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PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *SPHAERANTHUS INDICUS* LINN. FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Sphaeranthus indicus Linn (Asteraceae) is a common annual spreading herb found in rice field throughout India. Six crude extracts were prepared from the whole plant *Sphaeranthus indicus* using different solvents by cold maceration process. The extracts were subjected to screening to detect potential antimicrobial activity against *S. aureus, B. substillus, P. Gentamycin* and Nystatin as standard by cup plate agar diffusion method. The aim of our present study was to find out the antimicrobial activity of the different extracts of entire part including flower heads of *Sphaeranthus indicus*. The different extracts such as hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extract exhibits comparable antimicrobial activity with the standard.

INTRODUCTION: *Sphaeranthus indicus* Linn is a common annual spreading herb found in rice field throughout India, Srilanka, Ausralia and Africa. Essential oil [1], obtained by steam distillation of the whole herb contains ocimen, α- terpine, a- citral, geranion, a-ionone, β-ionone, d- cadinene, p- methoxy cinnamaldehyde [2], and an alkaloid spearanthine [3]. The alcoholic extract of powdered caputula contains stigmasterol, β- sitosterol, hentriacontane, sesque- terpinelactone[4], sphaeranthanolide [5], flavone and isoflavone glycoside. Plants have a great potential for producing new drugs of great benefit to mankind [6].

During last ten years the phase of development of new antimicrobial drugs has slowed down while the prevalence of resistance bacteria is no longer matched by expansion in the arsenal of agents [7]. Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, micro organisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds [8]. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [9-12]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [13].

Considering the aforesaid, one of the traditionally used medicinal plants belonging was screened for their antimicrobial properties, *Sphaeranthus indicus* which belongs to *Asteraceae* family. It is used in homeopathic medicine for the treatment of insomnia, epilepsy, tetanus, muscle spasms and leaves presented anxiolytic activity [14, 15]. *Sphaerathus indicus* Linn is an annual spreading herb used to treat hemicraniasis [16], jaundice, diabetes, hernia, haemorrhoids, helmenthiasis, skin diseases, nervine tonic etc. The bark ground and mixed with whey, is said to be useful in treating piles. 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 [17]. *Sphaeranthus indicus* was found to possess powerful medicinal properties to cure skin infections, diseases of the liver, jaundice, bronchitis. In view of the medicinal importance of *Sphaeranthus indicus* in the indigenous system, it was decided to work on the phytochemistry and antimicrobial investigations on *Sphaeranthus indicus* Linn.

MATERIALS AND METHODS/EXPERIMENTAL:

Materials used in experiment: Hexane, chloroform, ethyl acetate, methanol, ethanol and water. Gentamycin sulfate Nystatin.

Site and Year of Experimentation: Experiment conducted at Amrita School of Pharmacy, Amrita Vishwavidyapeetham University and Atlac Lab in April 2009.

Collection and identification of plant material: The whole plant was collected from the wet lands of Kozhikode Dist of Kerala in the month of September. The plant was identified and confirmed by a taxonomist; a voucher specimen was deposited at the herbarium in the institute.

Preparation of plant extracts: The whole plant including the flower heads was shade dried and coarsely powdered with electric blender. The powdered drug passed through sieve number 40 to obtain uniform powder and packed in airtight sealed envelopes for further studies. Then it was extracted by maceration at room temperature for 7 days with regular stirring after every 2 hrs in the order of increasing polarity with hexane, chloroform, ethyl acetate, methanol, ethanol and water. The extracts were collected and concentrated at 40^{o}C under reduced pressure using rotary evaporator. The extract was stored at 4ºC until further use for various evaluations. These extracts were used to conduct the

phytochemical and pharmacological evaluation of *Sphaeranthus indicus.*

Phytochemical screening test: Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailment. The phytochemical screening of the plant extract was carried out by the methods used [18-21] to detect the presence or absence of certain bioactive compounds. Antimicrobial activity was also conducted using Modified agar well diffusion method.

Pharmacological screening:

Antimicrobial activity:

Inoculum: The microorganisms were inoculated into SBCB and incubated at 35 ± 2^0 C for 4 hrs. The turbidity of the resulting suspension was diluted with SBCB to match with 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml.

Agar well diffusion method: The modified agar well diffusion method was employed [22].Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. As a final step the rim of the agar was also swabbed. After allowing the inoculum to dry at room temperature, 6-mm-diameter wells were bored in the agar.

Each extract was check for antimicrobial activity by introducing 100µL of 4000µg/ml concentration into triplicate wells. Simultaneously, Gentamycin sulfate and Nystatin were used as positive control at a concentration of 1µg/ml. and the dilution medium for the positive control was respective solvents. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 35±2°C for 24 hr.

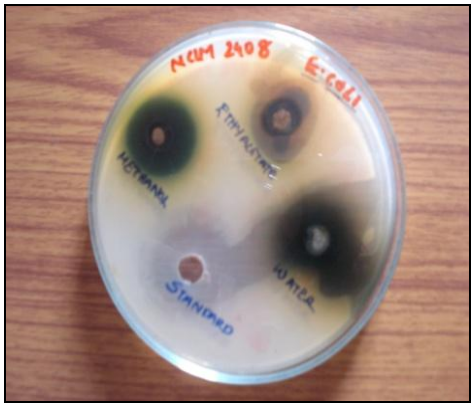
RESULTS AND DISCUSSION: The results are reported in Table 1. The present study deals with the preliminary phytochemical screening and comparison of the six extracts (hexane, chloroform, ethyl acetate, ethanol, methanol and water) of the plant *Sphaeranthus indicus* tested against gram positive *Staphylococcus aureus*, *Bacillus substilus*, gram negative bacteria *P. aeroginosa, E. coli* and fungus *C. albicans* against some common human pathogens. Out of the six extracts, chloroform, methanol and aqueous extracts showed high *in vitro* antibacterial activity against *S. aureus*, chloroform, methanol and ethanol extract against *P. aeroginosa*, methanol, chloroform and hexane against *B. substilus*, aqueous, methanol, ethyl acetate and chloroform against *E. coli*.

TABLE 1: ANTIMICROBIAL ACTIVITY OF *SPHAERANTHUS INDICUS* LINN.

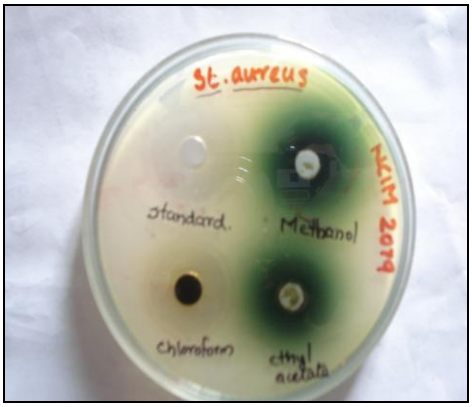
INHIBITION ZONES PRODUCED AGAINST FEW BACTERIAL STRAINS

Sphearanthus indicus (Asteraceae)	Extract	*S. aureus* NCIM2079	*P. aeroginosa* NCIM2200	*B. substilus* ATCC6633	*E. coli* NCIM2408	*C. albicans* ATCC1023
	AQ	21	14	18	22	3
	ET	14	21	13	17	4
	HX	19	18	19	18	2
	MT	22	20	23	21	3
	EA	18	14	19	22	5
	CH	22	21	20	21	3
Positive control						
Gentamycin (1 µg/ml)	NA	23	25	23	23	NT
Nystatin (1 µg/ml)	NT	NT	NT	NT	NT	17
Negative control						
AQ, ET, HX, MT, EA, CH	NA	0	0	0	0	0

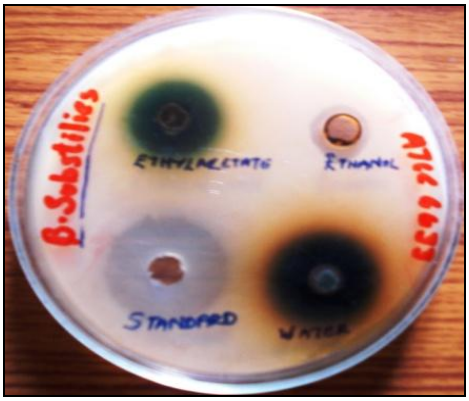
Used concentration: 100 µl of 4000 µg/ml of the extract; Gentamycin; Nystatin 1 µg/ml; AQ – Aqueous (Distilled water); EA - Ethyl acetate; NA-Not applicable; ET – Ethanol; CH – Chloroform; NT-Not tested; MT- Methanol HX- Hexane



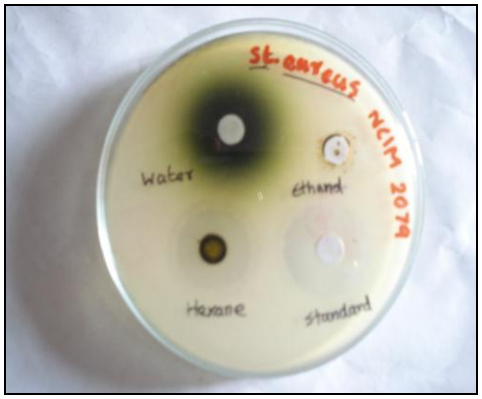
NCIM2408(Methanol,Ethylacetate,Water)



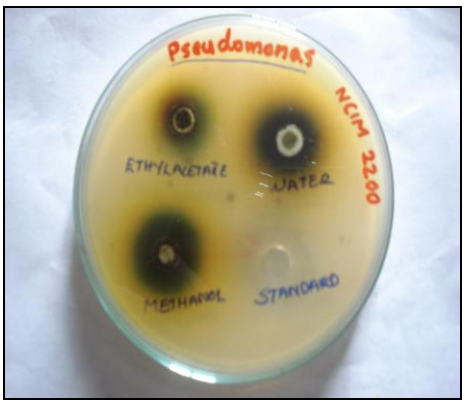
NCIM2079 (Methanol, Ethylacetate, Water, Hexane)



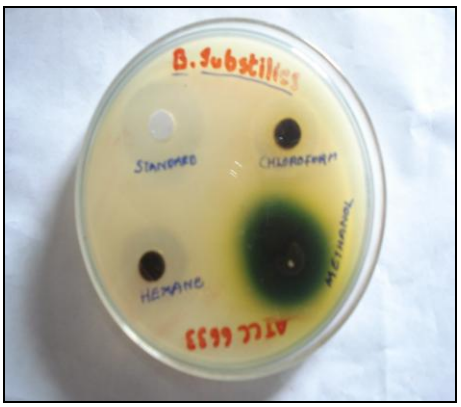
ATCC6633 (Ethylacetate, Water)



NCIM2079 (Methanol, Ethylacetate, Water, Hexane)



NCIM2200 (Ethylacetate, Water, Methanol)



ATCC6633 (Methanol, Hexane)

FIGURE 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF VARIOUS EXTRACTS OF *SPHAERANTHUS INDICUS* LINN.

Methanol extracts showed strong antibacterial activity when compared with the standard against the bacterial strain *B. substilus*. All the extracts showed significant activity against the tested bacterial strain. But the antifungal activity of the six extracts showed less inhibition against the standard nystatin (1 µg/ml). The preliminary phytochemical screening of all the *Sphaeranthus indicus* extracts showed the presence of carbohydrates, monosaccharides, reducing sugar. SIE, SIM, SIA showed the presence of proteins and amino acids, SIEA, SIE, SIM, SIA showed the presence of phenols and tannins, SIE showed the presence of alkaloids and flavones, SIE, SIM showed the presence of flavonoids , fats and oils. SIEA, SIE, SIM showed the presence of steroids, terpenoids shown only by SIE extract.

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