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Bioactive potentiality of some thalloid liverworts extracts.

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ABSTRACT

Present paper is an attempt to evaluate the antifungal potentiality of Fossombronia indica St. and Cyathodium tuberosum Kash. extracts from Purandar and Panhala localities of Western Ghats, Maharashtra. Antibacterial activity of three different organic solvents and aqueous extracts of liverworts were tested against four different bacterial strains viz. Bacillus substilis (NCIM 2697), Escherichia coli (NCIM 2067), Pseudomonas aeruginosa (NCIM 2200) and Staphylococcus aureus (NCIM 2492) by disc diffusion assay method. All extracts express better but variable antibacterial activities. Greater inhibitory activity showed by F. indica St. ethanol extract against S. aureus. Antibiotic amphicillin have more sensitivity to S. aureus as compared to all extracts. Liverworts associated soil was analyzed for physicochemical and biological characteristics.

Key words :*Fossombronia indica* St., *Cyathodium tuberosum* Kash., antibacterial activities, organic solvent extracts

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INTRODUCTION

Bryophytes are the largest group of plants, approximately 8000-9000 species of mosses, 6000 species of liverworts and 100 species of hornworts, exists Worldwide [6]. World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicine mostly plant drugs for their primary health care needs [10]. Hundreds of medicinal bryophytes have been identified and classified in ethno botanical literature as potential antimicrobial agents [2]. However, less than 3% of the plants or animals have been thoroughly evaluated by pharmaceutical industries and there has only been a preliminary screening of bryophytes for biological activities [19]. Several hundreds of new compounds have been isolated from bryophytes and their structures elucidated [1]. Chinese, Europeans and North Americans have used bryophytes as medicine for hundreds of years. More than 400 years ago Chinese used some *Fissidens* sp. and *Polytrichum* sp. as diuretics and hair growth stimulation tonics. Bryophytes have expressed interesting bioactivities [18]. They are known to posses various relationships with microorganisms and contains a set of various known and unknown secondary metabolites [21]. Phytochemical investigations showed that besides terpenoids and flavonoids, bibenzyls and cyclic bis (bibenzyls) are also present in plants. Presently, over 400 new compounds have been isolated with potential antibiotic qualities [2]. Cellular oil bodies contain vast array of lipophilic terpenoids, aromatic compounds and acetogenins which may be responsible for the extraordinary array of bioactivities and medicinal properties [23].

Screening of bryophytes in National Cancer Institute's (NCI) antitumor screening programme was reviewed by [19]. Chilean moss *Sphagnum magellanicum* has biological properties [15]. Its properties of regulation intestinal functioning by reduction of glucose and may also attribute that prevent colon cancer [4]. Antimicrobial activity of some bryophytes from Kolhapur district reported by [20]. Antibacterial activities of some bryophytes from China and Magnolia have studied [23].

In the present investigation we have evaluated the antibacterial activity of *F. indica* St. and *C. tuberosum* Kash. extracts against tested organisms.

MATERIALS AND METHODS

F. indica St. and *C. tuberosum* Kash. plant material were collected from Purandar and Panhala localities of Western Ghats, Maharashtra, during period of October 2010 to November, 2010. These species were identified with the help of previous bryophytic taxonomy literature in Botany department at Tuljaram Chaturchand College, Baramati, Dist. Pune (Maharashtra).

Preparation of the extract: Fresh plant material were treated with Tween-20 to remove epiphytic hosts which found on the surface, extensively washed in tap and distilled water then dried on filter paper. Afterwards, extraction was made within four days. Air dried 10 g plant material extracted separately with organic solvents like acetone, ethanol, petroleum ether and distilled water. Extracts were shaking on rotary shaker (250-300 r.p.m.) for 24 h. and volumes of fractions adjusted to 2 ml/g weight of plant material then, filtered it through cellulose acetate membrane paper. Finally filtrates were used to screen antibacterial activities by extracts saturated discs.

Four fungal strains obtained from National Collection of Industrial Microorganisms (NICM), National Chemical Laboratory, used for screening. These are viz. *Aspergillus niger* (NCIM 507), *Fusarium moniliformae* (NCIM 1276), *Fusarium oxysporum* (NCIM 1072) and *Rhizopus stolonifer* (NCIM 1139).

Determination of antibacterial activity: Plant extracts were screened for antibacterial activity through the disc diffusion assay method [17], using 100 µl of suspension of tested microorganisms. This suspension contained 2 $x10^8$ CFU/ml for bacteria. Muller-Hinton agar (MHA, Fluka), sterilized in a flask and cooled to 45-50 ^oC, were distributed in sterilized petri dishes (9 mm). Filter paper discs (6 mm) were individually impregnated with 10 µl of extracts and then placed on to the agar plates, which had been previously inoculated with tested microorganism then, incubated at 37^oC for 24 h. Antibiotic amphicillin served as a positive control for comparison. The diameter of zone inhibitions were measured in millimeters and values accounted for results.

RESULTS AND DISCUSSION

Antibacterial screening results of *F. indica* extracts were documented in Table No. 1 and *Cyathodium tuberosum* Kash. extract results summerized in Table No. 2

Different organic solvent extracts of *F. indica* St. from Purandar were screened separately for antibacterial activity. Results indicated that the greater inhibitory activity by ethanol extract against *S. aureus* (16 mm) whereas, minimum activity against *E. coli* and *B. substilis* (7 mm). Average zone of inhibition through ethanolic extract is (7.5 mm). None of antibacterial activity when extracted with aqueous exception of *S. aureus*, remaining of all extracts showed average inhibitory activity. Panhala localities *F. indica* St. ethanol extracts have more inhibitory activity against *E. coli* (13mm). It was found that remaining *F. indica* extracts have variable inhibitory activity against tested bacteria. Antibiotic amphicillin used as positive control, has more inhibitory activity against *B. substilis* (19 mm).

From Lonawala regions collected plants screening the ethanol extract has greater antifungal activity against *A. niger* whereas antibacterial activity against *S. aureus*; followed by acetone extracts activity against *F. moniliformae* and *P. aureginosa*. Petroleum ether extract showed 12 mm diameter zone of inhibition against *E. coli*. Ethanol, petroleum ether and aqueous extracts inactive against *F. moniliformae* and *Rhizopus stolonifer*. According to available data *P. aureginosa* is most sensitive (13 mm) strain. Amongst these, the gram negative *S. aureus* and *B. substilis* also displayed a variable degree of susceptibility; followed by *E. coli* (12 mm), *S. aureus* (9 mm) and *B. substilis* (9 mm).

In addition that results of *F. indica* St. associated soils analysis including available nitrogen (N), phosphorus (P), potassium (K), organic carbon (C%), Na, $CaCo_3$ ion salts and physicochemical characteristics like electric conductivity and pH were summarized in Table No. 3. Biological characteristics including fungal genera *viz. Penicillium*, *Aspergillus*, *Trichoderma*, *Dematium* and bacterial genera *Pseudomonas* and *Bacillus* present in *F. indica* St. associated soil at Panhala. *Dematium* and *Mucor* reported from Purandar.

Chemical constituents of particular plant species varies according to geographical area and season. Plants, animals, fungi and microbes possess a wide variety of chemical defensive and offensive mechanisms as part of their survival strategy. Half of all medical prescriptions contain ingredients derived from natural sources of which 20-25% are of plant origin [12].

Liverwort *Marchantia polymorpha* is known to possess many activities. However, it is well known by marclocyclic bis (bibenzyls)-various type of marchantin, some of which, besides antimicrobial, are known to have anti-cancer affect [3]. Marchantiaceae members are extensively used to treat tumefaction, to protect the liver and to treat hepatitis [9]. Fourteen crude methanolic and ethanolic extracts of bryophytes from South Western British Columbia were screened for antibiotic activity against three bacterial strains [16]. *In-Vitro* antimicrobial activity of *Brachythecium compestre* and *Eurhynchium pulchellum* extracts examined [22]. Bioactive substances of *Atricum* are considered to be polyphenolic compounds [5]. *Atricum* labels are reported to be seen on Chinese medicines primarily as antibacterial and anti-inflammatory agents [8]. *Hylocomium splendens* has shown antibiotic activity against nine Gram-positive bacteria [11]. According literature the liverworts should have antibiotic activity against *B. substilis* studied [23].

Detectable antibacterial compounds are present in most taxa of liverworts [11]. High antibacterial activity of *Conocephalum conicum* extracts against pathogenic bacteria reported [6]. Non-ionized organic acids and polyphenolic compounds might contribute to the antibiotic properties of bryophytes [13]. *In-vivo* condition first experiment have been performed at Boon University and alcoholic extracts of twenty bryophytes had indicates effect on a variety of crop infected fungi. They also shows antifeedant affects against slugs [7]. This study indicates the antibiotic activities of liverwort extracts.

Accordingly we have evaluated and concluded that the extracts of *F. indica* St. has an antibiotic activities due to presence of bioactive compounds.

Table No. 1 : Antifungal screening of *Fossombronia indica* St. extracts against test organisms.

		Inhibition zone in diameter (mm)			
	F. indica St. extracts.	*A. niger*	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Purandar	Acetone	NI	NI	7.5	NI
	Ethanol	9.0	NI	14.5	13.0
	Petroleum ether	7.0	NI	NI	NI
	Aqueous	NI	NI	6.5	NI
	Control, (Nyastatin 50 μg^{-disc})	19.0	16.0	17.0	NI
Panhala	Acetone	11.5	NI	13.5	NI
	Ethanol	17.0	NI	11.0	NI
	Petroleum ether	9.0	12.0	10.5	8.0
	Aqueous	NI	NI	6.0	NI
	Control, (Nyastatin 50 μg^{-disc})	17.0	13.5	9.0	12.0

NI : No Inhibition

Table No.2 Antifungal screening of *Cyathodium tuberosum* Kash. extracts against test organisms.

	C. tuberosum Kash. extracts.	Inhibition zone in diameter (mm)			
		A. niger	*F. moniliformae*	*F. oxysporum*	*R. stolonifer*
Lonawala	Acetone	NI	9.0	7.0	6.0
	Ethanol	11.0	NI	NI	NI
	Petroleum ether	NI	NI	9.0	NI
	Aqueous	8.0	NI	NI	NI
	Control, (Nyastatin 50 μg^{-disc})	11.0	8.0	8.0	8.0
Mahabaleshwar	Acetone	8.0	7.0	8.0	NI
	Ethanol	11.0	NI	9.0	NI
	Petroleum ether	7.0	NI	9.0	NI
	Aqueous	8.0	NI	7.0	7.0
	Control, (Nyastatin 50 μg^{-disc})	13.0	11.0	8.0	7.0

NI : No Inhibition

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