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Evaluation of Antimicrobial Activity of Seed Extracts of Arare Thar Desert Plant *Blepharis Sindica*T.Anders *in Vitro.*

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Abstract: *Methanolic and petroleumether seedextractsof Blepharis sindicawere tested against bacterial cultures Bacillus subtilis,Staphylococcus aureus,Streptomyces griseus,Pseudomonas aeruginosa and fungal speciesAspergillus niger, Fusarium oxysporium,Penicillium funiculosum andTrichoderma reesei. Best antimicrobial activity obtained amongst bacterial strains were againstStreptomycesgriseusfollowed byBacillus subtilis which showed some control only with petroleum ether seed extract. The maximum antifungal activity amongst the fungal species tested waswithTrichodermareesei with both the seed extracts followed by Penicillium funiculosum,.while Fusarium oxysporium was controlled only with petroleum ether seed extract. Among the two extracts taken for the study petroleum ether extract gave better results over methanolic extract.*

Key Words:*Antimicrobial activity, Methanolic seed extract, Petroleum ether seed extract,Blepharis sindica.*

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I. Introduction

Blepharis sindica is a perennial herbaceous plantof sandy tracts of Thar desert of India. It belongs to Acanthaceae family.It has huge amount of medicinal properties. Therefore rural people in the extreme deserts use it as home remedies for various ailments. The seeds of theplant are diuretic, expectorant, aphrodisiac, earache and tonic(Mohammed *et al.*,2004).Cream coloured seeds are boiled in milk and used as an invigorating tonic given to cattle to increase milk production(Lal*et al.*,2012 and Apurva *et al.*, 2015).The roots of plant is used for urinary discharge and dysmenorrhoea (Apurva*et al.*,2015). Powdered plant is applied locally on infections of the genitals and on burns.

Several plant species of Acanthaceae family have shown antimicrobial activities. The methanolic extracts of thirty-nine native plant species collected from Northern Argentina were tested for inhibition of microbial growth. Among which eight species showed good results against six bacterial species tested (Salvat *et al*., 2004).

Many Thar desert plants have been tested for their antimicrobial activity against various microorganisms.*Jatropha curcas,* which is a Thar shrub of Euphorbiaceae, whose numerous biologically active substances have been isolated and characterized from all parts of the plant. Their mechanism of action has also been studied (Prasad *et al*., 2012). Its latex exhibits antimicrobial activity against *Staphylococcus aureus,Bacillus subtilis,Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Candida albicans* (Oyi *et al.*, 2002).

Antimicrobial activity of *Blepharis sindica*seedswas studied with its methanolic and petroleum ether extracts. Four bacterial and fourfungal species were selected for the primary screening.

II. Materials And Methods

Microorganisms Used

Clinical laboratory bacterial isolates of*Bacillus subtilis, Staphylococcus aureus, Streptomyce griseus, Pseudomonasaeruginosa* and fungal isolates *Aspergillus niger, Fusarium oxysporium ,Trichoderma reesei*and *Penicillium funiculosum* were obtained from the stock cultures of Microbiology Laboratory, SMS Medical College, Jaipur, India.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude methanolicand petroleum ether extracts of seeds of *Blepharis sindica*was studied against gram positive and gram negative bacterial strains by the agar well diffusion method(Bonjar *et al*, 2004).Bacteriological agar from Hi Media, India, was used for the bacteriological medium. The methanolic and petroleum ether extracts of seeds of *Blepharis sindica* were extracted by Soxlet extraction method. The solvents were totally evaporated after final extraction and then the dried extracts were diluted in

100% Dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. Thebacteriological agar was melted and cooled to 48 - 50ºC and a standardized inoculum (1.5×108 CFU(colony forming unit)/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to prepare a solid plate. Wells (6 mm)were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well. The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extracts was determined for the bacterial species in terms of zone sizes around each well i.e. zone of inhibition (ZI). The diameters of zone of inhibition produced by the extracts were compared with those produced by the commercial control antibiotic, Streptomycin. For each bacterial strain controls were maintained, where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of Antifungal Assay

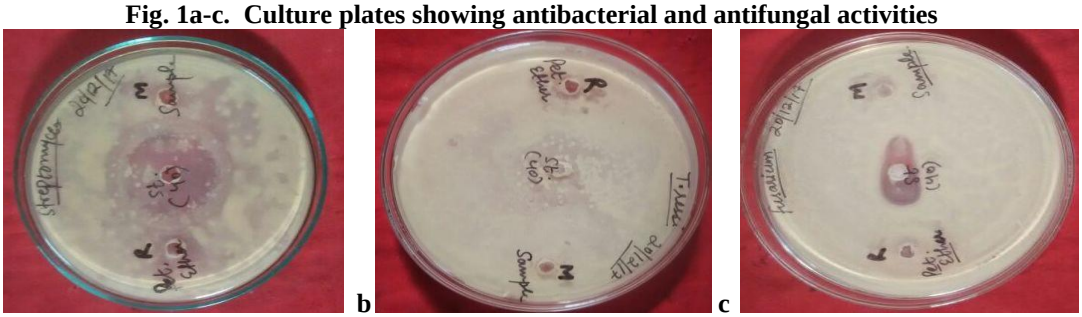
Anti fungal activity of the methanolic and petroleum ether seed extracts of *Blepharis sindica* was investigated by agar well diffusion method (Bonjar *et al*, 2004). The fungal test species*Aspergillus niger, Fusarium oxysporium, Trichoderma reesei,* and *Penicillium funiculosum* were subcultured onto Potato Dextrose Agar (PDA) medium andincubated at 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile Phosphate buffer saline solution and adjusted to a concentration of 106 spores/ml. By dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium inoculation. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 cm apart were punctured in the culture media using sterile glass tube. 0.1 ml of of diluted extracts (5mg/mL) in DMSO was administered in each well. Plates were incubated at 25°C. After incubation of 2-5 days depending on the fungal growth, bioactivities were determined by measuring the diameter of zone of inhibition (ZI) (in mm). All experiments were performed in triplicatesand means were calculated.

III. Results And Discussion

The results showed that the petroleum ether extract gave better results compared to the methanolic extract, among all the micro-organisms tested. The maximum antimicrobial activity obtained amongst the bacterial strains was against *Streptomyces griseus*(Fig. 1a, Table 1). The bacteria*Bacillus subtilis*was controlled only with petroleum ether seed extract to certain level, while against the other two bacteria there were no response (Table 1).

The antifungal activity amongst the four fungi tested, the highest antifungal activity was observed on *Trichoderma reesei* with both the seed extracts followed by *Penicillium funiculosum*(Fig. 1b and Table 1), while weak antifungal activity was shown by Petroleum ether extract against *Fusariumoxysporium*(Fig. 1c and Table 1).*Aspergillus niger*remained unresponsive with both the extracts (Table 1).

Fig. 1a-c. Culture plates showing antibacterial and antifungal activities



a **b** **c**

StreptomycesgriseusT.reesei ***Fusarium oxysporium***

Table 1: Table showing antimicrobial activity in terms of zones of inhibition (ZI) on the test microbial cultures due to petroleum ether and methanolic extracts of the seeds of *Blepharis sindica*

	Microbial strains	**Antimicrobial activity - ZI size(petroleum ether extract of seeds) mm**	**Antimicrobial activity - ZIsize(methanolic extract of seeds) mm**	**Rating**
	Bacteria			
1	*Bacillus subtilis*	6.0	Nil	*
2	*Staphylococcus aureus*	Nil	Nil	
3	*Streptomyces griseus*	12.0	6.0	***
4	*Pseudomonas aeruginosa*	Nil	Nil	
	Fungi			

5	*Aspergillus niger*	Nil	Nil	
6	*Fusarium oxysporium*	4.0	Nil	*
7	*Penicillium funiculosum*	8.0	6.0	**
8.	*Trichoderma reesei*	10	6.0	***

***-Sufficient, **- moderate,*- weak.

All the plants contain some or the other secondary metabolites which are responsible for its protection against biotic and abiotic stress. These secondary metabolites have medicinal and antimicrobial activity. The nature and quantities of these secondary metabolites vary with the edaphic, environmental and other internal and external factors that influence the normal growth of the plants. These secondary metabolites have various complex structures and bonding nature. Hence different secondary metabolites haveselective and differential solubility in different solvents. Therefore when different solvents are selected for extraction of the plant samples, qualitatively and quantitatively variable secondary metabolitesare extracted in different solvents and that leads to variability in the results in their activity as has been shown in the present study.

Several workers have studied the antimicrobial and other medicinal values of various plant species against various micro-organisms and ailments. Salvat *et al.* (2004) worked on thirty nine nativeplant species of Acanthacae family from Northern Argentina for their antimicrobial activity with their methanolic extracts.Among which eight species, *Astronium balansae*, *Geoffroea decorticans*, *Peltophorum dubium, Geoffroea spinosa*, *Lantana balansae*, *Prosopis kuntzei*, *Prosopis ruscifolia* and *Bulnesia sarmientoi*showed good antimicrobial activity against six bacterial species tested viz.*Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Enterococcus faecium*.

Root extract of *Hygrophila auriculata*(K. Schum) Heine (syn. *Asteracantha longifolia* Nees) of Acanthaceae family exhibited significant hepatoprotective and antioxidant activities of the aqueous extract of the roots when studied on CCl_4 -induced liver toxicity in rats (Shanmugasundaram and Venkataraman, 2006).

IV. Conclusion

The antimicrobial activity of seed extracts of *Blepharis sindica* with petroleum ether and methanol showed that petroleum ether extract was more effective compared to methanolic extract. Among the four bacterial species screened with the two extracts, the antibacterial activity was maximum on *Streptomyces* with both the extracts which was followed by *Bacillus subtilis,* only with petroleum ether seed extract. Similarly among the four fungal species tested against antibacterial activity by the two extracts,highest antifungal activity was observed on*Trichoderma reesei* with both the seed extracts followed by *Penicillium funiculosum*, while *Fusarium*oxysporium was controlled only with petroleum ether extract. However, when compared antifungal with antibacterial activity of the seed extracts, antifungal activity was more pronounced.

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