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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF LEAF OF *SPHAERANTHUS INDICUS* AGAINST SOME SELECTED HUMAN PATHOGENIC BACTERIA

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ABSTRACT

The evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine. This study explores the antibacterial activity of aqueous, Methanolic extracts and oil of *Sphaeranthus indicus* against different species of bacteria like *Staphylococcus aureus, Streptococcus faecalis, and Escheriachia coli.* Anti-bacterial activity of S.indicus extract was measured by Filter paper disc method. Aqueous extract of *S.indicus* leaf showed the largest zone of inhibition (25mm) against *S. faecalis* and 22 mm zone of inhibition against *S.aureus.* The present study demonstrates that the leaves of *S.indicus* are potentially good sources of antibacterial against the pathogens *(Staphylococcus aureus, Streptococcus faecalis).* They could be used as alternatives to common anti-microbial agents for treatment of bacterial infections. Clinical Pharmacist should inform the clinicians about these adverse reactions during each administration time. In India, Pharm D graduates are trained to become Clinical Pharmacist in hospital.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now-a-days, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Haidan Yuan, 2016). The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like phenolic compounds which are part of the essential oils, as well as in tannin. In recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases, microbial resistance has occurred. This has forced scientist to search for newer anti-microbial substances from various sources like the medicinal plants.

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of medicinal plants and out of the 4, 00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities (Shyamala, 2012). Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease

causing microbes either singly or in combination (Dhia, 2006).

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agents, a systematic investigation was undertaken to screen the local flora for antibacterial activity of *Sphaerantus indicus.* The leaves of *Sphaeranthus indicus* (Linn.) belonging to Asteraceae used traditionally in Ayurveda for hyperlipidemia, epilepsy, mental illness, jaundice, diabetes, leprosy, fever cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases, antimicrobial and AIDS. The reports showed that it is also used for hypertensive, anxiolytic, neuroleptic, immune-modulatory, anti-oxidant, anti-inflammatory, bronchodilator, anti-hyperglycemic and hepato protective. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is distributed throughout India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude (Ambavade, 2006).

MATERIALS AND METHODS

Plant material

The fresh leaves of *Sphaeranthus indicus* Linn was collected from Coimbatore district, Tamilnadu, India, identified and by authenticated by Dr. P Jayraman, Director of plant Anatomy Research Centre, and Chennai. A voucher herbarium specimen number SCOPS/SI/CP/01&2 was also preserved in the Sanjo College of pharmaceutical studies, Palakkad. The collected leaves were dried in shade and powdered to coarse consistency in cutter mill. The powder was passed through 60 # mesh particle size and stored in an airtight container at room temperature.

Preparation of Extract

The powdered leaves were subjected to batch extraction in Soxhlet apparatus. The solvent used as Methanol and water. The powdered leaves were evenly packed in Soxhlet extractor for extraction with solvent. The temperature was maintained on an. electric heating mantle with thermostat control. Appearance of brown solvent in the siphon tube was taken as the termination of extraction. The filtrate was concentrated using a rotary evaporator at low temperature (40-45^{o}C) and pressure and percentage yield was calculated.

Test for Phytochemical Analysis

The conventional chemical tests were carried out for the Methanolic and aqueous extracts of *Sphaeranthus indicus* to identify the presence of various chemical constituents (Khandelwal, 2008).

Human pathogenic bacterial species

The human pathogenic bacteria such as *Staphylococcus aureus, Streptococcus faecalis. Escheriachia coli,* were obtained from Vivek Laboratory, Coimbatore in Tamilnadu and were maintained in Muller – Hinton agar medium at 4ºC for experimental studies.

Composition of the medium (Muller Hinton agar medium)

Beef infusion	300 ml
Casein hydrolysate	17.5g
Starch	1.5g
Agar	10.0g
Distilled water	1 Litre

METHOD FILTER PAPER DISC METHOD[8-9]

Procedure

Emulsified the starch in a small amount of cold water poured into the beef infusion and added the casein hydrolysate and the agar. The volume was making up to 1 litre with distilled water. The constituents were dissolved by heating gently at 100^{o}C with agitation and filtered.. Adjusted the pH to 7.4 and dispensed in screw – capped bottles and sterilized by autoclaving at 121^{o}C for 20 minutes (James 1993 and Anandha narayanan, 1997).

Inoculation of Microbe

The sterilized Muller Hinton medium was heated on a water bath to melt the media. When the media was in lukewarm condition, it was poured into the sterilized Petri dish and allowed to solidify. The organisms Escherichia coli, Staphylococcus aureus, Streptococcus faecalis, were inoculated separately and poured aseptically into the sterile Petri dishes containing medium.

Preparation of sterile disc

Whitman's No.3 filter paper was punched into 5 mm disc form and they sterilized, each sterile disc was incorporated individually with 20 - 60l of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then they were stored at 4ºC.

Assay of antimicrobial activity using Disc diffusion method

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations (20, 30, 40, 50, 60µl). The filter paper discs impregnated with Methanolic, aqueous extracts and volatile oil and standard (Amikacin) were placed on the surface of the media. It was kept in an incubator for a period of 18 to 24 hours at 37^{o}C and the zone of inhibition on the growth of micro-organism was measured and the results were tabulated.

RESULTS

Preliminary phytochemical analysis

The leaf powder and various extracts such as Methanol and aqueous extract were subjected to preliminary phytochemical screening for their presence or absence of the constituents and the results were tabulated (Table 1).

Table 1. Preliminary Phytochemical Tests for Drug Powder and Methanolic and Aqueous Extracts of Leaf of *Sphaeranthus Indicus*

Test	Drug Powder	Methanol Extract	Aqueous Extract
Sterols	+	+	-
Terpenoids	+	+	-
Carbohydrates	+	+	+
Flavanoids	+	+	+
Proteins	-	-	-
Alkaloids	-	-	-
Glycosides	-	-	-
Saponins	-	-	-
Tannins	+	+	+
Mucilages	-	-	-
Volatile Oil	+	-	-

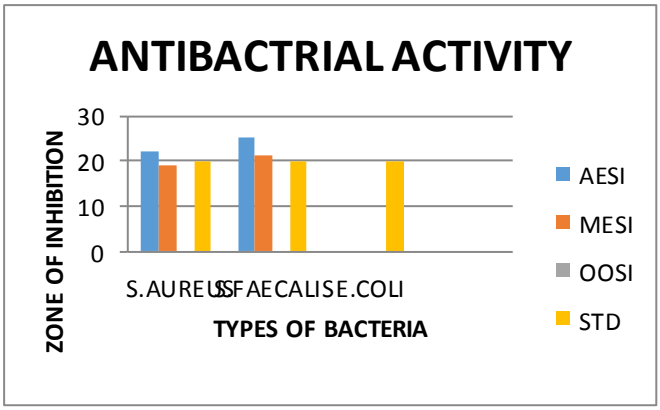
+ indicates positive reaction, -indicates negative reaction.

ANTIMICROBIAL ACTIVITY

Table: 2 - Comparison of Antibacterial Activity of Aqueous, Methanolic Extracts and Essential Oil.

S. No.	Organisms	Zone of inhibition in mm			
		Aqueous extract	Methanolic extract	Essential oil	Standard (10µg/ disc)
1.	Staphylococcus aureus	22mm	19mm	R	20mm
2.	Streptococcus faecalis	25mm	21mm	R	20mm
3.	Escheriachia coli	R	R	R	20mm

Figure- 1: **Comparison of Antibacterial Activity of Aqueous, Methanolic Extracts and Essential Oil**



AESI- Aqueous extract of *Sphaerantus indicus*

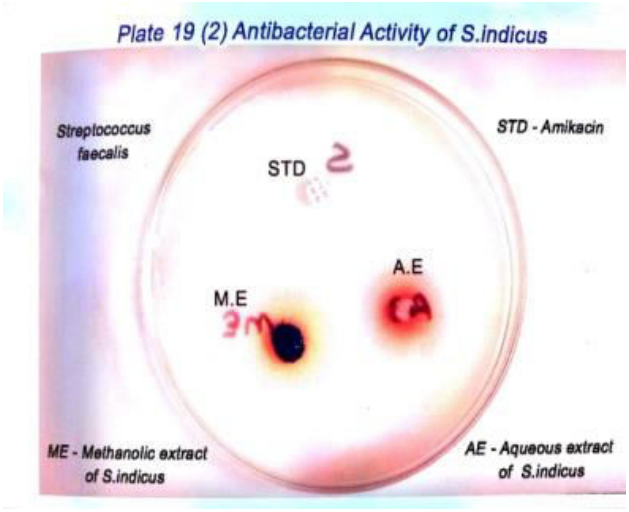
MESI- Methanolic extract of *Sphaerantus indicus*

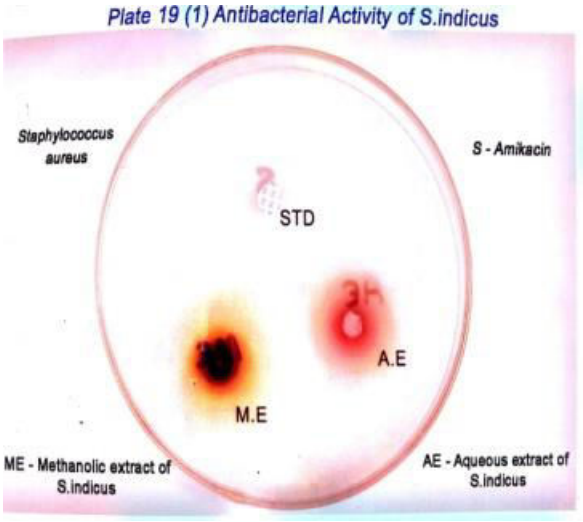
OOSI- Oil of *Sphaerantus indicus*

STD- Standard Amikacin

Aqueous extract of *S.indicus* leaf showed the largest zone of inhibition (25mm) against *S. faecalis* and 22 mm zone of inhibition against *S.aureus*, Methanolic extract of *S.indicus* leaf showed 21 mm and 19 mm zone of inhibitions against *S. faecalis* and *S.aureus* respectively. Aqueous and Methanolic extracts did not show zone of inhibition against *Escherichia coli*. Oil of *S.indicus* did not show any zone of inhibition against any of the tested microorganisms. Aqueous extract was found to be effective against both *staphylococcus aureus,* and *streptococcus faecalis*. The aqueous extract has shown more potent antibacterial activity than that of the standard Amikacin (20 mm).The phytochemical screening demonstrated that the presence of different types of compounds like terpenoids, tannins, deoxy sugars, sterols, and flavonoids which may contribute for the antimicrobial action of the *Sphaeranthus indicus.*

Figure 2, 3, 4: Anti-bacterial activity of Aqueous extract of *S.indicus against S.aureu, S.faecalis, E.coli*





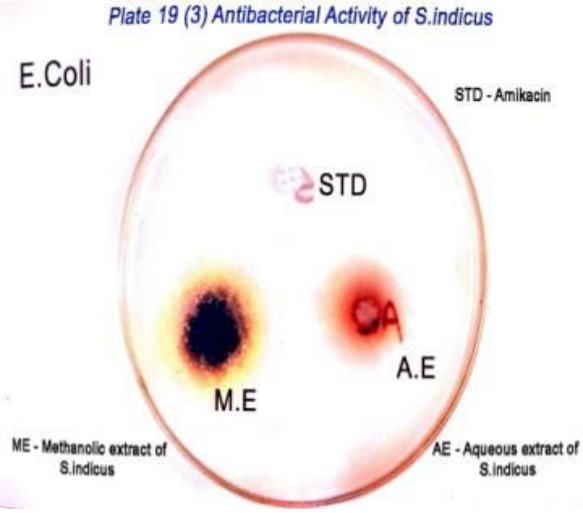
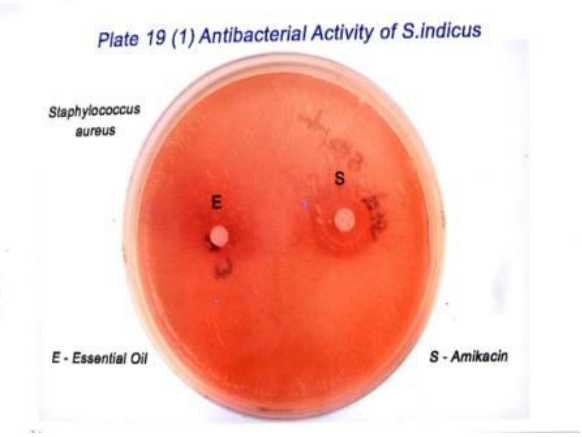
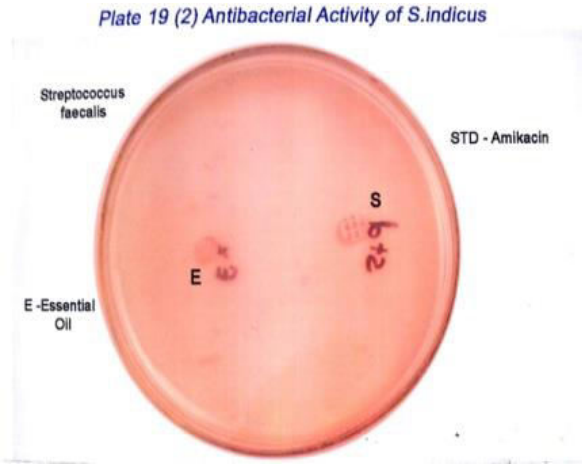
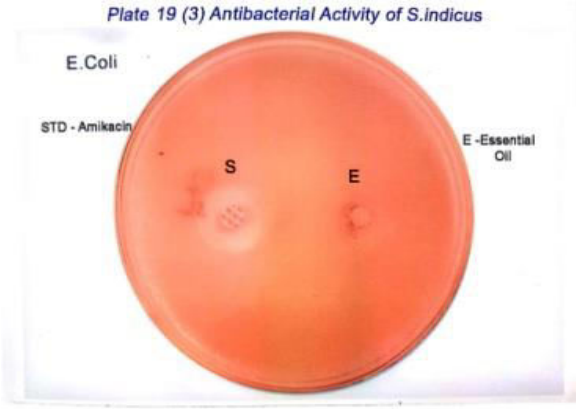


Fig 5, 6, 7: Anti-bacterial activity of Oil of *S.indicus against S.aureu, S.faecalis, E.coli*







DISCUSSION

In recent times there has been considerable significance in the use of plant material as an unconventional method to control pathogenic microorganism (Aqil *et. al.,* 2005) and many components of plants products have been shown to be particularly targeted against resistant pathogenic bacteria (Nostro *et. al.*, 2006). The appearance of multidrug resistant strain of many pathogens is a severe threat and makes chemotherapy more difficult. Furthermore, the current price of most of the chemotherapeutic agents is intolerable to the public particularly in developing countries like India (Gopalakrishna Sarala *et. al.*, 2010). Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. Therefore the present work was carried out to explore the antimicrobial property of *S.indicus*. The aqueous and Methanolic leaf

extract of *S.indicus* showed the activity against two tested microorganisms but the activity was very significant against *S.faecalis* .The plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The present study shows that the Methanolic and aqueous extract of leaves of *S.indicus* shows significant activity against S.aureus *and S.faecalis* which may be due to their phytochemical or secondary metabolites.

CONCLUSION

It is concluded that this study would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

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