



RESEARCH ARTICLE

Phytochemical screening and antioxidant potential of *Plagiochasma appendiculatum* Lehm. & Lindenb. and *Sphagnum fimbriatum* Wilson

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Abstract

In recent time bryophytes are gaining attention in terms of their unique phytochemistry. They can generate a wide range of bioactive phytochemicals. Though many of the bryophytes have been evaluated for this aspect yet majority of them are unexplored regarding their phytochemistry and bioactive potential. Hence, in present attempt 2 commonly growing species of bryophytes, viz., *Plagiochasma appendiculatum* (thalloid liverwort) and *Sphagnum fimbriatum* (moss) were evaluated for their phytochemical profiling using Folin-Ciocalteu and aluminium colorimetric methods. While antioxidant activity of selected species was evaluated by DPPH and NOSA. It was found that these species have higher levels of total phenols and flavonoids and have good antioxidant potential. Further, to check their possible role as antimicrobial agents, the plant extracts were tested against selected fungal and bacterial strains. The results suggested that these 2 bryophyte species have substantial antifungal and antibacterial activities. Based on this study, these plants appear as a good contender to evaluate further for future herbal formulations.

Keywords

Antioxidant, antimicrobial, bioactivity, bryophytes, secondary metabolites

Introduction

Bryophytes stand second largest class of terrestrial plants after angiosperms, and they are classified between pteridophytes and algae (1, 2). Bryophytes have medicinally significant bioactive chemicals, yet little is known about them. However, bryophytes are utilized as medications and cures to treat a variety of ailments all around the globe (3, 4). Bryophytes are thought to be a great repository of advanced bioproducts or secondary chemicals, some of them have metabolic processes. Many chemicals have been identified from bryophytes and their compositions have been determined. Four flavanols (myricetin, datiscetin, kaempferol and quercetin) and two flavones (flavones and luteolin) were found to have an inhibitory effect on methicillin-resistant *Staphylococcus aureus* among the flavonoids studied (MRSA). Five moss species yielded a total of seven pure flavonoids, which were separated and classified (5). All the flavonoids had significant antibacterial efficacy against the tested bacteria, with Saponaria having the highest activity. Some of these flavonoids have been found to have antibacterial properties. Moss bi-flavonoids have also been identified as potential antibacterial drugs (6).

The existence of several phenol contents like gallic, caffeic, 3-4 hydrobenzoic, chlorogenic, vanillic, p-coumaric as well as salicylic acid was discovered using reverse-phase high-pressure liquid chromatography for the moss, *Sphagnum magellanicum* (7). Bryophytes are the chosen grasses since they have long been utilized to treat liver and skin diseases (8-10). Mosses, despite having a wider diversity of species, have been examined less thoroughly for therapeutic reasons than liverworts. Terpenoids, bibenzyls, flavonoids, a few fatty acids and acetophenols are among the bioactive molecules present in bryophytes (11).

These bioactive compounds are well known for their antioxidant and antimicrobial activities (12). Besides this, there has been no known incidence of viruses infecting bryophytes, thus it's probable that the plants have a chemical defense against viruses. The bryophytes have been recognized as an advanced source of antiviral, are a bioactive compound source with antibacterial properties, and a huge majority of the bryophyte species have been found as having significant antiviral activity against PVX. Recent antiviral compound research has revealed that a bioflavonoid found in bryophytes can inhibit a wide range of viral pathogens (13).

The phytochemicals formed in bryophytes provide a much needed defense not only against the biotic stresses but also the abiotic stresses. Thus, the goal of this research was to investigate the phytochemical, antioxidant and antimicrobial properties of 2 bryophytes with different life forms.

Materials and Methods

Collection of plant and identification

In December (winter), samples of the selected bryophytes were collected, 2020 from the Nainital region of Uttarakhand (India) at an altitude of ca. 2084 msl. Between 11:20 AM and 1:30 PM (IST) all the samples were taken from their environment during the optimum level of environmental factors. The random sample approach was used during the collection within a stretch of 1 km. The identification was done based on available herbarium specimens and literature at Bryotechnology Laboratory, Banasthali Vidyapith (Rajasthan), India. The taxonomic data of the reference specimens [BURI-1396/2022 (*P. appendiculatum*) and BURI-1397/2022 (*S. fimbriatum*)] was accordingly submitted to the Herbarium, Banasthali University Rajasthan India (BURI).

Preparation of plant material

First, soil and other plant detritus were meticulously cleaned from the plants using clean water. Collected thalli were then set aside and placed in liquid nitrogen and transported to the research laboratory where they were preserved at a specific temperature of -80 °C till further study. Before extract preparation thalli of the samples were taken and it was pulverized after being air-dried at ambient temperature. The powder (5 g) was macerated in 80% methanol and left for 48 h in an orbital shaker at 50 °C. After filtration, the extract was kept at 4 °C until needed (14).

Determination of Total Phenolic Content

Colorimetric analysis was carried out using the Folin-Ciocalteu technique to determine total phenolic content (TPC) (15). Initially, 0.125 ml of the methanolic extract and 0.5 ml of water were filled in a test tube. In that order Folin-Ciocalteu reagent (0.125 ml), sodium carbonate solution (1.25 ml), and water (3 ml) were added and the mixture was left for 90 min. Thereafter, the absorbance was measured at 760 nm. Based on dry material (mg GAE/g dry weight of the sample) total phenol concentration was determined in Gallic Acid Equivalents (GAE). The data was expressed using the mean and standard deviation.

Determination of Total Flavonoid Content

A method for estimating total flavonoid Content (TFC) was adopted standard methodology (16). 1 ml extract, 0.3 ml sodium nitrite solution and 4 ml distilled water at a concentration of 5% sodium nitrite were mixed. After 5 mins, 2 ml of 1M sodium hydroxide was added to the mixture, followed by 0.6 ml of 10% aluminium chloride. A colored flavonoid aluminium complex forms when flavonoid reacts with aluminium chloride and sodium nitrite, which may be identified using a spectrophotometer at 415 nm. The tests were carried out using quercetin standards in concentrations ranging from 0 to 100 µg/ml. The results were expressed as mg QE/g (mg of quercetin equivalent per gm of dried material).

Antioxidant Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

A strategy for determining the influence on the DPPH free radical was established as per the standard procedure (17). DPPH solution was prepared by dissolving 4 µg DPPH in 100 ml of methanol. Thereafter, 2 ml of DPPH solution was taken and added to 200 µl of extract. After a 20 min incubation period, the decrease of the DPPH radical was quantified using a Spectrophotometer at 517 nm against a reagent blank. The antioxidant activity of the crude methanolic extract was measured using the IC₅₀ value, which is defined as the concentration of extract that causes 50% free radicals' reduction. The lower the IC₅₀ value, the higher will be antioxidant activity. Based on the % of decolorization of the sample, the calculation of the scavenging activity of the samples was done.

Nitric Oxide Scavenging Activity (NOSA)

The standard method (17) was used to study the NO scavenging activity of bryophyte samples. 0.5 ml extract, 0.5 ml phosphate buffer and 2 ml 20 mM sodium nitroprusside were incubated for 150 min at 25 °C. After incubation, 3 ml Griess reagent was added to the mixture and left for 30 min at room temperature. Thereafter, the absorbance of the mixture was measured at 540 nm.

Antimicrobial activity

Two fungal species *Fusarium solani* (Mart.) Sacc. (Fs; MTCC350), *Aspergillus niger* (An; MTCC282) and 2 bacterial species *Bacillus subtilis* (MTCC 619), *Escherichia coli* (MTCC 118) were collected from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chand-

garh (India). Bacterial and Fungal cultures were maintained on Nutrient Agar (NA) and Potato Dextrose Agar (PDA), respectively. 3-5 ml sterile distilled water was added to the agar tubes to prepare their spore suspension. Then, the obtained suspended spores were further shaken well to release the spores and were used as a base layer in Petri plates.

Disc diffusion method

The selected bryophyte species were tested for their antimicrobial efficacy using the disc diffusion method (18). Discs of the respective plant samples were placed in Petri plates with a lower base layer of PDA and NA and an upper base layer of fungal and bacterial spore suspension respectively. Fluconazole disc ($10 \mu\text{g ml}^{-1}$) and tetracycline ($10 \mu\text{g ml}^{-1}$) disc were inserted in the center of the Petri plate as positive control and subsequently incubated for 24 h at 28°C and 37°C , respectively. The zone of inhibition (ZOI) in mm was used to measure the activity.

Statistical analysis

The results are shown as means ($n = 3$) of triplicates. All the collected data were analysed using the IBM SPSS Statistics 20 software. The chosen variable was subjected to three-way interactions. Tukey's $p < 0.05$ post-test was used to compare the variance of data for each output variable multiple-comparison. All data are presented as a mean with standard error.

Results

Quantitative analysis and antioxidant assay

Initially, in this study, the gametophytes of selected bryophytes were analysed concerning their phytochemical contents, as they are rich in phenolics and flavonoids. These chemicals have antioxidant properties which help in neutralizing lipid free radicals and prevent hydroperoxides from decomposing into free radicals, contributing to plants all over antioxidant activities (19). A linear calibration curve of Gallic acid and Quercetin with a coefficient of determination $R^2 = 0.99$ was used to determine the TPC and TFC. The TPC of *P. appendiculatum* and *S. fimbriatum* extracts were found to be $30.04 \text{ mg} \pm 0.25 \text{ GAE/gdw}$ and $41.02 \text{ mg} \pm 0.33 \text{ GAE/gdw}$ (Mean \pm S.D, $n=3$) respectively. The TFC of *P. appendiculatum* and *S. fimbriatum* extracts were found to be $24.03 \text{ mg} \pm 0.22 \text{ QE/gdw}$ and $38.09 \text{ mg} \pm 0.35 \text{ QE/gdw}$ (Mean \pm S.D, $n=3$) respectively (Fig. 1A).

The radical scavenging activity of antioxidants lowers the absorption of DPPH which leads to a change in color from purple to yellow. NO is a signalling molecule that also serves as a vasodilator, neuronal messenger and other functions. Overproduction of this free radical harms metabolism and can lead to inflammation, cancer and other problems. The production of hydroxyl radical and nitric dioxide can be caused by NO (20). The antioxidant activity of *P. appendiculatum* and *S. fimbriatum* as IC_{50} value against DPPH and NOSA were found to be 56.07 ± 0.46 , 54.04 ± 0.44 and 54.01 ± 0.42 , 53.01 ± 0.42 ($\mu\text{g/ml}$; Mean \pm S.D, $n=3$) respectively. The higher the value of IC_{50} , the lower the antioxidant activity (Fig. 1B).

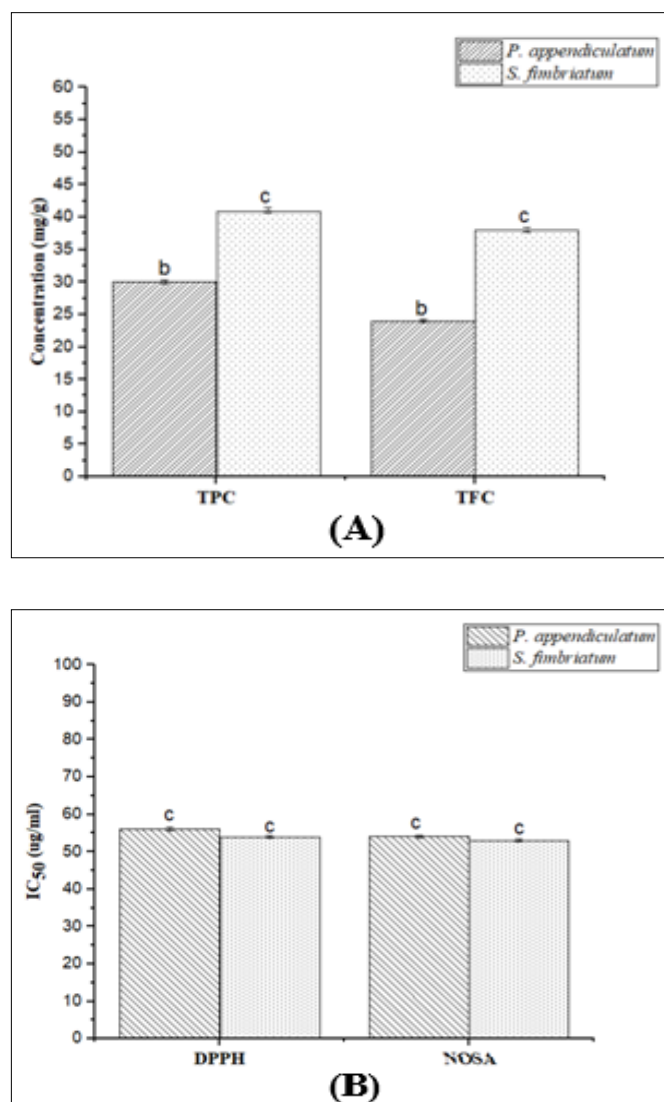


Fig. 1. (A) Quantitative analysis (B) Antioxidant assay of selected bryophyte species.

Antimicrobial activity

Two bryophyte species, viz., *P. appendiculatum* and *S. fimbriatum* were examined for resistance against two fungal and bacterial strains viz. *Fusarium solani*, *Aspergillus niger* and *Escherichia coli*, *Bacillus subtilis*, respectively. A synthetic fungicide fluconazole and bactericide tetracycline were employed as positive controls to compare their activity. The acquired data suggested that these two bryophyte species have antifungal and antibacterial activities (Table 1-2; Fig. 2).

Discussion

During this study, it was observed that both thalloid liverwort and moss are rich in phytoconstituents which have substantial antioxidant and antimicrobial activities. The findings are in accordance to the earlier reports in many bryophytes (20, 21). The observed antioxidant activity could be attributed to the abundance of total phenol and total flavonoid contents in these bryophytes. These flavonoids and phenolic compounds have noteworthy medicinal potential due to their action and have been shown to decrease free radical oxidation and increase antioxidative enzyme activity (22-25) which improves the overall metabolism.

Table 1. Antifungal activity of selected bryophyte species

Sl. No.	Species name	Zone of inhibition (Fungal species)					
		<i>Fusarium solani</i>			<i>Aspergillus niger</i>		
		PE	Ab	Control	PE	Ab	Control
1.	<i>P. appendiculatum</i>	14±0.21 ^b	21±0.45 ^c	08±0.10 ^a	12±0.15 ^b	22±0.20 ^c	07±0.05 ^a
2.	<i>S. fimbriatum</i>	12±0.13 ^b	23±0.38 ^c	07±0.06 ^a	11±0.22 ^a	22±0.29 ^c	08±0.06 ^a

PE (plant extract), Ab (fluconazole), Control (methanol), Data are the means and standard deviation of the mean for $n = 3$ independent experiments.

Table 2. Antibacterial activity of selected bryophyte species

Sl. No.	Species name	Zone of inhibition (Bacterial species)					
		<i>Escherichia coli</i>			<i>Bacillus subtilis</i>		
		PE	Ab	Control	PE	Ab	Control
1.	<i>P. appendiculatum</i>	11±0.21 ^b	24±0.42 ^c	07±0.10 ^a	09±0.15 ^b	21±0.21 ^c	05±0.04 ^a
2.	<i>S. fimbriatum</i>	10±0.13 ^b	23±0.38 ^c	09±0.06 ^a	09±0.22 ^a	22±0.29 ^c	08±0.04 ^a

PE (plant extract), Ab (tetracycline), Control (methanol), Data are the means and standard deviation of the mean for $n = 3$ independent experiments.

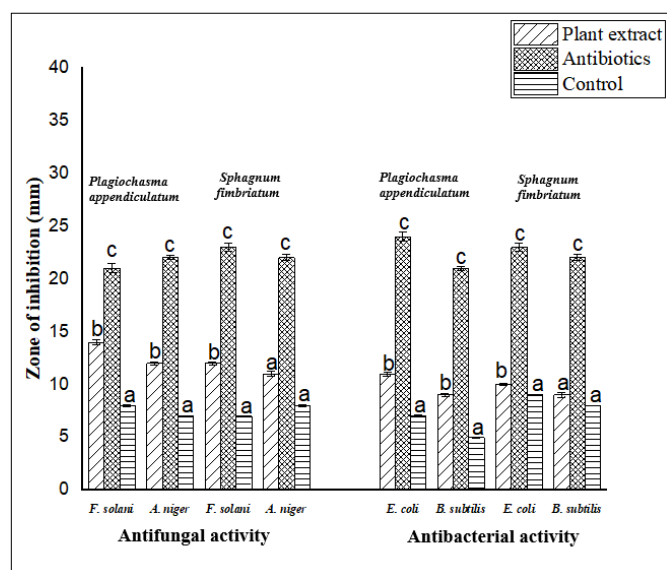


Fig. 2. Comparative study of antifungal and antibacterial activity of selected bryophyte species.

The *in-silico* crystal structure modelling showed that total phenol and total flavonoid contents, particularly flavonoids can be a good candidate for binding and inhibiting SARS-CoV-2 S and ACE-2 proteins (26). Likewise, another study showed that plant derived Quercetin has inhibitory potential against SARS-CoV-2 infection (27). Hence, it is widely assumed that the phytoconstituents of pharmaceutical significance can be used as anti-inflammatory and antioxidant compounds to lessen or avoid the severity of the various pathogenic diseases.

Consequently, many attempts have been made to assess medicinal and nutraceutical potential of bryophytes and it was shown that these amphibious plants are the precious reservoir of antioxidative, anti-cancerous, antimicrobial and antiviral compounds (21). Hence, the bioactivity of the selected bryophytes was evaluated against few microbial strains, and the extracts showed remarkable antimicrobial efficacies and emerged as the useful ingredients to be used in future remedies against the microbial attacks in an eco-friendly way.

However, in comparison to higher plants, the bryo-

phytes have been lesser known for their medicinal and nutritional significance due to their small size, difficulties in collection and subsequent identification an unavailability of desired biomass of single species. These constrains limit their worth economically though they have unparalleled and imperative ecological impact.

Conclusion

In conclusion, the extracts of the bryophytes in the present study exhibited substantial potential as an antioxidant and antimicrobial agent. The obtained results highlight the need for further study, particularly of bioactive compounds, which could be of use to the pharmaceutical, food industries and cosmetics. The hugely diverse second largest group of land plants is remained unexplored for phytochemistry and can serve as a hoard of valuable biologically active secondary metabolites in future.

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Authors contributions

SJ carried out the antimicrobial activity studies and drafted the manuscript. SS and RS carried out the phytochemical and antioxidant assay. SV and GPN participated in the design of the study and performed the statistical analysis. AA conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: There are no conflicts of interest relevant to this article, declared by the authors.

Ethical issues: None.

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