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RESEARCH ARTICLE

MICROBIOLOGY

ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF EPIPHYTIC MOSS STEREOPHYLLUM LIGULATUM JAEG.

B. L. CHAUDHARY AND PREM KUMAR*

Bryology laboratory, Department of Botany, MLS University, Udaipur – 313001, India.

PREM KUMAR

Bryology laboratory, Department of Botany, MLS University, Udaipur – 313001, India.

ABSTRACT

Epiphytic moss *Stereophyllum ligulatum* was collected from Maharastra. The plants were extracted in methanol, ethanol, petroleum ether, acetone and benzene. All the crude extracts tested for antibacterial activity against some pathogenic bacteria *viz. Bacillus cereus, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes* and *Klebsiella pneumoniae* by agar well diffusion method. The plant extracts showed remarkable inhibitory activity on the growth of microorganisms. Phytochemical screening was carried out using standard procedure to identify the constituents as described by Sofowora *et al*. (1998).

KEYWORDS

Antibacterial activity, Phytochemical, Epiphytic moss, Agar well method, Extract, Secondary metabolites.

INTRODUCTION

The investigation on various plant biological activities has reach lately great scientific interests since plant extracts are seen as a potential bio-pharmaceuticals but also could be used in agriculture (Gupta et al., 2010; Sumathi and Parvathi, 2010; Solomon *et al,* 2010; Karatas and Ertekin, 2010). In such studies, the vascular plants receive high attention while some other group of organisms like bryophytes remains neglected. They are recognized as the basal or first diverging lineage of land plants (Forrest et al., 2006). They are morphologically and biochemically diverse. Bryophytes (that is, liverworts, hornworts and mosses) expressed interesting bioactivities (Dulger *et al.,* 2005; Chobot *et al*., 2006; Sabovljevic et al., 2006, 2010; Milar *et al*., 2007; Singh *et al*., 2007; Tonguc and Mercili, 2007;Veljic et al., 2009, 2010; Ulka and Karadge, 2010). They are known to possess fungi as endobionts, as well as to develop mycorrhiza. However, relationships of bryophytes and fungi remain under-investigated. They are not common in the diet of other organisms and even more, most of the consumers avoid them. Besides antifeeding effect, bryophytes are known to possess various relationships with microorganisms (protozoas, fungi, bacterias, algae) (Ando and Matsuo, 1984; Castaldo-Cobianchi *et al*., 1988; Asakawa, 1990; Basile *et al*., 1998; Sabovljevic *et al*., 2001) and contains a set of various known and unknown secondary metabolites (Xie and Lou, 2009). Bryophytes, as a diverse group, are chemically still incompletely known although; many new compounds for science were described from them, mainly from liverworts (Sabovljevic and Sabovljevic, 2008). They are rather rarely used in the ethno-medicine as compared to vascular plants and rather a few uses are known in some traditional medicine. The reports on biological activities of bryophyte extracts and neglected and unknown potentials of these second biggest groups of land plants with about 25,000 to 28,000 species and much more infra-taxa worldwide are reviewed by Sabovljevic and Sabovljevic (2008, 2010). New and interesting compounds found in bryophytes emphasised them as new sources of agents, some of which can be extremely active e.g. Pejin *et al.* (2011) *in press* a, b.

Many bryophytes exhibit antimicrobial effects against fungi and bacteria (Frahm and Kirchhoff, 2002; Ilhan *et al*., 2006; Sabovljevic et al., 2006; Basile *et al*., 1998a, b; 1999; Scher *et al*., 2004; Subhisha and Subramoniam, 2005; Bodade *et al*., 2008; Dülger *et al.*, 2009). Almost all species of bryophytes are not damaged by insect larvae, fungi, bacteria, slugs, snails and mammals (Asakawa, 2001) because, biological compounds like oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenylquinone and aromatic and phenolic substances in bryophytes are protected against these organisms (Asakawa, 1981, 1984, 1990, 2000). Therefore, bryophytes have the potential for medical use. Traditional medical use of bryophytes in China started more than 400 years ago.

MATERIALS AND METHODS

Plant material

S. ligulatum was collected from different localities of Maharastra such as Bhimashankar (Pune District), Mahableshwar, Panchgani (Satara District). The Specimens were identified in the Bryology laboratory Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur 313001 India. The specimen was deposited at the

Herbarium, Department of Botany University College of Science Udaipur.

Extract Preparation

All plant material collected was washed with distilled water to remove any adhering soil or extraneous material. Whole plants were then air dried until water content was negligible and then ground into a fine powder. Powdered plants were extracted with 200 ml petroleum ether, benzene, acetone, methanol and ethanol for 96 hours at room temperature. Filtering it with whatman filter paper No.1 and the crude extract was obtained by evaporating the solvent in open air.

Test Microorganisms

Test microorganisms were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank IMTECH, Chandigarh, India.

Antibacterial assay and phytochemical screening

The agar well diffusion assay technique (Perez, Paul and Bazerque1990) was used to evaluate antibacterial activity .The bacteria used were *B.cereus, E.aerogenes, E.coli, K.pneumoniae* and *S.aureus.* All bacterial cultures were plated out on nutrient agar plates and incubated for 24 hours at 37º C and colonies from this fresh culture were used for making suspension. 100 µl of bacterial suspension was uniformly spread on nutrient agar medium in sterile petri plates. After solidification of nutrient agar, wells were made with a 6 mm sterile cork borer. Different concentrations of extracts were made with DMSO (Dimethyle sulfoxide) and 100 µl of it were poured in the wells. The plates were incubated at 37º C for 24 hrs and antibacterial activity of plant extract was observed by measuring the diameter zone of Inhibition and average was recorded.

Phytochemical Screening

Chemical test were carried out of all the extracts of *Stereophyllum ligulatum* using standard procedures to identify the constituents as described by Sofowora, Trease, Evans and Harborne.

***Alkaloids*:** About 0.2 g of the extracts was warned with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragendoffs reagent were added. Orange red precipitate indicates the presence of alkaloids.

***Tannins*:** Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

***Reducing sugars*:** One ml of each fraction was taken in separate test tubes. These were diluted with 2.0 ml of distilled water followed by addition of Fehling's solution (A+B) and the mixtures warm. Brick - red precipitate at the bottom of test tubes indicated the presence of reducing sugars.

***Saponins*:** About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Forthing (appearance of creamy miss of small bubbles) shows the presence of saponins.

***Flavonoids*:** Extract of about 0.2g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

***Steroids*:** 2ml of acetic anhydride was added to 0.5 g of the extract of each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski test): 0.2 g of the extract of the whole plant sample was mixed with 2ml of chloroform ($CHCl_3$) and conc. H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Table 1
Results of antibacterial activity of S.ligulatum by agar well diffusion method against some pathogenic bacteria.

Microorganisms	Extracts	Different Concentration of the Plant extracts (µg/ml)				
		1000	750	500	250	175
Bacillus cereus	Petroleum ether	22	21	20	18	17
	Benzene	26	25	23	20	17
	Acetone	25	24	22	19	17
	Methanol	23	22	21	19	18
	Ethanol	23	21	20	18	16
Staphylococcus aureus	Petroleum ether	23	20	18	15	14
	Benzene	24	22	21	19	16
	Acetone	22	19	16	9	5
	Methanol	18	16	14	13	12
	Ethanol	23	20	19	17	14
Escherichia coli	Petroleum ether	20	17	14	11	9
	Benzene	23	20	18	15	13
	Acetone	23	21	18	15	7
	Methanol	18	16	13	11	10
	Ethanol	22	19	16	14	12
Enterobacter aerogenes	Petroleum ether	21	19	17	13	6
	Benzene	22	21	19	17	16
	Acetone	24	23	21	19	18
	Methanol	24	22	21	18	16
	Ethanol	22	20	19	17	16
Klebsiella pneumoniae	Petroleum ether	21	19	18	16	15
	Benzene	21	18	17	15	13
	Acetone	21	19	18	16	11
	Methanol	23	21	20	18	17
	Ethanol	22	20	19	17	16

Values of zone of Inhibition included cup borer diameter (6.00) in mm and are mean of three replicates.

Table.2
Phytochemical screening of S. legulatum

Test	Results
Alkaloid	**+**
Flavonoids	**+**
Tannins	**-**
Saponins	**-**
Reducing sugars	**+**
Steroids	**-**
Terpenoids	**+**

(+) presence, (-) absence

RESULTS AND DISCUSSION

Antibacterial activity of *S.ligulatum* extract in different solvents on test bacteria are represented in table 1.All the extracts viz. petroleum ether, benzene, acetone, ethanol and methanol showed remarkable antibacterial activity against all test bacterial culture. Acetone extract showed maximum activity against *B.cereus* while methanol extract showed minimum activity against *S.aureus*.

Results of phytochemical screening indicate the presence of some secondary metabolite including alkaloids, flavonoids, and terpenoids and reducing sugars as shown in table 2. Some of these metabolites particularly the flavonoids were reported to the responsible for antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003).

PLATE - 1

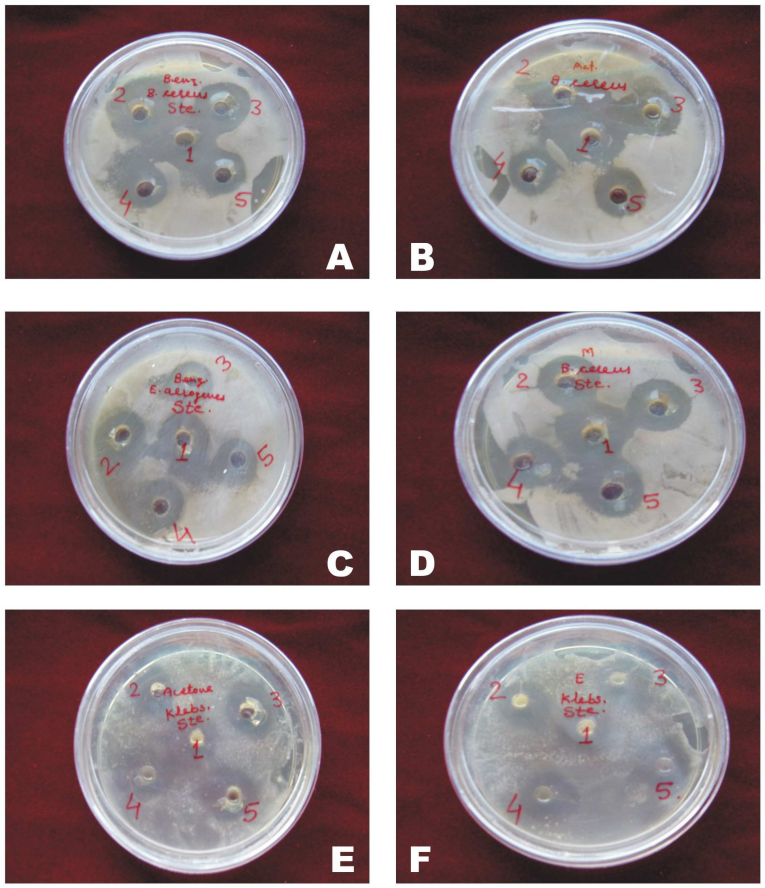

Fig. 1 Antibacterial activity of crude extract of *Stereophyllum ligulatum* Jaeg. (A)Benzene extract against *B. cereus* (B) Acetone extract *B. cereus* (c)Benzene extract against *E. aerogenes* (D) Methanol extract *B. cereus* (E) Acetone extract against *K. pneumoniae* ,(F) Ethanol extract against *Klebsiella pneumoniae*

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