



In Vitro Antimicrobial Activity Of Semecarpus anacardium Nuts Against human Pathogenic Bacteria.

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Abstract: *Semecarpus anacardium* is a toxic nature plant and it belongs to family Anacardiaceae. It is well known for its use in ayurveda and siddha as a potent medicinal herb since ancient times. According to Ayurveda *Semecarpus anacardium* is also named as Bhallataka and Dhobi nut. Phytochemical and pharmacological analyses of *Semecarpus anacardium* nut extracts shows a variety of bioactive compounds such as bioflavonoids, bharawanols, phenolic compounds, minerals and glycosides which shows various medicinal properties. The various extract of *Semecarpus anacardium* has been screened for phytochemicals and exhibit the presence of Phenols, Steroids, Alkaloids, Flavonoids, Saponins, Tanins and Phytosterols. Antimicrobial susceptibility was assayed by the agar well diffusion method and three concentration of plant extract were prepared in 10% DMSO. Each bacterial suspension was inoculated on Muller-Hinton agar plates. Disc of Streptomycin was used as positive control and 10% DMSO soaked filter paper disc was used as negative control. Plates were incubated for 18 hr at $35 \pm 2^\circ\text{C}$. After the incubation, the antibacterial activities of the extracts were determined by measuring the diameter of the inhibition zone around the well that was filled with the extract. The highest antibacterial activity was exhibited by the acetone extract against *Enterococcus faecalis* (IZ=27mm) and chloroform extract against *Proteus vulgaris* (IZ=23mm). Methanolic extract of nuts showed significant activity against five human pathogenic bacteria. The results revealed that various extracts of *S. anacardium* nuts showed significant antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. It provides preliminary information for further phytochemical and pharmacological analysis on the chemical constituency of the plant extracts. The results of the antimicrobial activity of various sequential extracts were in agreement with the uses of the extracts of *Semecarpus anacardium* in traditional medicine for the treatment of bacterial diseases.

Keywords : Antimicrobial, Bhallataka, Acetone Extract, Chloroform Extract, Agar Well Diffusion, In Vitro.

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1. INTRODUCTION

Ayurveda, the vedic science of life, it is a comprehensive system of natural health care which originated in India more than 5000 years ago. It is still widely used in India as a primary health care. Plants parts such as fruits, stems, flowers, spices and herbal medicines have been used to cure many diseases since ancient time. Synthetic drugs are readily available and highly effective in curing various ailments and more harmful side-effects than the traditional herbal medicines. *Semecarpus anacardium* belongs to Anacardiaceae family which contains 700 species distributed among 60 genera. It is a moderate sized deciduous tree having a height of approx. 10 to 25 meters and distributed in Sub- Himalayan region.¹ It is available in hotter regions up to the altitude of 3500ft. In India the species is distributed in the forest areas of West Bengal, Bihar, Orissa, Karnataka, Chhattisgarh, Madhya Pradesh, Tamilnadu and Maharashtra etc.² It is distributed at the outer Himalayas from Sulej to Sikkim and fairly at hotter parts of India as far as east Assam. The trees are not found under cultivation but in forests often occurring with Sal trees. It is a potent medicinal plant as 'Ardha -Vaidhya' in ayurveda³ 'Panacea'.⁴ It is commonly known as Bhallataka, Bhilwa, Biba and Dhobi nut etc. *Semecarpus anacardium* mature nuts has been classified in semi poisonous⁵ nature and found to be associated with contact dermatitis due to presence of a chemical urushiol. Ayurvedic pharmacopoeia has described the method of purification (known as shodhana) to remove the toxic substances from this plant.^{6,7} The fruit nuts of *Semecarpus anacardium* contains a number of alkaloids, flavonoids and other bioactive compounds.⁸ So many bioactive compounds mostly bharilawanols, bioflavonoids, phenolics and anacardic acid have been identified as a active constituents of *Semecarpus anacardium* nuts. Researchers reported that plant drug has antioxidative, anti-inflammatory, antiarthritic and anticancer activities.⁹ A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of problematic bacterial infections.¹⁰ Plants have natural antioxidants which is the sources of potentially safe, effective and cheap antioxidants. It has been found that *semecarpus anacardium* having polyphenolic compounds such as flavonoids possess antioxidant activity and potential beneficial effects on human health in fighting diseases.¹¹ According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs.¹² *Semecarpus anacardium* is medicinally very rich plant from the ancient time.^{13,14} The nuts of plants are used in various ailments, specially alimentary tract and dermatologic conditions. It has beneficial effect on neurological disorders, cancer, blood pressure and respiration problems.^{15,16} Many plants have been used because of their antimicrobial traits, which are due to photochemical synthesized in the secondary metabolism of the plant.^{17,18} Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties.^{19,20} A number of phototherapy manuals have mentioned various medicinal plants for treating infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infections. *Semecarpus anacardium* seeds have high priority and applicability for treating various ailments like asthma, nervous disability, rheumatism, epilepsy and tumors. The tribals of Simlipal biosphere reserve use it for curing headache, hydrocoel, anti-inflammatory, immunomodulatory and as

antiseptic²¹. The study evaluates the phytochemical screening of different sequential extraction and to determine the antibacterial activities of crude extracts of petroleum ether, chloroform, acetone, ethanol and methanol against human pathogenic bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* by using the agar well diffusion method and results have been shown in the zone of inhibition.

2. MATERIALS AND METHODS

2.1 Plant collection and Authentication.

Semecarpus anacardium was collected from the Jhargram forest area at West Bengal in the month of November to December. Plant materials was identified taxonomically by expert taxonomist at the Herbarium, Department of Botany and Forestry, Vidyasagar University, Midinapur, West Bengal. The collected sample specimen has been deposited in the institution herbarium for future reference. An authentication no. RUBL211734 from herbarium of Department of Botany, University of Rajasthan, Jaipur was received for the specimen. Plant material was washed with distilled water and then shade dried. Dried plant material was ground into fine powder using a mechanical grinder and kept in an air tight container for further analysis.

2.2 Preparation of plant extract.

20 gm plant material were soxhlet extracted sequentially with Petroleum ether, Chloroform, Acetone, Ethanol and Methanol for 72 hrs. Each of the resultant extract was filtered, dried in vacuo and weighed to calculate the extract value (%) on dry weight basis.²² The extracts were resublimized with 10% DMSO and stored at 4°C in air tight bottles for further studies.

2.3 Phytochemical screening

Phytochemical screening of the extracts were carried out by standard procedure.²³⁻²⁴

2.4 Bacterial strains

For the experimental purpose first of all prepare a pure cultures of the bacterial strains viz. Gram-negative bacteria such as *Pseudomonas aeruginosa* (MTCC733), *Proteus vulgaris* (MTCC744), *Klebsiella pneumonia* (MTCC109); Gram positive bacteria such as *Staphylococcus aureus* (MTCC0087), *Enterococcus faecalis* (MTCC439) were procured from the Institute of Microbial Technology, Chandigarh, India. The bacteria were subcultured on Muller Hinton Agar medium.

2.5 Preparation of Inoculums.

Staphylococcus aureus, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* cultured on Blood agar plate and kept for 24 hrs at 37°C. BAP is popular medium capable of growing a range of microorganisms of clinical significance. The medium have given good colonial appearances, haemolysis patterns and pigment production. Inoculum size of bacteria was adjusted using McFarland turbidity standard as reference.²⁵ The bacterial suspension prepared in peptone water compared to 0.5 McFarland Turbidity standards.

2.6 Antimicrobial Susceptibility Assays.

Antimicrobial susceptibility was assayed by the agar well diffusion method.²⁶ Three concentrations (2 µg/ml, 5 µg/ml and 10 µg/ml) of plant extract were prepared in 10% DMSO. Each bacterial suspension was inoculated on Muller-Hinton agar plates and the plates were then allowed to dry for 5 to 10 minutes. A disc of Streptomycin was used as positive control

and 10% DMSO soaked filter paper disc was used as negative control. Plates were incubated for 18 hr at 35±2°C. After the incubation, the antibacterial activities of the extracts were determined by measuring the diameter of the inhibition zone around the well that was filled with the extract. The plant extracts having antimicrobial property inhibit bacterial growth in the media surrounding the wells and thereby yielded a clear distinct area that are defined as the zone of inhibition which were expressed in millimeter.

$$\text{Activity Index} = \frac{\text{Inhibition zone produced by extract}}{\text{Inhibition zone produced by standard}}$$

2.7 Minimum inhibitory concentration

The antibacterial assay was carried out by serial microdilution method in order to determine the antibacterial activity of methanol and ethanol extracts of *S. anacardium* against human pathogenic bacteria.²⁷⁻²⁸ The bacterial suspension were adjusted with sterile saline to a concentration of 1.0×10^7 cfu/ml. All experiments were repeated thrice. The ethanol and methanol extracts of the plant nuts were tested for determining the minimum concentration effective in showing inhibition of any visible growth of bacteria termed as the minimum inhibitory concentration. For these 5 different concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml) of extract has been prepared and tested for the exhibiting zone of inhibition around them.

2.8 STATISTICAL ANALYSIS

Experimental results are expressed as the inhibition zone

(mm) including the diameter of the well. All measurements were replicated three times. Activity index of different extracts were showed in comparative graphs. MIC determination of Methanol and Ethanol samples of *S. anacardium* nuts at concentration ranging from (6.25 to 100 mg/ml) exhibited in Table 3. Photographs of antimicrobial activity of different extracts of nuts against various pathogenic bacteria depicted in figure 2.

3. RESULTS.

3.1 Phytochemical screening

The results of phytochemical screening of pet-ether extract, chloroform extract, ethanol extract and methanol extracts of nuts depicts the presence of bioactive compounds like alkaloids, flavonoids, saponins, phenolic compounds, phytosterols, glycosides, triterpenoids, steroids and tanins. The results have been shown in Table 1.

Table 1: Phytochemical screening of nut extract of <i>Semecarpus anacardium</i> .				
Phytoconstituents	Pet Ether extract	Chloroform extract	Ethanol extract	Methanol extract
Alkaloids	-	-	-	+
Flavanoids	-	-	+	+
Saponins	-	-	-	+
Phenol	+	-	-	+
Triterpenoids	+	-	-	+
Phytosterols	-	-	+	+
Glycosides	-	-	-	+
Steroids	+	+	-	+
Tanins	-	-	-	+
Fixed oil & fats	+	-	-	-
Ascorbic acid	+	+	+	+

(+) = Present, (-) = Absent

The results exhibited, that presence of primary and secondary metabolites may be responsible for curative agent for various ailments. So the primary tests may be helpful in determining the bioactive principles and may lead to the drug discovery and development. The antimicrobial activity of different extracts of *Semecarpus anacardium* nuts viz. pet ether extract, chloroform extract, acetone extract, ethanol extract and methanol extract were tested against five different pathogenic bacteria. The results observed on antibacterial activity of different extract of *Semecarpus anacardium* nuts at two different concentration (5 and 10 µg/ml) were summarized in Table 2. Fig. 1 and 2 showed a comparative graph of activity index of various extracts and

photographs of zone of inhibition in mm respectively. The results revealed that methanolic extracts showed the maximum activity against *Staphylococcus aureus* (IZ=23mm) at 10 µg/ml concentration. Acetone extracts exhibited maximum antibacterial activity against *Enterococcus faecalis* (IZ=27mm) at 5 µg/ml concentration. Both methanolic extract and acetone extract showed moderate inhibitory activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* at 5 µg/ml concentration. Chloroform extract showed maximum activity against *Proteus vulgaris* (IZ=23mm) at 5 µg/ml concentration. Pet ether extract did not show any inhibitory activity against *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 2: Antibacterial activity of various solvent extracts has been shown in the zone of inhibition (mm).

Name of the bacteria	Zone of Inhibition and Activity Index of the extracts										
	Std.	Pet ether		Chloroform		Acetone		Ethanol		Methanol	
	IZ (mm)	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
<i>Staphylococcus aureus</i>	24	-	-	-	-	21	0.87	18	0.75	23	0.95
<i>Enterococcus faecalis</i>	18	12	0.66	-	-	27	1.5	22	1.2	23	1.2
<i>Klebsiella Pneumonia</i>	20	-	-	-	-	12	0.6	-	-	12	0.6
<i>Proteus vulgaris</i>	29	10	0.34	23	0.79	-	-	10	0.34	22	0.75
<i>Pseudomonas aeruginosa</i>	29	-	-	10	0.34	11	0.37	14	0.48	15	0.51

IZ= Inhibition zone (in mm), AI= Activity Index

The antibacterial assay of different extracts of plant nuts on observation after 24 hr incubation exhibited various zone of inhibition given in Table 2. From the above data obtained via agar well diffusion method, some of the extracts showed high inhibitory activity against growth of various pathogenic bacteria. The highest inhibitory activity has been exhibited by acetone extract, ethanol and methanol extract respectively

against *Staphylococcus aureus* and *Enterococcus faecalis*. Chloroform extract have been exhibited highest inhibitory activity against *Proteus vulgaris*. Moderate inhibition effect was also observed from pet ether extract against *Enterococcus faecalis* and *Proteus vulgaris*. So the plant was found to have least effective against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

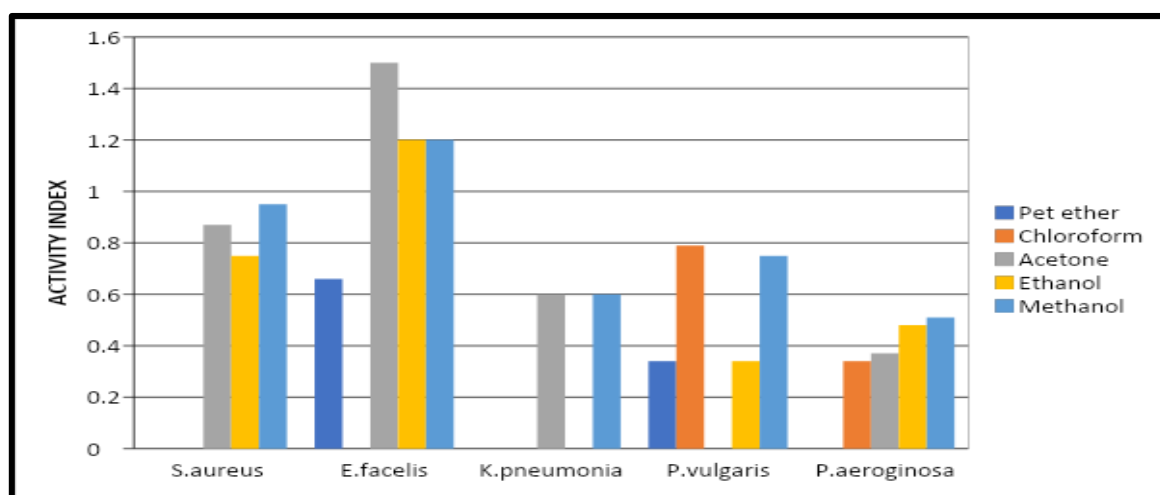
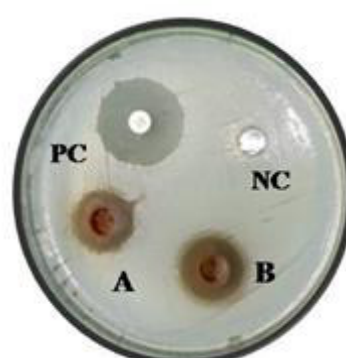


Fig 1. Comparative graph of activity index of various extracts on different bacterial strains.



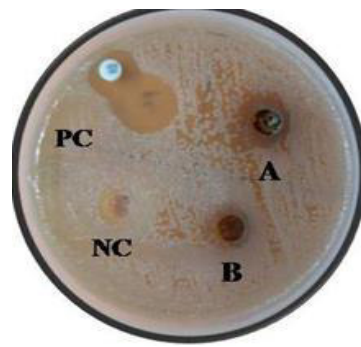
(a) Acetone extract-*Staphylococcus aureus*



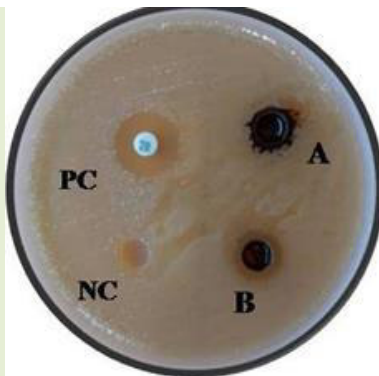
(b) Ethanol extract-*Staphylococcus aureus*



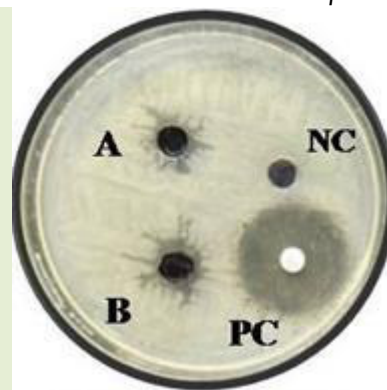
(c) Methanol extract-*Staphylococcus aureus*



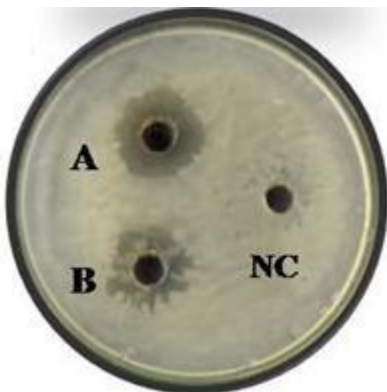
(d) Acetone extract-*Klebsiella pneumoniae*



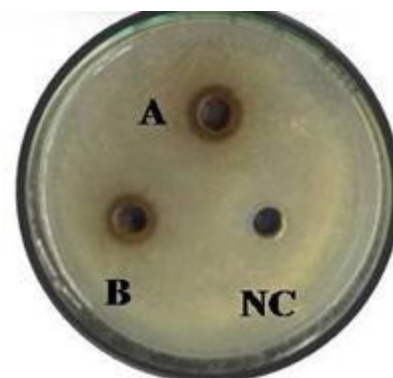
(e) Methanol extract-*Klebsiella pneumoniae*



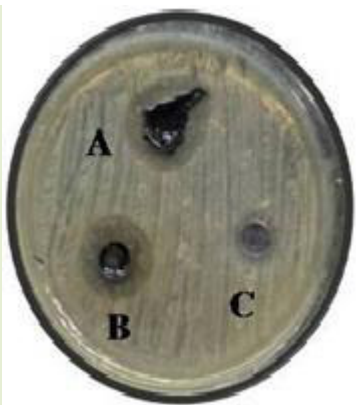
(f) Pet ether extract-*Proteus vulgaris*



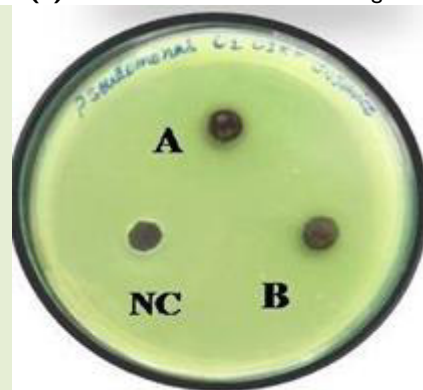
(g) Chloroform extract-*Proteus vulgaris*



(h) Ethanol extract-*Proteus vulgaris*



(i) Methanol extract-*Proteus vulgaris*



(j) Chloroform extract-*Pseudomonas aeruginosa*

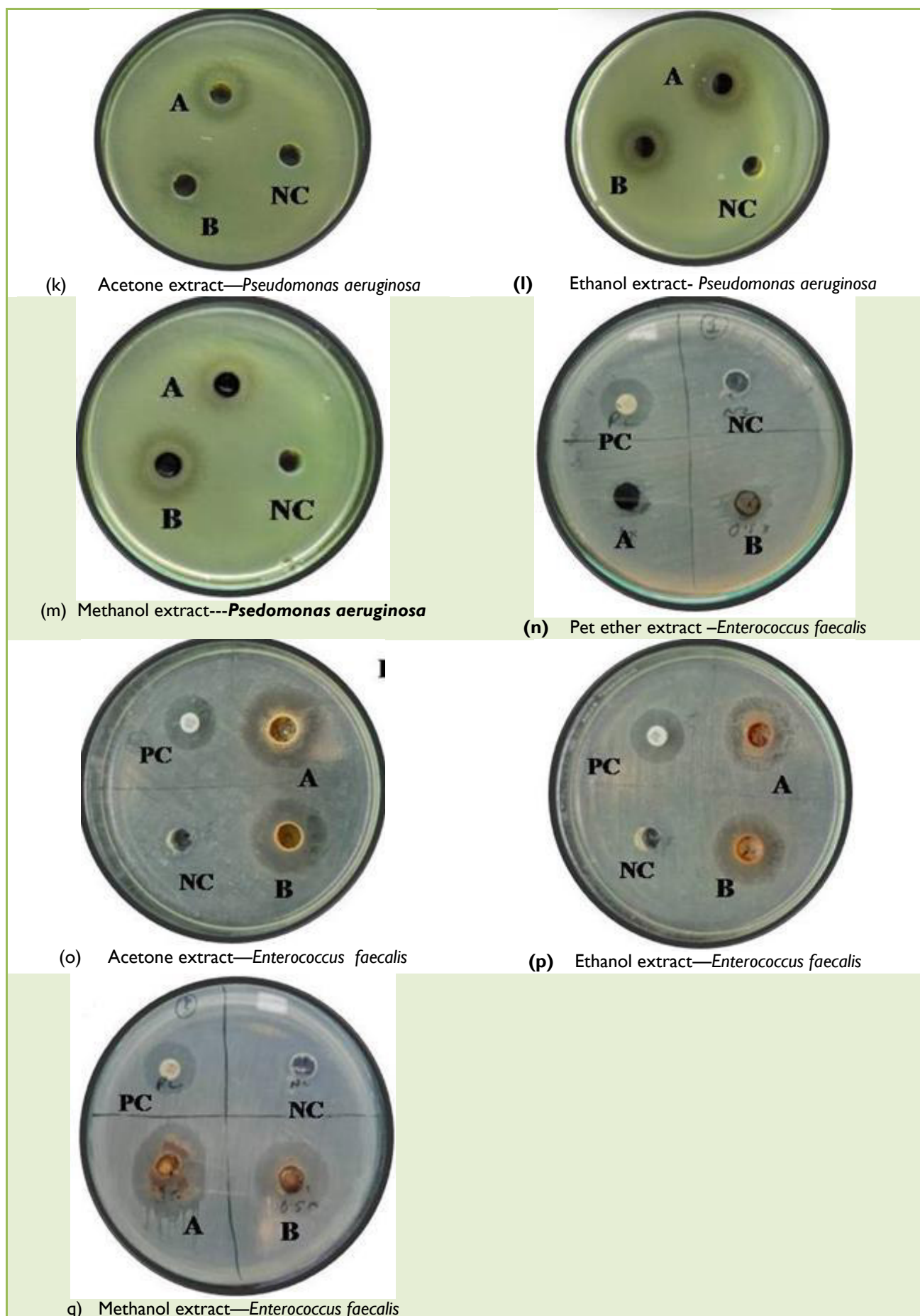


Fig 2. Photographs of antimicrobial activity of different extracts of nuts against various pathogenic bacteria: PC-Positive control (Streptomycin), NC-Negative control (DMSO), A-(5mg/ml concentration) of plant sample, B-(10mg/ml concentration) of plant sample.

Table 3: MIC determination of Methanol and Ethanol samples of *S.anacardium* nuts at concentration ranging from (6.25 to 100mg/ml)

Test Bacteria	Minimum Inhibitory Concentration (mg/ml)	
	Methanol	Ethanol
<i>S.aureus</i>	6.25	6.25
<i>P.aeruginosa</i>	12.5	12.5
<i>K.pneumoniae</i>	100	100
<i>E.facelis</i>	6.25	6.25

The methanolic extract of the nuts of *S.anacardium* showed a significant level of inhibitory potential against all bacterial strains. *Klebsella pneumoniae* has been found to be sensitive to both methanol and ethanol extract of nuts at the concentration of 100 mg/ml.

4. DISCUSSION

The invention of novel drugs from the medicinal plants is an important alternative to overcome the increasing levels of drug resistance by pathogens. The traditional Indian system of medicine can be broadly classified into the empirical forms of folk medicine which are village based, region specific, indigenous herb based, local resources based and in many cases, community-specific. The vast agroclimatic conditions of India make it an ideal place for the luxuriant growth of flora and fauna. There has been a tremendous upsurge in the demand for phytopharmaceutical, raw medicinal herbs and vegetable drugs of Indian origin from the Western nations. There is also an increase in domestic demand for raw material used for pharmacies, perfumeries and biopesticidal units. The demand for traditional herbal drugs is also increasing rapidly mainly because of the harmful effects of synthetic chemical drugs and expansion of pharmacies manufacturing natural drug formulations²⁹. Most of Indian medicinal plants are source of large amounts of drugs, which have the properties of anti-inflammatory, anti-bacterial, anti-fungal, anti-diabetic, anti-cancer, anti-hypertensive and anti-mitotic activities the antibiotic properties and have no side effects.^{30,31} Antimicrobial activity of medicinal plants and their bioactive compounds have been deliberated in the late 19th century. Antimicrobial activity and phytochemical screening of oils and nuts of *Semecarpus anacardium* has been studied by Ekta and Mohanta et al.³²⁻³³ The results of the present investigation suggested the bactericidal potential of different extracts of *Semecarpus anacardium*. The results obtained from the conducted experiment have established that the nuts of *Semecarpus anacardium* display *in-vitro* antimicrobial potential to varying extent against various bacterial strains. The antimicrobial potential was found better against methanolic extract. The methanolic extract of the plant has comparatively efficient antibacterial activities which may be due to presence of phenolic compounds and active compounds such as flavonoids and phytosterols. The results revealed that various extracts of plant nuts showed antibacterial activity against significant activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*. It provides preliminary information for further phytochemical and pharmacological analysis on the chemical constituency of the plant extracts. Analysis of different solvent nut extracts of plant demonstrated the presence of phytochemicals such as flavonoids, alkaloids, phenolics, terpenoids and glycosides. These phytochemicals have antimicrobial activity, which may contribute to the antimicrobial action of the above medicinal plant.³⁴ Phenolic compounds are toxic for microorganism,

inhibiting the enzymes which are necessary for the growth of microorganism. Phenolic compounds degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane, damage membrane protein and interfere with membrane integrated enzymes.^{35,36} From our results, petroleum ether, chloroform, acetone, ethanol and methanol extract of *Semecarpus anacardium* has successfully controlled the growth of five human pathogenic bacteria which were tested. Secondary metabolites are responsible for antimicrobial, anti fungal, antispasmodic, anticancerous activities.³⁷⁻⁴³ The results of the antimicrobial activity of various sequential extracts were in agreement with the uses of the extracts of *Semecarpus anacardium* in traditional medicine for the treatment of bacterial diseases. The increase in prevalence of multiple drug resistance has showed down the development of new synthetic antimicrobial drugs, and has necessitated the search for new antimicrobials from alternative sources. Natural compounds are a source of numerous therapeutic agents.

5. CONCLUSION

In the present study, the use of this plant as traditional drug suggest that some of the plant parts mainly nuts extracts possess bioactive components with good antibacterial properties which can be used for the development of new antimicrobial drugs. *Semecarpus anacardium* is significantly active against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The preliminary phytochemical analysis of plant nuts revealed the presence of alkaloids, flavonoids, triterpenoids, phenolic compounds, phytosterols and steroids which may be responsible for effective antibacterial activities.

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8. AUTHORS CONTRIBUTION STATEMENT

The submitted research work is guided by Dr. R.A. Sharma and laboratory research work, experiments were carried out by Dr. Alka Sharma.

9. CONFLICT OF INTEREST

The author declares no conflict of interests regarding the presented manuscript

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