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Original Article

Sphaeranthus indicus: A Promising Antimicrobial Agent

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ABSTRACT

Objectives: The aim of the present study was to determine the antimicrobial property of the solvent extracts of flowers and aerial parts of *S. indicus*. **Methods:** The flowers and the aerial parts of *Sphaeranthus indicus* were extracted with n-hexane, benzene, chloroform, ethyl acetate and acetone respectively. The extracts were analyzed for the antimicrobial effect by disc diffusion method at concentrations of 5, 2.5 and 1.25 mg/disc. The Minimum Inhibitory Concentration (MIC) was tested using broth micro dilution method at concentrations ranging from 5 to 0.039 mg/ml. **Results:** There was a significant antibacterial and antifungal activity in hexane extract of flower and aerial parts. The MIC was seen at 0.15 mg/ml against *Staphylococcus aureus* and the highest MIC (5 mg/ml) was noted for *S. epidermidis*. The n-hexane extracts of flower and aerial parts showed MIC as 0.15 and 1.25 mg/ml respectively against *Candida albicans*. **Conclusion:** Concluding it can be said that the *S. indicus* flower n-hexane extract showed promising antimicrobial agent.

Key words: *Sphaeranthus indicus*, Antibacterial activity, Minimum inhibitory concentration, Ethyl acetate, Acetone, n-hexane.

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INTRODUCTION

Herbal medicines have been used by the mankind since time immemorial. The Ayurvedic system of medicine is the oldest traditional system of medicine. The system reveals that ancient Indians had a rich knowledge of medicinal plants.[1] The Indian subcontinent is enriched with rich flora owing to the extreme variations in climate and geographical conditions. Many of the crude drugs of traditional system have been investigated scientifically. The modern world of drug discovery across the globe focuses upon the plants-based drugs.[2] *Sphaeranthus indicus* Linn. is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments. 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.[3] Synonyms of *S. indicus* Linn are *Sphaeranthus hirtus* Willd and *Sphaeranthus mollis* Roxb, *Sphaeranthus indicus* L. is a medicinal plant belonging to family. The drug has been previously used for the treatment of skin infection, bronchitis, jaundice and nervous depression. The roots and seeds of the plant has shown anthelmintic activity. It has also been found to be useful in the treatment of indigestion, asthma, leucoderma and dysentery.[4] A novel isoflavone glycoside from leaves and a new sesquiterpene glycoside and sphaeranthanolide were isolated from the flowers of *S. indicus* which was found to be an immune stimulant. The Medicinal information obtained from the tribal healers indicated that the plant is traditionally used to treat skin disease, cough and fever. The bark when mixed with whey is found to be useful in treating piles.[5] The Flowers are found to be having tonic properties. The leaf juice is boiled with milk and sugar-candy is prescribed for cough. An aqueous extract of the whole plant was found to be toxic to American cockroaches.[6] The present study aims to determine the antimicrobial property of the solvent extracts of flowers and aerial parts of *S. indicus*.

MATERIALS AND METHODS

Plant material

S. indicus was collected from paddy fields of Jabalpur (Madhya Pradesh), India. The plant was identified and confirmed by a taxonomist and the voucher specimen (ERIB-D-73) was deposited in the herbarium at Entomology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India.

Preparation of plant extracts

The flowers and the aerial parts were separated, shade dried and coarsely powdered with electric blender. 200g powder of flowers and aerial parts were soaked separately in 600 ml of n-hexane, in an aspirator bottle for 72 h. The extracts were collected and concentrated at 40°C under reduced pressure using rotary evaporator. The extractwas stored at 4°C until further use. The remaining plant residue was subsequently extracted with benzene, chloroform and ethylacetate similar manner.[7]

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Test concentrations

The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) and extracts were loaded on the 6 mm dia. sterile disc (Himedia, Bombay) with the concentrations of 1.25, 2.5 and 5mg/disc.[8]

Antimicrobial assay

Test cultures

Bacteria: *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* MTCC 3615, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*27853 and *Klebsiella pneumoniae* ATCC 15380.

The bacterial cultures were maintained in Nutrient Agar (NA) at 4°C. The bacterial cultures were inoculated in Mueller Hinton (MH) broth and incubated at 37°C for 18 h at 150rpm.[9] The bacterial inoculum was standardized to 0.8 OD at 660 nm and it was used for disc diffusion method. The final inoculum size of 1x105 CFU/ml for bacteria and 1x104 CFU/ml for *Candida* were used for broth micro dilution technique.[10]

Disc diffusion method

Preliminary antibacterial screening was carried out using disc diffusion method. Discs with different concentrations of plant extracts were placed on the pre inoculated Mueller Hinton Agar (MHA)plates with respective cultures and were incubated at 37°C for 24 h. Streptomycin (10 µg/disc) and DMSO were used as positive and negative control, respectively. The inhibition zone around the disc (diameter) was measured and recorded.[11]

Minimum Inhibitory Concentrations (MIC)

Broth micro dilution method was used to determine the MIC. This was carried out in 96 well microtitre plates containing 200 µl Mueller Hinton Broth with different concentrations of plant extracts. The final concentration of DMSO was maintained at 0.1% in the test broth. Triplicates were maintained along with the negative control. Plates were incubated at 37°C for 12 h for bacteria and at 27°C for fungi. MIC was determined as the complete inhibition of growth at lowest concentration.[12]

RESULTS

Flower extracts with hexane gave pale yellow colour and other extracts were pale brown to brown in colour. Extracts from aerial parts were dark green to dark brown in colour. The yield of the flower extract fell in the range of 0.5-1% (w/w) and that of aerial parts were in the range of 1-2% (w/w) of the dried material. 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) (Table 1). 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. The hexane extract of flowers showed MIC at 0.31 mg/ml for *B. subtilis,* 0.15 mg/ml for *S. aureus* and 5 mg/ml for *S. epidermidis*. On the other hand, aerial parts showed higher MIC at 2.5 mg/ml for *B. subtilis*5mg/ml for *Staphylococcus* spp. and 5 mg/ml for *E. faecalis* compared to flower. Most of the gram-negative bacteria showed higher MIC (>5 mg/ml) for both the extracts of flower and aerial parts (Table 2). 100% growth inhibition was observed at 0.625 mg/ml concentration and determined as MIC (Table 3).

DISCUSSION

In the study, the results obtained indicated that the hexane extract of the *S. indicus* inhibited the growth of the test isolates except *S. typhi*. This, therefore, shows that the extract contains substance (s) that can inhibit the growth of some micro-organisms. Hexane extracts of flowers and aerial parts of *S. indicus* exhibited antibacterial activity. A sesquiterpene lactone, 7-hydroxyfrullanolide isolated from *S. indicus* had antimicrobial activity. The significance of antibacterial activity is due to the presence of alkaloids, tannins and flavonoids which have been shown to possess antibacterial properties. The exhibited antibacterial properties imply the

Table 1: Antimicrobial activity of flower extracts of *Sphaeranthus indicus* by disc diffusion method.

Tested organism	Zone of inhibition in diameter (mm)															
Bacteria	Streptomycin 10 µg/disc	Hexane (mg/disc)			Benzene (mg/disc)	Chloroform (mg/disc)		Ethyl acetate (mg/disc)			Acetone (mg/disc)					
		1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5
Bacillus subtilis MTCC 441	13	11	19	23	-	7	10	-	10	12	-	10	12	-	10	12
Staphylococcus aureus ATCC 25923	15	11	17	19	-	10	11	-	10	11	-	12	13	-	-	10
Staphylococcus epidermidis MTCC 3615	-	12	16	19	-	9	14	-	-	11	-	13	14	-	-	11
Enterococcus faecalis ATCC 29212	-	9	11	13	-	-	-	-	-	-	-	-	11	-	-	10
Escherichia coli ATCC 25922	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia ATCC 15380	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

use of drug in traditional medicine. It has been reported that previously the extracts of the plant was used in sore and wound healing, as ear drop for boils in the ear and treatment of boils. It has also been reported to be used in the control of diarrhoea and dysentery. The large zones of inhibition exhibited by the extract against *S. aureus* and *P. aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, bores and open wounds. *S. aureus* and *P. aeruginosa* have been implicated in cases of boils, sores and wounds. It is also seen that also growth inhibition against bacteria's signifies the use in the control of diarrhoea and dysentery. The inability of the extract to inhibit *Salmonella typhi* may be that it possesses a mechanism for detoxifying the active principles in the extract Some bacteria are known to possess mechanisms by which they convert substances that inhibit their growth to non-toxic compounds for examples *S. aureus* produces the enzyme penicillinase which converts the antibiotic penicillin to penicillinoic acid which is no longer inhibitory to its growth. The inhibition zone of antibiotic streptomycin (10 µg/disc) was comparable with both the flower extract (1.25 mg/disc) and aerial parts extract (2.5 mg/disc) against *B.subtilis* and *S. aureus*.[13] Similar antibacterial activity was observed in other plants of the same family.[14] Higher inhibition zone was observed in *B. subtilis* at 5 mg/disc for hexane flower extract.[15] The inhibition zone was directly proportional to the concentration used. Hexane flower extract showed MIC at 0.31 mg/ml for *Bacillus* sp. whereas the aerial part showed higher MIC at 2.5 mg/ml.[16] From the above results it can be concluded that plant *Sphaeranthus indicus* extracts have great potential as antimicrobial compounds against micro-organisms and that they can be used in the treatment of infectious diseases caused by resistant micro-organisms. Antibiotic resistance has become a global concern. There has been an increasing incidence of multiple resistances in human pathogenic micro-organism in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants common to tropical countries. It can grow to a height of 40 cm. The stem is slender and often reddish in colour, covered with yellowish bristly hairs especially in the younger parts. The *S. indicus* hexane extracts of flower and aerial parts showed good antibacterial activity against gram positive organisms. Flower extracts were more active than the aerial parts. It also possessed strong antifungal activity against Candida and other tested fungi. The findings of the present research may lead to the development of natural antimicrobial agents.

Table 2: Minimum Inhibitory Concentration (MIC) of hexane extracts of *Sphaeranthus indicus* by broth micro dilution method.

Tested organisms	Minimum Inhibitory Concentration (mg/ml)	
	Flower	Aerial Part
Bacillus subtilis MTCC 441	0.33	2.6
Staphylococcus aureus ATCC 25923	0.14	4.9
Staphylococcus epidermidis MTCC 3615	1.25	5.0
Enterococcus faecalis ATCC 29212	5.0	5.0
Escherichia coli ATCC 25922	> 5.0	> 5.0
Klebsiella pneumonia ATCC 15380	> 5.0	> 5.0
Pseudomonas aeruginosa ATCC 27853	> 5.0	> 5.0

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Table 3: Antimicrobial activity of the extracts of aerial parts of *Sphaeranthus indicus* by disc diffusion method.

Tested organism		Zone of inhibition in diameter (mm)														
Bacteria	Streptomycin 10 µg/disc	Hexane (mg/disc)			Benzene (mg/disc)			Chloroform (mg/disc)			Ethyl acetate (mg/disc)			Acetone (mg/disc)		
		1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5
Bacillus subtilis MTCC 441	13	11	15	22	-	7	10	-	10	12	-	10	12	-	10	12
Staphylococcus aureus ATCC 25923	15	11	16	18	-	10	11	-	10	11	-	12	13	-	-	10
Staphylococcus epidermidis MTCC 3615	-	12	12	17	-	9	13	-	-	11	-	13	14	-	-	11
Enterococcus faecalis ATCC 29212	-	9	10	12	-	-	-	-	-	-	-	-	11	-	-	10
Escherichia coli ATCC 25922	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia ATCC 15380	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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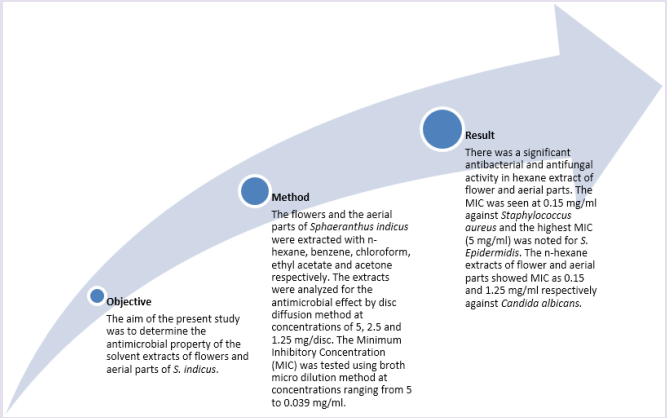
GRAPHICAL ABSTRACT



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SUMMARY

In this study it was aimed to determine the antimicrobial property of the solvent extracts of flowers and aerial parts of *S. indicus*. There was a significant antibacterial and antifungal effect in all the parameters. Concluding it can be said that the *S. indicus* flower n-hexane extract showed promising antimicrobial agent.

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