MOLECULAR APPROACH"

[*]R.PADMA PRIYA,[1*] T.A. ANITHA,AND [2]G. RUBALAKSHMI

[* & 1*] Department of Biochemistry, MGR College, Hosur.

[2] GRD Bioclinical Research, Rasipuram, Namakkal, Tamilandu, India

[*1]E-mail:anithasaravan@gmail.com

ABSTRACT

In India various indigeneuous system such as siddha, unani, ayurvedha, and allopathy uses number of plants to treat different ailments. Phytomedicine by definition stated that herbal based traditional medicine practice that uses various plant materials in modalties considered both preventive and therapeutic .*Indigofera tinctoria* is an valuable medicinal plant belonging to the family Fabaceae .This is the first report of the antioxidant and antithrombolytic analysis of silver nanoparticles from root of *Indigofera tinctoria*.The aim of the study was to investigate the effect of silver nanoparticles from root of *Indigofera tinctoria* by measuring the antioxidant and antithrombolytic activities. *In vitro* free radical scavenging activity was analyzed from the plant by DPPH scavenging, LPO, SO and reducing power assays. The results showed that the silver nanoparticles from root of *Indigofera tinctoria* were found to possess good antioxidant and antithrombolytic properties. The percentage inhibition is found to increase dose dependently.The findings of the present study suggest that the silver nanoparticles from root of *Indigofera tinctoria*possess excellent antioxidant and antithrombolytic potential that may be used for therapeutic purposes of various disease treatment. This study is a positive demonstration of the utility of screening South Indian endemic Fabaceae member for its food and medicinal uses.

Key words: *Indigofera tinctoria*, Free radical scavenging, Antithrombolytic, Bioinformatics.

AB-05

A GREEN APPROACH FOR PRODUCTION AND PURIFICATION OF BIO ACTIVE COMPOUND SAPONIN FROM ROOT OF *CURCUMA ANGUSTIFOLIA* AND IT'S ANTIINFLAMMATORY, MEMBRANE STABILIZING POTENTIAL

[*]R.SATHYA, [1*]ANITHA T.A, AND [2]G. RUBALAKSHMI

*** & 1* Department of Biochemistry, MGR College, Hosur.**
[2] GRD Bioclinical Research, Rasipuram, Namakkal, Tamilandu, India

***E-mail:anithasarvan@gmail.com**

Abstract

In today's world the percentage of people using chemical drugs increases with their side effects. "The boon given to our earth is the herbs". *Curcuma angustifolia* is an indigenous medicinal plant belonging to the family Zingiberaceae. This is the first report of the separation of saponin, antiinflammatory and insilico analysis of *C.angustifolia*.Saponins are a course of chemical compounds which is found in different plant species. More particularly, they are amphipathic glycosides gathered phenomenologically by the soap-like froth they create when shaken in fluidarrangements, and basically by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroid derivative. Free radical scavenging activity was measured by DPPH scavenging and LPO assays. The anti-inflammatory properties were evaluated by RBC lysis and membrane stabilization assays. It appears that the membrane stabilizing effect exhibited by the compound might be playing the signifigant role in its anti inflammatory activity.Asia is one of the most promising regions for discovering novel biologically-active substances from its flora. More efforts are needed to explore potent anti inflammatory plants from the mother earth and save humans around the world. The findings of the present study suggest that the saponin from *Curcuma angustifolia*possess excellent antioxidant potential that may be used for therapeutic purposes of anti inflammatory treatment.

Key words: *Curcuma angustifolia*, saponin, anti-inflammatory,Bioinformatics.

AB-06

ANTI CANCER ACTIVITY OF CHLOROGENIC ACID IN KB CELLS ON ORAL CARCINOMA

AKILA PALANIYANDI*, KAVIARASI EASU, MONISHA KANAGARAJ, SUGANTHI SUBRAMANIYAN.

Assistant Professor, PG and Research Department of Biochemistry
Theivannai Ammal College for Women (Autonomous), Vlluppuram, Tamilnadu, India.

ABSTRACT

Chlrogenic acid (CGA) is one of the most abundant polyphenol compounds in coffee and it also present in fruits, vegetables and plants. Intent of the present study has been made to appraise the anti cancer effect of CGA on KB oral cancer cells. We demonstrated the effects of CGA on the cell growth and apoptosis in KB cells were analyzed by the generation of reactive oxygen species (ROS), DNA damage and apoptotic morphological changes were analyzed by AO/EtBr staining, cytotoxicity effect of CGA on KB cells measured by MTT assay. Further, effect of CGA on antioxidant status of SOD, CAT, GPx and TBARS level in KB cells were also analyzed. Our results indicated that CGA induces apoptosis as evidenced by loss of tumor cell viability, enhanced ROS, lipid peroxidation results in altered mitochondrial membrane potential, increased DNA damage in KB cells. Therefore, CGA might be used as a therapeutic agent for the treatment of oral cancer.

AB-07

EVALUATION OF BIOACTIVE COMPOUNDS AND ANTIBACTERIAL PROPERTIES OF METHANOLIC SEED EXTRACT OF *HELIANTHUS GIGANTEUS*

***S.SHALINI, [1*] A. KALAIVANI AND [2] G. RUBALAKSHMI**

*** &[1]*Department Of Biochemistry,PGP College of Arts and Science, Namakkal.**
[2] GRD Bioclinical Research, Rasipuram, Namakkal.
***E-mail: tamilpts@gmail.com**

ABSTRACT

A recent report of world health organization estimate that as many as 80% of the human population depend on plant based medicines to ameliorate their health problem. A great number of plant materials and plant extracts are extensively used in present day pharmaceutical industries and the therapeutic value of the bioactive compounds are under thorough investigation .One such plant is *Helianthus giganteus*, is an indigenous medicinal plant belonging to the family Asteraceae. There is no systematic work that has been undertaken on this plant. The objective of the present study is to elucidate the phytochemical screening and antimicrobial properties of methanolic seed extract of *Helianthus giganteus*. The methanol extract of the leaves showed better antioxidant activity followed by the aqueous,chloroform and petroleum ether extracts. The methanolic seed extract of *Helianthus giganteus* tested has showed better inhibition towards *Propiniobacterium acidipropionici, Streptococcus pyogenes*, Methicillin Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the maximum effect. This study is the first report of the bioactive and antimicrobial properties of methanolic seed extract of *Helianthus giganteus*. The methanolic seed extract can be used as an effective antioxidant and antibacterial agent to combat various ailments caused by the free radicals and the bacterial species.

Key words: *Helianthus giganteus*, seeds,medicinal plant, antimicrobial, bioactive compounds.

AB-08

STRUCTURAL INVESTIGATION AND INSILICO CHARACTERISATION OF PROTEINS OF *HELIANTHUS GIGANTEUS* AND ITS ANTIHEMOLYTIC PROPERTIES- AN INDIGENOUS MEDICINAL PLANT

[*]DHANAPAL. P, [1*]TAMILMANI .P AND [2]G. RUBALAKSHMI

[*&1*] Department of Biochemistry, PGP College of Arts &Science, Namakkal.

[2] GRD Bioclinical Research, Rasipuram, Namakkal.

***E-mail: tamilpts@gmail.com**

ABSTRACT

The presence of bioactive constituents in medicinal plants plays a major role in healing as well as curing the diseases. *Helianthus giganteus* has been used in various folk medicinal preparations and its chemical composition and pharmacological activities have been elaborated recently.In the study ten proteins of *Helianthus giganteus* were analysed using different bioinformatics tools and anti thrombolytic analysis . By using the tools like Expasy Protparm server, SOPMA, SOSUI, TMHMM, structural predictions and functional characterization were done. The primary information like molecular weight ,PI, etc., were obtained by protparam and secondary Structure like prediction showed that α – helix, random coil, β – turn and extended strand obtained by SOPMA, Transmembrane protines were identified by SOSUI and TMHMM. Plants of different family showing identity 80% and above were selected and its sequences retrieved, aligned using Clustal Omega. phylogenetic tree was constructed for the aligned sequence. Homology modeling was done using Swiss model and finally Rasmol was to visualize the tertiary structure of the proteins.

Key Words: *Helianthus giganteus,* anti-coagulant, thrombolysis, Computational tools, Expasy, Swiss model, Rasmol.

AB-09

ECO-FRIENDLY SYNTHESIS OF GOLD NANOPARTICLES, CHARACTERIZATION AND ANTICANCER ACTIVITY AGAINST LUNG AND LIVER CANCER CELLS

DR. M. GOVINDARAJ*, G. SANDHRA, T. NILA, R. SIVARANJANI & K. UDHAYAKUMAR

PG & Research Department of Chemistry, Thanthai Hans Roever College (AUTONOMOUS), Perambalur-621220, Tamil Nadu, India.

***Corresponding Author. Email: mgrajchemist@gmail.com**

ABSTRACT

Tremendous growth in nanomedicine has introduced new methods for drug delivery to improve therapeutic efficiency of exotic physicochemical properties as anticancer drugs. Gold nanoparticles (AuNPs) have variety of applications in clinical translation in biomedical field. Treatment of tumors using nanoparticles is one of the most promising research arenas in the field of nanotechnology by improving delivery of anticancer agents. Green-synthesis employing emerged novel methods as a simple and alternative way to chemical synthesis of nanoparticle. Eco-friendly synthesis of AuNPs was studied using Polyscias scutellaria leaf extract. The AuNPs was formed as ruby red colour in the reaction mixture and SPR band centredat 545 nm, optimized incubation time was at 3 hours. In present study, the processes praemter were optimized to confirm the size of the nanoparticle, by energy dispersive X-ray (EDX), X-ray diffraction (XRD), Transmission electron microscopy (TEM), Scanning electron microscope (SEM) and Fourier transform infrared (FTIR) spectroscopy. EDX supports of gold nanoparticle in the synthesized nanoparticles was indicates presence. Nanoparticle morphology was analyzed by TEM shows that size ranging from 5 to 13 nm with mostly spherical in shape and SEM image confirmed with 100nm size. XRD pattern indicates crystalline nature of nanoparticles confirmed by the strong four intense peaks in XRD image. FTIR indicates the active functional groups in the culture interaction with AuNPs. Furthermore, we investigated the anticancer activities against HepG2 and A549 cells at 50 µg concentration of nanoparticles. A deeper understanding of these nanotechnologies large scaled up for development of targeted anticancer activity using gold nanoparticles AuNPs and their possible long-term effects in biological systems is needed for further clinical translation in biomedical applications.

KEYWORDS: Anticancer activity; Eco-friendly; Gold nanoparticles (AuNPs)

AB – 10

ASSESSMENT OF ANTIOXIDANT AND ANTI-CHOLINESTERASE ACTIVITY OF METHANOLIC EXTRACT OF *NIGELLA SATIVA* LINN.

S.SUDHA[1] ANDS.ARIF NISHA[2*]

[1]Ph.D Scholar, Department of Biotechnology, SrimadAndavan Arts and Science College (Autonomous),(Affiliated to Bharathidasan University), Tiruchirappalli – 600 005, Tamilnadu, India.
[2]Assistant Professor, Department of Biotechnology, Srimad Andavan Arts and Science College (Autonomous),(Affiliated to Bharathidasan University), Tiruchirappalli – 600 005, Tamilnadu, India.E -Mail: nisha.neo24@gmail.com, nisha@andavancollege.ac.in

ABSTRACT

Medicinal plants are the potential source of unlimited chemical compounds that are of great interest in pharmacological preparations. Black cumin *Nigella sativa* Linn. (Family Ranunculaceae) has been historically used for the treatment of a number of disorders, diseases in respiratory system, digestive tract, kidney and liver function, cardio vascular system and immune system. A number of pharmacological actions of *N. sativa* have been investigated in the past few decades, which includes antibacterial, anti-fungal, anti-diabetic, anti-cancer, anti-inflammatory, anti-analgesic, immunomodulatory activities. However, research on neuroprotective efficacy of *N. sativa* is still at its infancy. Therefore, the present study aims at evaluation of anti-Alzheimer's potential of *N. sativa*. The antioxidant and anti-cholinesterase activity was assessed in methanolic extract of *N. sativa*. The phytochemical constituents were analyzed by GC-MS analysis. The results of the present study suggest that the methanolic extract of *N. sativa* seeds exhibited excellent antioxidant and anti-cholinesterase activities consistofdifferent inhibitory activities.At the concentration of 500 µg/ml, *N. sativa* exhibited excellent AChEinhibitory activity of93±85.7756%.The results of antioxidant assay suggest that the extract showed noticeable hydrogen peroxide scavenging activity of 97±81.8044%,DPPH radical scavenging activity of 99 ±67.50%.The results of GC-MS analysis shows the presence of Linoleic acid in the methanolic extract, which may contribute for the above mentioned activities. Since the extract showed excellent cholinesterase inhibitory activity and antioxidant activities, upon further characterization, *N. sativa* could be a potential lead therapeutic compound for Alzheimer's disease.

Key words:*Nigella sativa*, Phytochemical screening, Antioxidant activity, Anti-cholinesterase activity, Alzheimer's disease

AB-11

BIOCHEMICAL ANALYSIS OF *CARDIOSPERMUM HELICACABUM LINN.*

ABIRAMI S*, ANNAPOORANI B, SURIYA P*,

Department of Biochemistry, Annai college of Arts and Science, Kumbakonam.
***Head, Department of Biochemistry, Annai College of Arts and Science, Kumbakonam, Tamilnadu, India. Correspondance: suriya.boichem@yahoo.com**

Abstract

Plant synthesis hundred of chemical for functions including defence against insects, fungi, diseases, and herbivorous mammals.The world health organization [WHO] notes that of 119 plant derived paramedical medicine, about74% are used in model medicine in ways that correlated directly with the traditional uses as plant medicines by native cultures. The present study, we deal with the role of *cardiospermum helicacabum* is an important medicinal plant belongs to sapindaceae family and its widely spread in tropical and sub tropical Asia and Africa in India *C.halicacabum* leaves are commonly consumed as leafy vegetable. Substance derived from the plant remain the basis for the large proportion of the commercial medication used today for the treatment of arthritis, heart disease, high blood pressure, pain, asthma, cancer and other problems.

Keywords:*C.Halicacabum*, medicinal plants, arthritis.

AB-12

ANALYSIS OF BIOCHEMICAL OF *AZADIRACHTA INDICA*

SARUMATHI S, ARUL JOTHI N, SURIYA P*

Department of Biochemistry, Annai college of Arts and Science, Kumbakonam.
***Head, Department of Biochemistry, Annai college of Arts and Science,**
Kumbakonam, Tanilnadu, India.
Correspondance: suriya.boichem@yahoo.com

Abstract

Neem oil extracted from the leaves of the neem tree and has insecticidal and medicinal properties due to which it has been used in pest control in rice cultivation. Neem (leaf and leaves) extracts have been found to have insecticidal properties. It is used as foliar spray and in rice cultivation.Neem bark and roots also medicinal properties. The abnormities in carbohydrate, fat, and protein metabolism. When ketones body is present in the blood or urine, it is called ketoacidosis, hence proper treatment should be taken immediately, else it can leads to other diabetic complications. Diabetes mellitus has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, strock and peripheral vascular disease) complications.

Keywords: Neem, medicinal plants, Diabetes mellitus.

AB-13

SCREENING OF ANTIBACTIRIAL ACTIVITY AND PHYTOCHEMICALS ANALYSIS OF ocimum sanctum LEAF EXTRACT

KIRUTHIGA.R.,

Assistant Professor, Department of Microbiology, Srinivasan College of Arts and Science, Perambalur, Tamilnadu, India.

ABSTRACT

The aim of this study was to investigate the antibacterial effects of ethanol extracts of O. sanctum against *E.coli, streptococcus sp., pseudomonas sp.*,to determine their potentials as antibacterial agent for evaluate the potentials antibacterial agents. In ethno-botanical literature of India, several hundreds of plants are used to treat many diseases and one of the most popular one is ocimum sanctum.In the traditional system of medicine, different parts (leaves, stem, flower, root, seeds, and even whole plant) of O. sanctum have been recommended for the treatment of many diseases because of enormous bioactive compounds and are also responsible for antimicrobial activity. O. sanctum leaves were powdered by using mechanical blender and quality was checked.ethanolic extract were prepared from the leaf powder by using "cold extraction method**".**Preliminary Phytochemical screening and antimicrobial test were performed by disc diffusion method inVarious concentrations like 5µg, 10µg, 15 µg, 20µg of extract.the highest zone of inhibition was found to be 20mm in *pseudomonas sp.,*hence O. sanctum to possess antibacterial property and it can be recommended as antibacterial drug instead of synthetic chemical.

Key words: *E.coli,* ocimum sanctum, ethanolic extract.

AB-14

STUDIES ON *IN VITRO* ANTI-CANCER ACTIVITY OF HERBAL PLANT *Mentha piperita*

PENNARASI MOHANRAJ[1]*, AKBARSHA MOHAMMAD ABDULKADER[2], SUTHIRA SELVAM[3], THIRUMURUGAN RAMASAMY[1]

[1,3]National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli.
[1,3]Department of Animal Science, Bharathidasan University, Tiruchirappalli.
[3]Research Coordinator,National College , Tiruchirappalli
[3]Department of Biotechnology, Bharathidasan University, Tiruchirappalli.
e-mail id:suthirabiotech@gmail.com

ABSTRACT

Lung cancer is the deadliest cancer in the world and also ranks top in our country. In Indian scenario, the number of cancer patients is doubled every year. *Mentha piperita*, a herbal plant which is also called as peppermint widely used in cooking and also used extensively in pharmaceutical industries due to its medicinal properties. The herbal extracts have antimicrobial, antioxidant properties which have been studied widely. The herbal extracts of peppermint such as methanol, ethanol and aqueous has flavonoids. It is responsible for the anti-cancer activity of a compound. The cytotoxicity of the compounds were tested in A549, lung cancer cell line and compared with L132, normal embryonic lung cell line by MTT assay. The cell viability test shows that the compounds has toxic effect against A549 cell line and has negligible toxicity against L132 cell line. The apoptosis mediated cell death occurred in A549 cell line is analysed by staining. Hence, the result shows that herbal extracts of peppermint possess anticancer property.

Key words: cytotoxicity, MTT assay, apoptosis.

AB-15

DAILY FOODS AND CANCER

V. MADHUBALA

M.Sc Biotechnology, Bharathidasan University, Tiruchirappalli- 620 024, Tamilnadu, India

ABSTRACT

Cancer is a leading cause of death worldwide and cancer deaths are projected to continue to raise with an estimated 12 million death in 2030 according to World Health Organisation (WHO). Public health officers and medical experts should collaborate toward the design of disease prevention diets for nutritional intervention. Most studies suggest a link between red meat and a higher risk of colorectal cancer. There are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. The AICR/WCRF study found these meats increase colorectal cancer risk. We take a simple survey from various hospitals,we create a datasheets and Biostatistics analysis. No single food or food component can protect you against cancer by itself. But research shows that a diet filled with a variety of vegetables, fruits, whole grains, beans and other plant foods helps lower risk for many cancers.

Keywords: World Health Organisation, AICR/WCRF, Biostatistics analysis

AB-16

IDENTIFICATION OF FRAMESHIFT AND BASE PAIR SUBSTITUTION MUTATIONS OF *SALACIA CHINENSIS* ROOT EXTRACT USING RAT LIVER MICROSOMAL ENZYMES

M. REDDI NAGESH

Part time Ph.D Scholar& Research Scientist, Diligence Bio Private Limited, Pondicherry,
Email:reddi51@gmail.com

ABSTRACT

Salacia chinensis common medicinal plant, has multiple uses in traditional system of medicine and in particular it is used as a anti diabetic agent for centuries. *S. chinensis* roots have biologically active compounds like triterpenes, phenolic compounds, glycosides which shows nemorous medicinal properties. The root extract shows various activities like antioxidant, antiobesity, antiulcer and antidiabetic. Ethanol extract of *salacia chinensis* roots has been evaluvated to for its mutagenic activity in *Salmonella typhimurium* tester strains as per the Organisation for Economic Co-operation and Development guideline.Ethanol extracts of *Salacia chinensis* was tested up to 5 mg/plate in *Salmonella typhimurium*, TA98, TA100, TA102, TA1537 and TA1535 strains along with vehicle control and appropriate strain specific positive controls both in the absence and presence of S9 mix(rat liver homogenate consists of microsomal enzymes) . The result shows that extract of *Salacia Chinensis* was not mutagenic in ames study, either in the presence and absence of metabolic activation.

Keywords: *Salacia chinensis*, ethanol extraction, Mutagenicity, *Salmonella typhimurium*.

AB-17

BACTERIAL REVERSE MUTATION ASSAY WITH SIMAROUBA GLUCA LEAF EXTRACT IN SALMONELLA TYPHIMURIUM TESTER STRAINS

SIVA SELVI. D,

Part Time PH.D Scholar&Senior Research Scientist, Vanta Bioscience Private Limted, Chennai, Tamilnadu, India
Email:sivamano80@gmail.com

ABSTRACT

Simarouba gluca, a folk medicinal plant, was evaluated for mutagenic potential via in vitro assays.The Simarouba gluca leaf extract, was evaluated in a Bacterial Reverse Mutation Assay as per OECD Guideline No. 471. "Bacterial Reverse Mutation Test" Adopted on 21st July 1997. Simarouba gluca leaf extract, was tested for its ability to induce reverse mutations at the histidine locus in tester strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537 and TA102) in the presence and absence of an exogenous mammalian metabolic activation system (S9).The test item was found to be soluble in Type 1 Milli Q water at a concentration of 5 mg/mL and no precipitation was observed at the highest tested concentration i.e.5000 µg/plate. On the basis of solubility and precipitation tests, the Cytotoxicity Test was performed at 5, 2.5, 1.25, 0.625, 0.3125 mg/plate, both in the presence and absence of a metabolic activation system. The positive controls used in the study exhibited significant increase in mean number of revertant frequency respective to their strains, indicating that the sensitivity of the tester strains towards specific mutagens and confirmed that the test conditions were adequate and that the metabolic activation system functioned properly. On the basis of the results of this study, it is concluded that Methanol extract of Simarouba glauca is non-mutagenic as it did not induce (point) gene mutations at histidine locus by base pair changes or frame-shift in the presence and absence of metabolic activation system in all five tester strains of Salmonella typhimurium TA1537, TA1535, TA98, TA100 and TA102.

Keyword: Simarouba gluca, Methanol Plant extract,, Bacterial reverse mutation assay, Salmonella

AB-18

ANTI-INFLAMMATION AND ANTI-CANCER MECHANISM OF ACTION OF SECONDARY METABOLITES FROM BORNEON BIODIVERSITY

CHARLES SANTHANARAJU VAIRAPPAN

Laboratory of Natural Products Chemistry, Institute For Tropical Biology and Conservation, Universiti Malaysia Sabah, 88450, KOTA kinabalu, Sabah, Malaysia.

ABSTRACT

Borneo, known as a biodiversity hotspot in the South East Asia, is rich with high diversity of terrestrial and marine flora/fauna. As part of our ongoing research, we have continually reported the diversity of secondary metabolites in plants and animals collected from Borneo. A total of 500 compounds were isolated and their diversity was studies interms of chemical structure, source and geographical location. These compounds were subjected to biological activity such as anti-inflammation and anti-cancer. Inflammation is a powerful innate immune system defense that is an orchestrated maneuver designed to eliminate cellular treats. Chronic inflammatory response plays an important role in cancer development and resistance to chemotherapy. Mechanism of action responsible for inflammatory diseases is not fully understood and there is a need to better understand their mechanism.In this study, we have identified novel secondary metabolites that showed potent activities in both the assays. Compounds were isolated and their structures elucidated based on spectroscopic data. Apoptosis mechanism was evaluated using Sub-G1 proportion, microscopic technique, Bax, Bcl-xl, Cleaved Capcase 3 and β-actin. In addition, we also investigated the microarray gene expression of these compounds on the clinical cancer cells to identify the genes that were up/down regulated during this study.

AB-19

ADVANCES IN MOLECULAR BIOLOGY

DR.K.SABITHA

Associate Professor, Department of Molecular Oncology, CancerInstitute, Adyar, Chennai, Tamilnadu, India

ABSTRACT

Over the last few years detection and treatment of cancer has a significant progress that leads to longer patient survival and improved quality of life.Advances from targeted therapies to immunotherapy had seen little success. Targeted therapy is a treatment that targets specific molecules in or on cancer cells such as differentially expressed genes or proteins, mutated or altered proteins or chromosomal abnormalities result in fusion gene (Fusion protein) or in the tumor's immediate surroundings. It uses materials either made by the body (Dendritic cells) or in a laboratory (Monoclonal antibodies) to improve, target, or restore immune system function. These technologies also allow us to identify new biomarkers for early detection of cancer and new methods of diagnosis.

AB-20

A BOON IN ALTERNATIVE MEDICINE FOR DIABETES: EFFECT OF ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF AN ENDEMIC INDIGENOUS MEDICINAL PLANT *COLEUS FORSKOHLII*

[1]*KABILRAJ.R, [1]R.PRAVEENKUMAR [2] G. RUBALAKSHMI,

[1*]Department of Biotechnology, Arunai Engineering College.
[2]GRD Bioclinical Research, Rasipuram, Namakkal, Tamilnadu, India.
***E-mail: kabilrajan526264@gmail.com**

ABSTRACT

Herbal medicine is also called phytomedicine or phytotherapy**.** *Ayurveda* and plant – based remedies for herbal care through day-to-day life experiences are part of the cultural heritage in India. In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their back bone. The aim of the present study is to promote a potential new herbal extracts for antioxidant and antidiabetic properties at a low cost. The objective of the present study is to elucidate the antioxidant, antidiabetic and in silico bioinformatic approaches of saponin from *Coleus forskohlii* Briq.In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs Plectranthus Amboinicus Andr. (Syn. Coleus forskohlii Briq.)is a perennial herb, belonging to the family Lamiaceae. Its tuberous roots are found to be a rich source of forskohlin (coleonol) used as a potential drug for hypertension, congestive heart failure, eczema, colic, respiratory disorders, painful urination, insomnia, and convulsions. Clinical studies of the plant further support these traditional uses, indicating therapeutic benefit in asthma, angina, psoriasis and prevention of cancer metastases. Forskolin directly activates almost all hormone sensitive adenylate cyclases in intact cells, tissues and even solubilised preparation of adenylate cyclase.The saponin extract of the plant showed better antioxidant activity. The saponin from root of *Coleus forskohlii* tested has showed better inhibition of hypoglycaemic and antidiabetic properties. This study is the first report of the antioxidant and antidiabetic properties of saponin from root of *Coleus forskohlii*. These plant extract can be used as an effective antioxidant, hypoglycemic and antidiabetic agent to combat various ailments caused by the free radicals.

Keywords: Saponin, antioxidant, antidiabetic, *Coleus forskohlii* and insilico Bioinformatics