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Original Research Article

In vitro evaluation of antibacterial activity in ethanolic extract of whole plant *Sphaeranthus indicus* Linn.

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ABSTRACT

Background: *Sphaeranthus indicus* Linn is a widely used medicinal plant in Indian traditional system of medicine against human pathogens. Alarming bacterial resistance is urging scientist to search for newer anti-microbial substances from the medicinal plants. The objective of the study was to evaluate the antibacterial activity of ethanolic extract of the whole plant *Sphaeranthus indicus* Linn (Asteraceae).

Methods: The antibacterial activity of ethanolic extract of whole plant of *Sphaeranthus indicus* Linn was done against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* in Mueller Hinton Agar (MHA) and compared with ciprofloxacin as standard by disc diffusion method.

Results: The study revealed that there was no zone of inhibition in doses of 100 mcg, 200 mcg and 300 mcg of ethanolic whole plant extract of *Sphaeranthus indicus* in MHA plates compared with ciprofloxacin 30 mcg.

Conclusions: Ethanolic extract of *Sphaeranthus indicus* does not have antibacterial activity. Further studies are needed in different extracts and parts of the plant. Simultaneous studies can be done in different places to evaluate environmental factors and regional variations.

Keywords: *Sphaeranthus indicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, In vitro, Disc diffusion

INTRODUCTION

Herbal medicines are naturally occurring, plant-derived products having minimal or no industrial processing which are available as local and regional treatment modalities. Different societies across the world have shown great interest in curing illnesses using plants/plant-based drugs.¹ *Sphaeranthus indicus* L. is a medicinal plant found in India and Malaysia belonging to Asteraceae family which has 1,100 genera worldwide and 19,000 species. It is a herb called as mundi, nearly 30 cm or 1 foot high with spreading branches, seen all over India, especially in hills, as weed in the paddy fields. All parts of the plant are used for medicinal purpose. The roots and seeds are used by traditional practitioners as an anthelmintic in doses of 40 grams daily in the form of

powder. Powder of the roots and seeds is given along with honey, in cough. The root-bark grounded and mixed with whey is very useful in bleeding piles, and is also used as paste topically. The oil extracted from the roots by steam distillation, and boiled in sesame oil, is recommended by traditional practitioners as a valuable aphrodisiac. Other uses are in glandular swellings in the neck and also in jaundice. This drug is also useful in urethral discharges. *S. indicus* has long been used in the treatment of skin infection, bronchitis, jaundice and nervous depression.² The herb is also reported to be useful as a tonic to treat indigestion, asthma and dysentery.³

The whole plant and its anatomical parts have been reported with different types of secondary metabolites

which include eudesmanolides, sesquiterpenoids, sesquiterpene lactones, sesquiterpene acids, flavone glycosides, flavonoid C-glycosides, isoflavone glycoside, sterols, sterol glycoside, alkaloid, peptide alkaloids, amino acids and sugars.⁴ A bicyclic sesquiterpene lactone isolated from the petroleum ether extract of the aerial part of the *S. indicus* was reported to have antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Fusarium sp.* and other microorganisms.⁵ Antimicrobial activity of alkaloidal and nonalkaloidal fractions of alcoholic extract of flowers has also been reported.⁶ The objective of this study was to evaluate the antibacterial activity of ethanolic extract of whole plant of *S. indicus* in three concentrations (100 mcg, 200 mcg, 300 mcg per disc) in vitro by using disc diffusion method. The antibacterial activity of whole plant ethanolic extract of *S. indicus* was compared with ciprofloxacin as the standard.

METHODS

Plant collection

The whole plant *S. indicus* was obtained locally and was authenticated by the Pharmacognosy unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram, Kerala, India. The procured plant material was disease free and also not contaminated with other plants. Distilled water was used to clean the whole plant material and surface sterilization was done with 70% ethanol. They were cut into small pieces and shade drying was done. Finally, they were completely made into powder form.

Preparation of ethanolic extract

It was pre extracted on petroleum ether in order to defatten the material. Soxhlet extraction was done with 90% ethanol. After that the extract was distilled and then dried in a previously weighed beaker in order to get rid of solvent. Whatman no.1 filter paper was used for filtration. They were stored at 4°C for further use. Three increasing concentrations of 100 mcg/ml, 200 mcg/ml, 300 mcg/ml were prepared in dimethylformamide purchased from chemical store which was manufactured by Qualigens Fine Chemicals, division of Glaxo smith Kline Pharmaceuticals Limited, Mumbai

Antibacterial strains

Antibacterial strains used were *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus* obtained from the Microbiology department in Government Medical College, Thiruvananthapuram.

Antibacterial activity

Antibacterial activity was done using culture media, plates, filter papers, standard drug and bacterial strains obtained from Department of Microbiology, Medical College, Thiruvananthapuram. *Pseudomonas aeruginosa*,

Escherichia coli, *Proteus mirabilis* and *Staphylococcus aureus* were sub cultured on peptone water at 37 °C for 24 hours. Ethanolic extract of whole plant of *S. indicus* in three concentrations (100 mcg, 200 mcg, 300 mcg per disc) were prepared in sterile dimethyl formamide (DMF). Control tests was done using equivalent amounts of the solvent (DMF). Ciprofloxacin, a quinolone in a dose of 30 mcg was used as the standard. The discs (6 mm diameter was cut from whatman No:1 filter paper) were autoclaved first and then soaked with the three increasing doses of the extract and dried. Next they were applied to the surface of Mueller Hinton Agar (MHA) plates seeded with bacteria and then incubated at 37 °C for 24 hours. The plates were observed for the presence or absence of zone of inhibition at the end of 24 hours and it was also repeated for 5 days. The test was done in triplicate.

RESULTS

As shown in (Figure 1 to 4) and as summarized in (Table 1), in this in vitro study, it was observed that the ethanolic whole plant extract of *Sphaeranthus Linn* didn't exhibit antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* and *Staphylococcus aureus*. The effect of *S. indicus* on the growth of bacteria was compared with ciprofloxacin as standard drug.

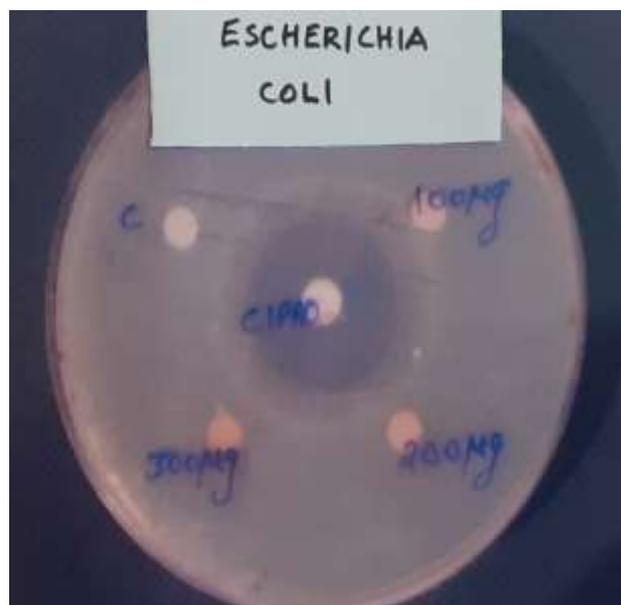


Figure 1: Effect of ethanolic extract of whole extract of *S. indicus* Linn, control and ciprofloxacin on growth of *E. coli*.

In this disc diffusion method of study with ciprofloxacin as standard drug, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* demonstrated a zone of inhibition of 30 mm, 35 mm, 32 mm and 29 mm respectively, whereas 100 mcg, 200 mcg and 300 mcg doses of whole plant ethanolic extract of *S. indicus* demonstrated no zone of inhibition.



Figure 2: Effect of ethanolic extract of whole extract of *S. indicus* Linn, control and ciprofloxacin on growth of *Pseudomonas aeruginosa*.



Figure 3: Effect of ethanolic extract of whole extract of *S. indicus* Linn, control and ciprofloxacin on growth of *Proteus mirabilis*.

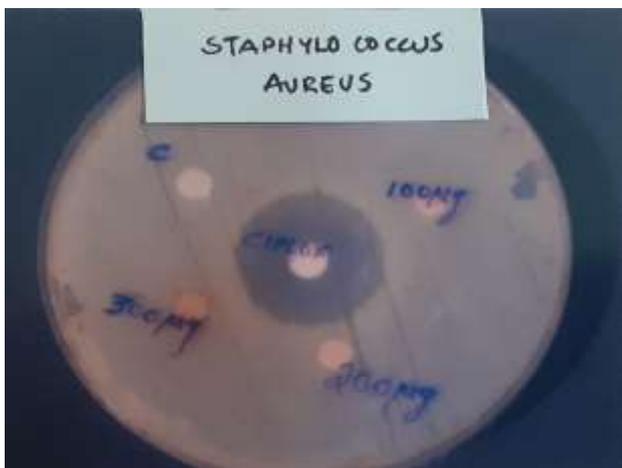


Figure 4: Effect of ethanolic extract of whole extract of *S. indicus* Linn, control and ciprofloxacin on growth of *Staphylococcus aureus*.

Table 1: Effect of *S. indicus* on the growth of bacteria in MHA plates compared with ciprofloxacin.

Organism	Zone of inhibition (mm)				
	Standard ciprofloxacin (30 mcg)	Control DMF	Extract		
			100 mcg	200 mcg	300 mcg
<i>E. coli</i>	30	Nil	Nil	Nil	Nil
<i>P. aeruginosa</i>	35	Nil	Nil	Nil	Nil
<i>P. mirabilis</i>	32	Nil	Nil	Nil	Nil
<i>S. aureus</i>	29	Nil	Nil	Nil	Nil

DISCUSSION

In recent times there has been considerable significance in the use of plant material as an unconventional method to control pathogenic microorganism, and many components of plants products have been shown to be particularly targeted against resistant pathogenic bacteria.^{7,8} Furthermore, the current price of most of the chemotherapeutic agents is on the rise hence steps should be taken to develop effective natural, non-toxic drug for treatment.⁹ Hence the present study was performed to explore the antimicrobial property of *S. indicus*. The whole plant ethanolic extract of *S. indicus* failed to demonstrate any antibacterial property against *E. coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus* in 3 doses (100 mcg, 200 mcg and 300 mcg when compared with ciprofloxacin (30 mcg) as standard drug. This is in contrast to a study in which the antimicrobial activity of methanolic, ethanolic, chloroformic and aqueous extract of *S. indicus* L. were tested against the uropathogenic organisms *E. coli*, *Klebsiella pneumoniae*, *P. mirabilis*, and *Pseudomonas aeruginosa*. It was found that out of the four extract the methanolic extract possessed higher degree of antibacterial activity against *Escherichia coli*. However, against *Klebsiella pneumoniae* ethanolic extract of plant showed higher activity than any other extract, whereas against *Proteus mirabilis*, chloroformic extract show similar inhibition zone as that with Kanamycin. It was also observed that against *Pseudomonas* methanolic and ethanolic extract shows similar activity, however it was higher than chloroform extract.²

The aqueous and methanolic leaf extract of *S. indicus* have shown significant activity against *S. faecalis*.¹⁰ The hexane extract of *S. indicus* flowers possessed more antibacterial activity and antifungal activity than the aerial parts against gram positive organisms.¹ Hexane extract of *S. indicus* (flowers and aerial parts) showed antimicrobial activity against most of the bacteria tested. Flower extract showed higher activity than the aerial parts against Gram positive bacteria such as *B. subtilis*, *S. aureus*, *S. epidermidis* and *E. faecalis* which were comparable with antibiotic Streptomycin (10 µg/disc). Benzene, chloroform, ethyl acetate and acetone extracts of flower and aerial parts showed some activity at higher

concentration (5 mg/disc) against gram positive bacteria.¹ Another in vitro study demonstrated that *S. indicus* essential oil showed maximum activity against the *E. coli*. Both gram positive and gram-negative bacteria were sensitive to this oil.¹¹

Ethanol extract of *S. indicus* has antibacterial activity against enteropathogens.¹² The fruits of *S. indicus* exhibited excellent antibacterial activity against gram positive as well as gram negative bacteria.¹³ It also possesses antifungal property.¹⁴ The 50% ethanolic extract of plant was reported to have hypoglycemic activity.¹⁵ Ethanolic extract of aerial part of *S. indicus* was evaluated for wound healing activity in guinea pigs which enhanced the rate of wound contraction and the period of epithelialization and was comparable to neomycin.¹⁶ The plant was also found to exhibit anticancer activity and antiprotozoal activity against *Entamoeba histolytica*.¹⁵ Chloroform, methanol and aqueous extracts of *S. indicus* has shown high in vitro antibacterial activity against *S. aureus*, chloroform, methanol and ethanol extract against *P. aeruginosa*, methanol, chloroform and hexane against *B. subtilis*, aqueous, methanol, ethyl acetate and chloroform against *E. coli*.¹⁷ In another study lowest MIC values were recorded in leaf extracts of *S. indicus* followed by stem and root extracts against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, *Klebsiella pneumoniae*.¹⁸ Similar antibacterial activity was observed in other plants of the same family.¹⁹

The ethanolic extract of *S. indicus* has demonstrated a dose dependent increase in urine volume in rat. The maximum diuretic and kaliuretic activity were observed at the dose of 300 mg/kg of the extract which confirms the basis for folklore use of *S. indicus* Linn as a diuretic agent.²⁰

Bioactive molecules in plants vary in potency hence efficacy of the extracts obtained also vary in their antibacterial activity.²¹ The chances of absence of antibacterial activity by the plant extracts were noted earlier also. An example is the reported resistance of *E. coli* against ethanolic extracts from neem leaves.²² Therefore, synergistic studies of plant extracts to identify a novel combination with much desired efficacy are needed.²³

CONCLUSION

Previous literature observations had demonstrated antibacterial activity in various parts and extracts of *S. indicus* Linn. This study had demonstrated that the ethanolic whole plant extract of *S. indicus* lack antibacterial property. The reason can be attributed to environmental factors, regional differences that can affect the growth and chemical composition of plants. It can also be due to the method of extraction used. Further studies are needed to standardize the methods of extraction and also in vitro testing done to confirm the findings in this

study. Meticulous attention to these issues should be considered for the emergence of a new era of antibacterial treatment that can be cost effective by using plant-derived principles.

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