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Antimicrobial Activity of *Sphaeranthus indicus* L.

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Abstract

Aerial parts and flowers of *Sphaeranthus indicus* were extracted with n-hexane, benzene, chloroform, ehtylacetate and acetone. The extracts were screened for their antimicrobial activity using *in vitro* 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. Significant antibacterial and antifungal activity was observed in hexane extract of flower and aerial parts. The flower extract showed MIC as 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.* In conclusion, the *S. indicus* flower n-hexane extract seems to be a promising antimicrobial agent.

Key words: antibacterial activity, antifungal activity, minimum inhibitory concentration, *Sphaeranthus indicus*.

1. Introduction

Different societies across the world have shown great interest in curing illnesses using plants/plant based drugs. *Spheranthus indicus* L. (Asteraceae) a medicinal plant is wide spread in India and Malaysia. *S. indicus* has long been used in the treatment of skin infection, bronchitis, jaundice and nervous depression (Nadkarni 1976). The roots and seeds are considered anthelmintic. The herb is also reported to be useful as a tonic to treat indigestion, asthma, leucoderma and dysentery (Chopra *et al.* 1956). A novel isoflavone glycoside from leaves (Yadava and Kumar 1999) and a new sesquiterpene glycoside and sphaeranthanolide were isolated from the flowers of *S. indicus* and it was found to be an immune stimulant (Shekhani *et al*. 1990). Medicinal information from tribal healers indicated that *S. indicus* is used to treat skin disease, cough and fever. The bark, ground and mixed with whey, is said to be useful in treating piles. Flowers are credited with alterative, depurative and tonic properties; leaf juice is boiled with milk and sugar-candy and prescribed for cough. An aqueous extract of the whole plant was slightly toxic to American cockroaches (Chopra *et al.* 1958). The present study was undertaken to assess 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 from Kancheepuram district of Tamil Nadu, India. The plant was identified and confirmed by a taxonomist and the voucher specimen (ERIB-D-73) was deposited in the herbarium at Entomology Research Institute, Loyola College, Chennai.

Preparation of plant extracts

The flowers and the aerial parts were separated, shade dried and coarsely powdered with electric blender.200 g 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 40ºC under reduced pressure using rotary evaporator. The extract was stored at 4ºC until further use. The remaining plant residue was subsequently extracted with benzene, chloroform and ethylacetate similar manner.

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 5 mg/disc.

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 and fungi; *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 1344 and Botrytis *cinerea*.The National Committee for Clinical Laboratory Standards (NCCLS 1999) recommended antimicrobial susceptibility test strains acquired from Christian Medical College, Vellore were used in this study. The bacterial cultures were maintained in Nutrient Agar (NA) and fungal cultures were maintained in Sabouraud Dextrose Agar (SDA) slants at 4°C.

The bacterial cultures were inoculated in Mueller Hinton (MH) broth and incubated at 37ºC for 18 h at 150 rpm. 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 $1x10^5$ CFU/ml for bacteria and $1x10^4$ CFU/ml for *Candida* were used for broth micro dilution technique. Antifungal screening was carried out by broth micro dilution method; the final inoculum size was 1×10^4 spores/ml.

Disc diffusion method

Preliminary antibacterial screening was carried out using disc diffusion method (Bauer *et al*. 1966). Discs with different concentrations of plant extracts were placed on the preinoculated Mueller Hinton Agar (MHA) plates with respective cultures and were incubated at 37ºC 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.

Minimum Inhibitory Concentrations (MIC)

Broth micro dilution method (NCCLS 1999) 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.

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 and fungi 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). The fungi *Aspergillus niger*, *Botrytis cinerea* and *C. albicans* were inhibited by the extracts of both the flower and aerial parts. 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 3).

The fungus *C. albicans* showed MIC at 0.15 mg/ml for flower extract and 1.25 mg/ml for hexane extracts of aerial parts. The hexane extract of aerial parts of *S. indicus* showed promising antifungal activity against *B. cinerea*. 100% growth inhibition was observed at 0.625 mg/ml concentration and determined as MIC (Table 2).

Discussion

Hexane extracts of flowers and aerial parts of *S. indicus* exhibited antibacterial and antifungal activity. The essential oil of *S. indicus* has been reported for its antifungal activity against plant pathogenic fungi (Rao et al., 1971). A sesquiterpene lactone, 7-hydroxyfrullanolide isolated from *S. indicus* had antimicrobial activity (Atta-ur-Rahman et al., 1989; Perumalsamy et al., 1999). 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*. Similar antibacterial activity was observed in other plants of the same family)(Roose et al., 1998). Higher inhibition zone was observed in *Bacillus subtilis* at 5 mg/disc for hexane flower extract. 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. The extracts of flower and aerial parts showed inhibition against gram positive organisms but not against gram negative organisms. Similar results were observed in *Chrysanthemum coronarium* flower extract (Urzua and Mendosa, 2003). Sesquiterpene lactones from *Vernonia colorata*, possessed high antibacterial activity primarily against Gram positive and low activity against Gram negative species (Rabe et al., 2002) similar to our findings here.

The flower extract showed MIC at 0.15 mg/ml and aerial parts showed MIC at 1.25 mg/ml against *C. albicans*; this is the first report on anti fungal activity against *Candida* as per the available literature. The hexane extract of flower showed complete inhibition against *A. niger* and *B. cinerae*; the MIC was determined as 1.25 and 0.625 mg/ml respectively. The hexane extract of aerial parts showed MIC as 2.5 and 0.625 mg/ml against *A. niger* and *B. cinerea* respectively. The antifungal activity against *Trichophyton* spp., *Epidermophyton floccosum* and *Microsporum cooki* was reported in thiophene compound isolated from *Tagetes patula* (Asteraceae) (Romagnoli et al., 1998). Antifungal activity was also reported for the ethanol extract of underground parts of *Leuzea*

carthamoides (Asteraceae) against *C. albicans*, *A. fumigatus* (Chobot et al., 2003), and *Centaurea hermanni* (Asteraceae) (Sur-Altiner et al., 1997). which is similar to our results.

Conclusion

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

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

Tested organisms	Zone of inhibition in diameter (mm)															
	Streptomycin	Hexane (mg/disc)			Benzene (mg/disc)			Chloroform (mg/disc)			Ethyl acetate (mg/disc)			Acetone (mg/disc)		
	10 µg/disc	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**
Bacteria																
Bacillus subtilis MTCC441	13	12	18	22	-	9	12	-	9	10	-	9	12	-	10	12
Staphylococcus aureus ATCC 25923	12	11	15	17	-	11	12	-	9	10	-	11	13	-	-	10
Staphylococcus epidermidis MTCC 3615	-	12	16	19	-	9	14	-	-	10	-	12	13	-	-	10
Enterococcus faecalis ATCC 29212	-	8	10	12	-	-	-	-	-	-	-	-	10	-	-	9
Escherichia coli ATCC 25922	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia ATCC 15380	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fungi																
Candida albicans MTCC 227	-	9	10	12	-	-	-	-	-	-	-	-	-	-	-	-

-, no activity

Table 2. Antimicrobial activity of the extracts of aerial parts of *Sphaeranthus indicus* by disc diffusion method.

Tested organisms	Zone of inhibition in diameter (mm)															
	Streptomycin	Hexane (mg/disc)			Benzene (mg/disc)			Chloroform (mg/disc)			Ethyl acetate (mg/disc)			Acetone (mg/disc)		
	10 µg/disc	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**	**1.25**	**2.5**	**5.0**
Bacteria																
Bacillus subtilis MTCC 441	13	10	13	18	-	-	11	-	-	12	-	9	14	-	-	13
Staphylococcus aureus ATCC 25923	12	11	13	16	-	-	10	-	-	10	-	8	11	-	-	12
Staphylococcus epidermidis MTCC 3615	-	9	12	14	-	9	12	-	9	11	-	-	13	-	-	9

Enterococcus faecalis ATCC 29212	-	8	10	13	-	-	-	-	-	9	-	8	11	-	-	-	
Escherichia coli ATCC 25922	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Klebsiella pneumonia ATCC 15380	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pseudomonas aeruginosa ATCC 27853	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Fungi																	
Candida albicans MTCC 227	-	8	10	11	-	-	-	-	-	-	-	-	-	-	-	-	

-, no activity

Table 3. 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***
Bacteria		
Bacillus subtilis MTCC 441	0.31	2.5
Staphylococcus aureus ATCC 25923	0.15	5.0
Staphylococcus epidermidis MTCC 3615	5.0	5.0
Enterococcus faecalis ATCC 29212	1.25	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
Fungi		
Candida albicans MTCC 227	0.15	1.25
Aspergillus niger MTCC 1344	1.25	2.5
Botrytis cinerea	0.625	0.625

ATCC - American Type Culture Collection Centre; MTCC - Microbial Type Culture Collection, India