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In-Vitro Antimicrobial Activity of Extracts of *Passiflora edulis (*Passifloraceae) and *Sphaeranthus indicus* (Asteraceae)

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ABSTRACT

The organic extracts of *Passiflora edulis* and *Sphaeranthus indicus* were evaluated for antimicrobial activity against clinically important bacteria viz., *Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 734, *Staphylococus aureus* MTCC 737, *Bacillus subtilis, Streptococcus pyogens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Aspergillus flavus, Pencillium restrictum and Trichoderma viride.* The in-vitro antimicrobial activity was performed by agar disc diffusion method. *Streptococcus pyogens* was the most resistant bacterial strains against
the methanol extracts.

Key words: Antimicrobial activity, Petroleum ether, Methanol, Benzene, Chloroform, Ethyl acetate, hot water *Passiflora edulis* and *Sphaeranthus indicus*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine (.R. Nair, et al.,2005) . Plants have a great potential for producing new drugs of great benefit to mankind (PAREKH et al., 2005). During last ten years the pace 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 (D.A. Akinpelu and T.M. Onakoya ., 2006). Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds ((Janovska et al., 2003). The need of the hour is to screen a number of medicinal plants for promising biological activity. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Chung et al., 2004; Nair R, Chanda SV., 2004; De Boer HJ et al., 2005; Nair R et al., 2005). 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 (Essawi T, Srour M .,2000). Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to

occur. Considering the aforesaid, the three traditionally used medicinal plants belonging to three different families were screened for their antimicrobial properties. *Passiflora edulis (*Passifloraceae)and *Sphaeranthus indicus* (Asteraceae). Passiflora edulis (climber) Sims belongs to Passifloraceae. It is used in homeopathic medicine for the treatment of insomnia, epilepsy, tetanus, muscle spasms and leaves presented anxiolytic activity (L.N. Yuldashevaet al.,2005).*Sphaeranthus indicus* Linn (herb) belongs to family *Asteraceae* . The plant is distributed through out the plains and wet lands in India, Sri Lanka and Australia.

All the parts of the plant have medicinal uses. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias. The whole herb is used in ayurvedic preparations to treat epilepsy and mental disorders. It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. The oil prepared using the plant roots is reportedly useful in treating scrofula and as an aphrodisiac. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. It also treats piles and hepatitis (Ambavade SD et al.,2006).The purpose of this study was to screen for the organic solvent extracts of these medicinal plants that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

MATERIAL AND METHODS

Plant material

Fresh plant samples were collected from The Nilgiris, Tamilnadu, India. Whole plant of *Spheranthaus indicus* and leaves of *Passiflora edulis* were taken for investigation of antimicrobial property. The taxonomic identities of these plants were confirmed by Botanical Survey of India, Southern Circle, Coimbatore, India and the voucher specimen of the plants were preserved. Fresh plant material were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of extracts:

For solvent extraction, 50 g of air-dried powder was taken in 250 ml of organic solvent (Petroleum ether, chloroform, benzene, ethyl acetate methanol and hot water) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated at room temperature. The repeated procedure was followed all the other solvents and water. The extracts were stored at 4^0C in airtight bottles.

Microorganisms:

In vitro antimicrobial activity was examined for all the organic extracts from two medicinal plants used by traditional healers. *Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 734, *Staphylococus aureus* MTCC 737 was obtained from IMTECH, Chandigarh, India. All the other clinically important pathogens were obtained from Department of Microbiology, RVS College of Arts and Science, Coimbatore, Tamilnadu, India.

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 following methods used by Amarasingham *et al*.,(1964),Das and Bhattacharjee, (1970),Gibbs, (1974), Trease and Evans (1978), Santaram and

Harborne (1984) to detect the presence or absence of certain bioactive compounds.

Antibacterial Assay

Plant extracts of *P.edulis* and *S.indicus*, which was prepared with different solvents like Petroleum ether, Benzene, Chloroform, Ethyl acetate, Methanol and Water were used to test their antibacterial activity.

Antibacterial activity was demonstrated using a modification of the method originally described by Bauer *et al* (1966) which is widely used for the antibacterial susceptibility testing .Liquid nutrient agar media and the Petri plates were sterilized by autoclaving at 120^{o} C for 30 minutes. Under aseptic conditions in the laminar airflow chamber, about 20ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media the bacterial strains were swabbed on the surface of the agar plates.

Whatmann No.1 filter paper was cut into small discs of diameter 0.4cm and autoclaved. These discs were dipped into the different plant extracts of each three concentrations namely (20mg/ml, 10mg/ml, 5mg/ml). The dipped discs were placed on the appropriate swabbed Petri plates such that each Petri plates have three concentration of each plant extract. It was then incubated at 37^{o} C for 24 hours the antibacterial drug which was used here was chloramphenicol for positive control and DMSO was used for negative control. After incubation the zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

The data reported in Table 1 presents the antimicrobial activity of the organic extracts of*P.edulis* and *S.indicus*. The results indicate that the extracts from the medicinal plants studied showed inhibition of growth of some of the tested micro organisms with to various degrees. The antibacterial activity of *S. indicus* and *P.edulis* leaf extract of all the solvents against *Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 734, *Staphylococus aureus* MTCC 737, *Bacillus subtilis, Streptococcus pyogens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Aspergillus flavus, Pencillium restrictum and Trichoderma viride* was evaluated . The methanolic extract of both plants (*P.edulis* and *S.indicus*) showed considerably more activity when compared to other all the solvents. Maximum antibacterial activity was shown against Streptococcus pyrogens, followed by*S. aureus* and *B.subtilis*. From the above results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. *S. indicus* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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Table 1. Phytochemical screening of *Sphaeranthus indicus*.

Extracts	AL	SA	TP	FL	ST	CG	CH	TE	AA	GL	OF
Petroleum ether	-	-	-	-	-	-	-	-	-	-	-

Benzene	-	-	+	-	-	-	+	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	+	-	+	-	-	-	-	-	-
Ethanol	-	-	+	+	+	+	-	-	-	-	+
Water	-	+	+	-	_	-	+	-	-	-	-

Table 2. Phytochemical screening of *Passiflora edulis* (leaves).

Extracts	AL	SA	TP	FL	ST	CG	CH	TE	AA	GL	OF
Petroleum ether	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	+	-	-	-	-	-	-	-	-
Ethanol	-	+	+	+	+	-	-	-	-	-	+
Water	-	+	+	-	+	-	-	-	-	-	-

'+' - Positive '-' - Negative

AL - Alkaloids
TP - Tannin & Phenolic compound
FL - Flavonoids
ST - Steroids
TE - Terpenoid
AA - Amino acids
GL - Glycosides
OF - Oil& Fat
CG - Cardioglycosides
CH - Carbohydrates

Table 1a. Antibacterial activity of *Sphaeranthus indicus* (Whole plant).

Extracts	Concentration (mg/ml)	*B. s*	*S. a*	*S. p*	*E. coli*	*S. t*	*K.p*	*P.a*
Petroleum ether	10	8	8	7	-	-	-	-
	5	-	7	-	-	-	-	-
	2.5	-	-	-	-	-	-	-
Benzene	10	10	10	11	8	-	-	-
	5	9	9	9	7	-	-	-
	2.5	9	8	8	7	-	-	-
Chloroform	10	8	7	8	-	-	-	-
	5	7	-	7	-	-	-	-
	2.5	7	-	7	-	-	-	-
	10	8	7	10	7	-	-	-

Ethyl acetate	5	7	-	7	7	-	-	-
	2.5	-	-	-	-	-	-	-
Methanol	10	10	8	12	8	8	-	-
	5	9	7	10	7	7	-	-
	2.5	8	7	8	7	7	-	-
Water	10	7	-	9	7	7	-	-
	5	-	-	8	-	-	-	-
	2.5	-	-	7	-	-	-	-

Table 1b. Antibacterial activity of *Passiflora edulis* (Leaves).

Extracts	**Concentration (mg/ml)**	***B. s***	***S. a***	***S. p***	***E. coli***	***S. t***	***K. p***	***P. a***
Petroleum ether	10	9	-	7	8	8	7	8
	5	-	-	-	7	-	7	7
	2.5	-	-	-	7	-	-	7
Chloroform	10	8	7	8	-	-	8	8
	5	7	7	7	-	-	7	7
	2.5	-	-	-	-	-	-	7
Ethyl acetate	10	10	9	8	7	7	7	7
	5	9	8	7	7	-	7	7
	2.5	8	-	7	-	-	-	-
Methanol	10	9	10	10	8	9	8	8
	5	8	9	9	7	8	7	7
	2.5	7	8	7	-	7	7	7
Water	10	8	-	8	-	-	9	7
	5	7	-	7	-	-	8	7
	2.5	-	-	-	-	-	7	-

*Zone of inhibition in mm (Test were done in duplicate)

Standard

Extracts	**Concentration (mg/disc)**	***B. s***	***S. a***	***S.p***	***E. coli***	***S. t***	***K.p***	***P.a***
Chloramphenicol	30	24	15	20	21	20	14	17

B.s – Bacillus subtilis , S.a - Staphylococcus aureus , S.p-Streptococcus pyrogens, E. coli –Eschershia coli , S.t – Salmonella typhi , K.p-Klebsiellae pneumoniae, P.a-Pseudomonas aeruginosa