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Phytochemicals and antimicrobial activity of Bryophytes

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ABSTRACT

The bryophytes possess some bioactive compounds and therefore are medicinally very useful plants. They contain pools of secondary metabolites, which are unique. The antimicrobial activity was assessed using agar well diffusion method against the two bacterial strains from the bryophytes viz *Anthoceros erectus*, *Asterella angusta*, *Cyathodium tuberosum*, *Plagiochasma articulata* and *Targionia hyphophylla* the studies of phytochemical constituents revealed presence of alkaloids, flavonoids, coumarins, phenols, tannins, steroids, and sugars. The antimicrobial activity is due to the presence of secondary metabolites. It was highest in *Targionia hyphophylla* and least in *Cyathodium tuberosum*. In this Research we study antibacterial activity and phytochemical screening of the bryophytes. The above observations suggest that due to significant antibacterial activity of the bryophytes they have good potential in drug development.

Keywords: Antimicrobial activity, Phytochemical screening, Bryophytes

INTRODUCTION

Bryophytes are the oldest land plants, which include liverworts, hornworts and mosses. These groups of vascular plants include about 25,000 to 28,000 species and they grow in shady locations. They are herbaceous and absorb water and mineral nutrients mainly through leaves. They have an interesting feature, which is about not getting attacked by bacteria, fungi or pests. They are potential source of medicine as they contain secondary metabolites. The antibiotic properties are due to the biologically active compounds. Bryophytes are a rich reservoir of biological active compounds such as terpenoids, flavonoids, alkaloids, glycosides, saponins, anthroquinons, sterols and other aromatic compounds. They also possess anticancer and antimicrobial activity due to their unique chemical constituents. In the present investigation on Phytochemicals and antimicrobial activity of Bryophytes. In this paper we study antibacterial activity and phytochemicals screening of the bryophytes. This study wills investigation of the use of bryophytes in drug development.

Over the centuries, bryology and bryologists have remained neglected due to inadequate commercial aspects of bryoflora. Initially, the bryologists by and large concentrated and even restricted to morpho-anatomical work only. Consequently, after Hedwig (1793), apart from the first and biggest landmark of life sciences related to non-Mendelian inheritance, sex chromosomes in plants, continuity of chromosomes during mitosis and introduction of UV-mutagenesis to genetic research, most of the research findings were published on systematics of these plants. But any significant boom in bryology was yet to report. Later on, bryologists confessed that for their sustenance and identify something innovative has to be done, so that bryology can also compete with the other vibrant branches of botany. At present, it is evident that commercial viewpoint is the key for any development; hence, several bryophytes have been assessed for unexplored aspects, especially for their medicinal properties. Subsequently, in last few decades, situation seems to be changing, various recognised and important publications have appeared related to innovative bryological work (Reski, 1998; Reski *et al*., 1998).

Tedela *et al*. (2014), investigated the antibacterial activity of acetone, ethanol, methanol and hexane extracts of *Calymperes erosum* C. Mull and *Bryum coronatum* Schwaegr against twenty clinically important bacteria pathogens. Agar dilution method was used to assess the effectiveness of the extracts on the test organisms. *Enterococcus feacalis* ATCC 29212, *Bacillus pumilis* ATCC 14884 and *Enterobacter cloaca* ATCC 13047 in decreasing order are most sensitive to the extracts while Proteus vulgaris KZN, S. aureus OK2 and Shigella sonnei ATCC 29930 were resistant to the extracts. Ethanolic extract was the most effective among the extracts followed by acetone extract.

Chauhan *et al*. (2014), evaluated antibacterial activity of acetone and methanol extracts of moss against various bacteria. The maximum zone of inhibition was found against Gram negative pathogens whereas weak inhibitory activity was investigated against S. aureus. The combined effect of chloramphenicol with acetone and methanol extracts exhibited pronounced antimicrobial effect against *B. subtilis, Proteus mirabilis* and *E. coli*.

MATERIAL AND METHODS

Collection of Bryophytes

The epiphytic mosses were collected from the Visakhapatnam region of Araku, the Visakhapatnam District, Andhra Pradesh. Collected samples were identified by using "Gangulee's mosess of eastern India and adjacent regions" (1969-1980). The identification was mainly based on habit, morphology, leaf structure, presence or absence of costa, pattern of cells in base, middle and terminal portion, leaf margin, arrangement of chloroplast, distribution, etc. Using this features collected, they were identified as *Pogonatum microstomum* (Schwaegr.) Brid, *Fissidens brevinervis* (Broth.) and *Pallavicinia lyelli* (Hook).

Extract preparation

The bryophytes collected were separately washed with distilled water to remove the adhering soil or extraneous dust particles. The shade dried plants were further ground into a fine power. Organic solvents such as 95 % (v/v) acetone, 95 % (v/v) Ethyl acetate and (Nice, Cochin) distilled water were employed for the extraction of different bioactive principles. In this study, cold extraction (percolation) was done. Powdered plants (50 g) were extracted with 100 mL respective solvents for 96 h at room temperature. The crude extract can be prepared by filtering the extracts with what man filter paper No. 1 followed by evaporating the solvent in open air. Then the extract was collected and stored at 4oC and checked for their antimicrobial property.

Antibacterial activity

Eight pathogenic bacterial strains procured from Microbial Type Culture Collection (MTCC, Chandigarh, India) were employed in the present study to investigate the antibacterial properties. The Gram negative organisms such as *Escherichia coli* (MTCC 585), *Klebsiella pneumoniae* (MTCC 3040), *Vibrio chole*ra (MTCC 3906), *Pseudomonas aeruginosa* (MTCC 7925), *Shigella flexneri* (MTCC 1457 and Gram positive organisms such as *Bacillus subtilis* (MTCC 428), *Staphylococcus aureus* (MTCC 3160) were used as the test pathogens. Nutrient agar and nutrient broth were used for storage and sub-culturing of the bacterial pathogens. Muller Hinton Agar was used for antibacterial assay.

Assay of antimicrobial activity

The antimicrobial activity was assessed using the Agar well diffusion assay (Perez *et al*., 1990). All bacterial cultures were plated out on Nutrient agar plates and were incubated for 24 h at 37 ±0.5 C and colonies from this fresh culture were used for making suspension. Fresh inoculums of approximately 10o CFU (colony forming units)/mL of tested microorganisms were used for the study. 100 µL of the bacterial suspension was uniformly spread on sterile Muller Hinton Agar plates. After solidification of the agar, wells were made with a 6 mm sterile cork borer. Different concentrations of the bryophyte extracts were made with 99% (v/v) DMSO (Dimethyl sulfoxide) and 100 µL of the extract were poured in the wells. The plates were incubated for 24 h at 37 ± 0.56 C and antibacterial activity of the plant extract was observed by measuring the diameter zone of inhibition in millimeters. Negative controls were made by DMSO alone and Positive controls were made by the antibiotic Streptomycin (25 µg /mL) at the centre of the plate. The experimental data were expressed as mean ± SD of triplicates using Microsoft Excel Software Programme.

Preliminary phytochemical analysis

The extracts of the collected bryophytes using different solvents were screened for the qualitative analysis of different classes of natural compounds, using the standard methodology of Sofowora (1982). The major pharmaceutically valuable phytochemical compounds like alkaloids, carboxylic acids, coumarins, flavonoids, phenol, proteins and amino acids, quinones, resins, saponins, sterols, tannins, xanthoproteins and sugars were investigated in the present study.

RESULT AND DISCUSSION

The bryophytes were identified as *Pogonatum microstomum* (Schwaegr.) Brid, *Fissidens brevinervis* (Broth.), *Pallavicinia lyelli* (Hook) and authenticated from the literature (Table 1). The antibiogram studies against the test pathogens to find out the Penicillin resistance by using commercial Penicillin discs are given in Table 2. The antibiogram studies revealed that all the tested microorganisms are resistant against Penicillin. The antibacterial activity of the bryophyte extracts are given in Table 3. Among the liverworts tested, the maximum antibacterial activity was reported by the aqueous extract of the liverwort, *Pallavicinia lyellii*, against Vibrio cholera, (27±1.5 mm). The ethyl acetate extract of the bryophyte *P. microstomum* (Schwaegr) Brid reported moderate antibacterial activity against *Staphylococcus aureus* (MTCC 3160). The result of this research highlights the activity of both the organic solvent and aqueous extracts. The antimicrobial principles were either polar or non-polar and they were extracted only through the solvent medium (Locher *et al*., 1995; Kenneth 2009; Eloff 1998).

The antibacterial activity has been depicted in Fig. 1. The results reveal that the bryophytes *Asterella angusta* had greater antibacterial activity in methanol extract and in *Targionia hyphophylla* and *Plagiochasma articulata* the antibacterial activity was greater in ethanol extract. In *Anthoceros erectus* the antibacterial activity of both methanol and ethanol was less and least in *Cyathodium tuberosum* the phytochemical screening is depicted in Table 1.

Table 1: Phytochemical analysis of selected bryophytes

Phytochemical constituents	*Anthoceros erectus*	*Asterella angusta*	*Cyathodium tuberosum*	*Plagiochasma articulata*	*Targionia hyphophylla*
Alkaloids	+	+	-	-	+
Carboxylic acids	-	-	-	-	-
Coumarins	+	+	-	+	+
Flavonoids	+	+	-	+	+
Phenols	+	+	+	+	+
Resins	-	-	-	-	-
Saponins	-	-	-	-	-
Steroids	+	-	-	+	+
Tanins	-	-	-	+	+
Sugars	+	+	+	+	+

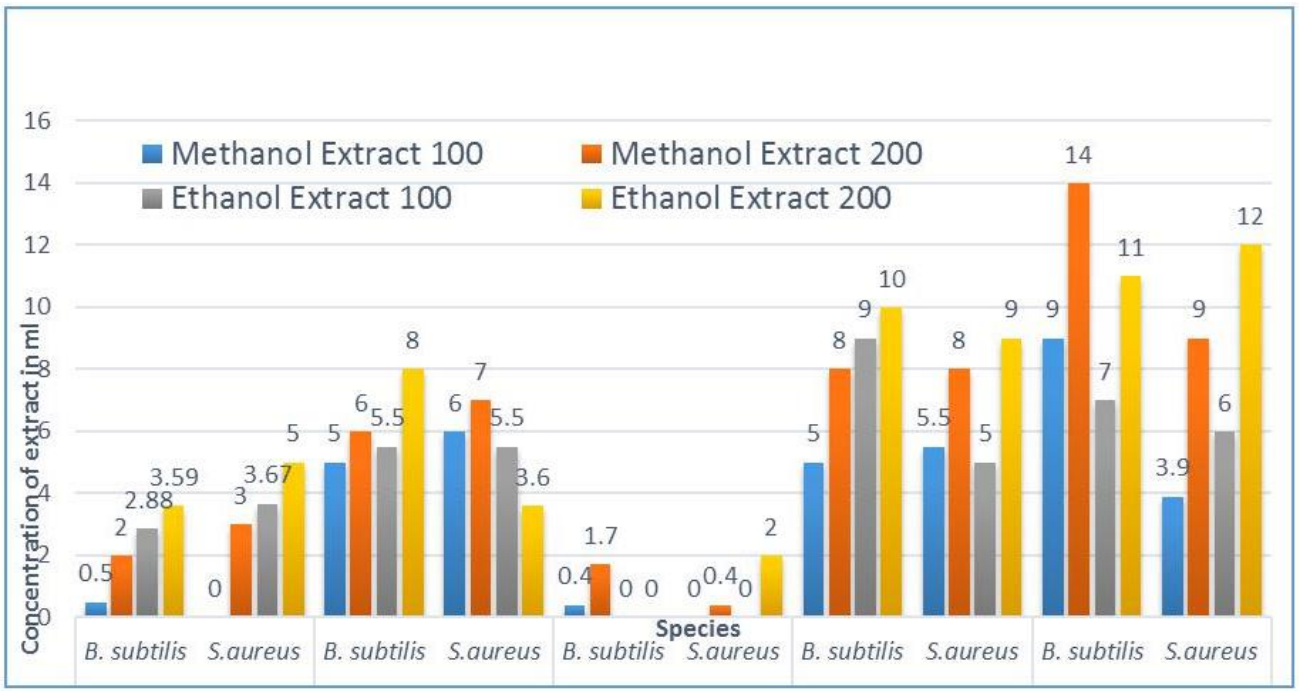

Fig. 1: Antibacterial activity of methanol and ethanol extracts of some bryophytes

It indicated the presence of secondary metabolites like alkaloids, phenols, tannins, flavonoids, coumarins, steroids, and sugars; whereas carboxylic acid, resins, saponins were not present.

DISCUSSION

The present study reports that there is varying level of activity in the test species. This suggests that the extracts in both the solvents have a broad spectrum activity; hence there is a need to investigate further for their use as for antibacterial agents, which could help in new drug development (Sawant *et al*., 2010). The antimicrobial activity carried out by Oyesiku and Caleb (2015) in three mosses also reveal that ethanol extract was found to be more active than the other two and are of the opinion that these extracts show antibiotic activity. This suggests that specific antibacterial compounds isolated by ethanol are more effective against specific bacteria. Deora and Rathore (2013) have revealed that *Plagiochasma articulata* crude extract adversely affected the bacterial growth.

Bodade *et al*. (2008) also have observed that the ethanolic extract was more active than other fractioned extracts and *Plagiochasma articulata* was most active against bacteria and fungi. They are of the opinion that antimicrobial activity may be due to secondary metabolites. Similar observations have been made by Nikolajeva *et al*. (2012) in different bryophytes. They have found that 73% ethanolic extracts exhibited antibacterial activity and it was also higher as compared with aqueous extracts. Studies carried out by Singh *et al*. (2011) also reveal that all the four different bryophyte, namely, *Plagiochasm aappendiculatum, Conocephalum coricum, Mnium marginatum,* and *Byrum argenteum* have antibacterial activity and their findings support the use of bryophytes in traditional medicine for treating burn infections.

The results of the phytochemical screening reveals that the phytochemical compounds may be responsible for antimicrobial activity of the bryophytes and they are alkaloids, flavonoids, quinones, resins, steroids, tanins and sugars. Batish *et al*. (1997), Ahmed *et al*. (1998), and Kumaraswamy and Satish (2008) are of the opinion that the activity may be due to presence of various secondary metabolites. Deora (2015) has observed that selected bryophytes showed the presence of terepenoids, flavonoids, steroids, and glycosides were present whereas, whereas alkaloids, saponins and anthroquinons were not present and have further stated that these chemical compounds could be potent antimicrobial agents to treat plant diseases.

CONCULSION

In this research work the antimicrobial activity in bryophytes was assessed using agar well diffusion method against the two bacterial strains. The bryophytes considered were *Anthoceros erectus, Asterella angusta, Cyathodium tuberosum, Plagiochasma articulata and Targionia hyphophylla*. The studies of phytochemical constituents revealed presence of alkaloids, flavonoids, coumarins, phenols, tannins, steroids, and sugars in the selected bryophytes. The antimicrobial activity is due to the presence of secondary metabolites. It was observed to be highest in *Targionia hyphophylla* and least in *Cyathodium tuberosum*. The research study suggests that due to significant antibacterial activity observed in the bryophytes they have good potential in drug development.

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