



Trade Science Inc.

BioTechnology

An Indian Journal

SHORT COMMUNICATION

BTAIJ, 1(3), 2007 [87-89]

In vitro* Antimicrobial Activity Of Essential Oil And Extracts Of *Sphaeranthus Indicus

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Received: 25th July, 2007 ; Accepted: 30th July, 2007

ABSTRACT

The biological activity of methanolic extracts of *Sphaeranthus indicus* L. (Asteraceae) against bacteria and fungi was evaluated. The crude extract exhibited strong antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus luteus*, *Micrococcus roseus*, *Candida albicans* and *Candida tropicalis*. The present study confirms that the folk claim is potential in *in vitro*.

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KEYWORDS

Sphaeranthus indicus;
Methanol extracts;
Antimicrobial activity.

INTRODUCTION

The use of plants as a source of medicine is based on the experience of many generations of traditional physicians and herbal practitioners found in different ethnic societies. Numerous wild and cultivated plants play a vital role among these cultures and this inter-relationship has evolved over generations of experience and practices. The consequent divorcement of aboriginal people from dependence upon their vegetal environment for their necessities of life has been set in motion, resulting in disintegration of knowledge of plants and their importance^[1]. With the development of scientific knowledge in pharmacology it has become necessary to provide scientific bases as to whether or not it is justified to use a plant and its active principles for specific diseases.

Many useful drugs from plants have been isolated and characterized. Of them 119 plant derived chemicals are used globally as drugs^[2] were discovered as a result of chemical studies designed to isolate the active principle responsible for the use of plants in traditional medicine^[3]. There are several factors for the continued popularity of traditional drugs, and one is their ready availability and economical importance as compared to the modern medicine, besides the adverse effects of synthetic drugs^[4]. The drugs derived from plants still form the mainstay of medical treatment in the developing countries and according to WHO it is estimated that 80 per cent of the world inhabitants still rely chiefly on traditional medicine for primary health care^[5], however, only 25 percent of modern medicines are derived from plant products^[6].

Sphaeranthus indicus L. belongs to the family

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Asteraceae and habituated in swampy places around tanks. The leaves are used as external application for scabies by the aboriginal tribes of Eastern Ghats^[7].

MATERIALS AND METHODS

Preparation of crude extracts

Fresh matured leaves were collected, washed with distilled water and shade dired. These dried leaves were powdered and extracted with methanol using Soxhlet apparatus for 6hr. The extract was filtered and concentrated under reduced pressure below 40°C to dryness.

Antimicrobial screening

The antimicrobial activity of the extract was assayed by using disc diffusion method^[8] and minimum inhibitory concentrations (MICs) were determined by standard method^[9]. Triplicates were carried out for each concentration.

Disc diffusion method

The antimicrobial assay of the methanolic extract was performed individually against *Pseudomonas aeruginosa*(MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Micrococcus luteus*(1541), *Micrococcus roseus*(MTCC 2522), *Bacillus subtilis*(MTCC-1429), *Candida albicans*(MTCC 183), and *Candida tropicalis*(MTCC 184). These were obtained from Institute of Microbial Technology(IMTECH), Chandigarh, India. All bacteria were cultured overnight at 37°C in nutrient agar medium and yeasts were cultured overnight at 30°C in Sabouraud dextrose agar.

A suspension of the each microorganism (0.1ml of 10⁸ cells per ml) was seeded on to the respective media plates. Sterile Whatman No.1 filter paper discs (6mm in diameter) were impregnated with 50µl of the oil, are placed on the inoculated plates. These plates after staying at 40°C for 2h, were incubated at 37°C for 24h for the bacteria and 30°C for 48h for the yeasts. The diameter of the inhibition zones around the discs were measured in millimeters. Standard antibiotics viz., ampicillin for bacteria and ketoconazole for yeasts were used as positive controls.

Determination of minimum inhibitory concentration (MIC)

TABLE 1: Antimicrobial activity of essential oil of *Sphaeranthus indicus*

Microorganism	Methanolic extract		Antibiotic 30µg/disc (in mm)
	Disc diffusion (in mm)	MIC (mg/ml)	
<i>Pseudomonas aeruginosa</i> (MTCC 1688)	20	0.25	24
<i>Staphylococcus aureus</i> (MTCC 737)	21	0.25	24
<i>Micrococcus luteus</i> (MTCC 1541)	24	0.5	22
<i>Micrococcus roseus</i> (MTCC 2522)	24	0.5	22
<i>Candida albicans</i> (MTCC 183)	20	0.25	22
<i>Candida tropicalis</i> (MTCC 184)	19	0.25	22

The extract was further diluted in appropriate amounts of respective solvents to get different concentrations at starting concentration of 64mg/ml to 0.25mg/ml respectively. The plates were incubated at 37°C for 24h for bacteria and 30°C for 48h for the yeasts.

RESULTS AND DISCUSSION

The methanol extract obtained from leaves of *Sphaeranthus indicus* was microbiologically evaluated. The antimicrobial activity of the extract was assessed against 8 pathogenic bacterial strains(TABLE 1). The TABLE 1 lists the zones(mm) and minimum inhibitory values (MIC) for the bacterial and fungal isolates studies. Of the 8 pathogenic test organisms studied in the disc diffusion assay, one(*Bacillus subtilis*) showed no inhibitory effect. The growth of tested microorganisms ranged from 0.25 to 0.5mg/ml (w/v) with the lowest MIC value against *Pseudomonas aeruginosa*, *Candida albicans* and *C.tropicalis* at 0.25mg/ml(w/v). For *Micrococcus roseus* and *M.luteus* the extract generally follows a concentration dependent antimicrobial activity.

The correction between the two different screening methods was examined and generally larger zones of inhibition correlated with lower minimum inhibitory concentration(MIC) values. Some variations for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida tropicalis* did occur, where the MIC value is lower that what would be expected when observing inhibition zones. This variation between methods^[10] can

be attributed mainly to the variation on extract on disc, disc size, agar composition as well as the volatility of extract in an open air system. Recently, it was illustrated that the MIC values of extracts were lowered two to eightfold when evaporation was prevented.

The activity could scientifically explain some of the uses of *Sphaeranthus indicus* in folk medicine. The inhibiting action of extract against all the microorganisms indicates its potential use as a natural medicine and with the comparative evaluation of the activity of the extract, the traditional use for antimicrobial therapy is confirmed.

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