Research Article

Phytochemical Profiling of *Bryum argenteum* Hedw. and *Dumortiera hirsuta* (Sw.) Nees.

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ABSTRACT

Aim: Bryophytes are the second largest group of land plants. This study involved phytochemical profiling on a liverwort representative from a primitive group and one moss specimen from an advanced group. **Materials and Methods:** The Powdered thallus of *B. argenteum* and *D. hirsuta* underwent successive solvent extraction based on polarity. The resulting crude extracts were subjected to preliminary qualitative and quantitative analysis. The phytochemical profiling was conducted using Thin Layer Chromatography. **Results and Discussion:** Both plants contain phenols, flavonoids, alkaloids, terpenoids, and tannins, according to their preliminary phytochemical profiles. The total phenolic concentration in *B. argenteum* is 24.90±0.12 mgGAE/g and in *D. hirsuta* is 26.70±0.25 mgGAE/g. TLC profiling with the mobile phase hexane: ethyl acetate: chloroform: formic acid (3:1:0.5:0.5 v/v/v/v) gives the highest separation. Among various solvent extracts of *B. argenteum* ethyl acetate shows the presence of 8 compounds having R_f of 0.16, 0.56, 0.70, 0.73, 0.76, 0.80, 0.83, 1.66 similarly there are 7 differentiated bands obtained from ethyl acetate extract of *D. hirsuta* with R_f values 0.73, 0.76, 0.63, 0.8, 0.46, 0.43 and 0.03. Every R_f represents a compound within the plant extracts. **Conclusion:** Thus the present investigation shows that these two plants contain various potential metabolites having different therapeutic values. Therefore, there is no need to neglect these tiny plants because they are a rich pool of medicinally important components.

Keywords: Bryophytes, Phytochemical profiling, Physicochemical parameters, Thin layer chromatography.

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INTRODUCTION

Secondary metabolism refers to the metabolic pathways and small molecules generated by an organism that is not essential for its growth or reproductive processes, in contrast to primary metabolism.[1] They play a vital role in different development within the plant body, signaling and defense.[2, 3] Numerous metabolites are advantageous to human health in various ways.[4, 5] Alkaloids, flavonoids, phenolics, terpenes, and steroids are major secondary metabolite groups within plants.[6] Nowadays, researchers are interested in the field of plant metabolism and metabolic products due to the high demand for green products. Industries use naturally derived metabolites as medicine, food supplements and perfumes.[7] Like the other group of plants bryophytes also possess a vast array of metabolites within their thallus. These wide varieties of metabolites protect the cells of an ancient class of terrestrial plants from environmental factors and pathogens.[8] More than 14,000 mosses (Bryophyta), 6,000 liverworts (Marchantiophyta), and 300 hornwort species (Anthocerotophyta) are among the three categories of bryophytes.[9] Many cultures across various continents have employed bryophytes as a means of treating various health issues. Indian and Chinese medicine claims that their uses vary greatly, ranging from curing fevers to healing skin infections and as a pain reliever.[10,11] Traditional Chinese medicine uses different bryophytes to cure skin diseases and eye infections.

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Marchantia polymorpha, *Rhodobryum giganteum* and *Sphagnum* are some of these.[12, 7] Some tribal communities in India use bryophytes for similar diseases and hair treatment.[13] The simplicity in morphology doesn't occur in the internal plant body, they are chemically diverse in nature.[14] This study intends to highlight bryophytes as a prospective reservoir of chemicals with a wide range of possible applications, as well as significant resources for future research.

MATERIALS AND METHODS

Plant Material Collection

The plant materials *B. argenteum* and *D. hirsuta* were collected from different areas of Kodaikanal, Tamil Nadu. The Bryology Division of the Department of Botany of the University of Calicut recognized and authenticated the specimens. Voucher specimens were deposited in Calicut University herbarium (CALI) bearing collection numbers 194671 (CALI) and 194673 (CALI) respectively. The plant material was washed and dried in a shady environment at room temperature before being crushed to powder using a mixer grinder.

Preparation of Plant Extracts

The plant material was subjected to a series of solvent extractions with petroleum ether, chloroform, ethyl acetate, ethanol, and distilled water in order. The extracted components were concentrated using a rotary evaporator and kept in sealed containers at 4ºC for later use.

% yield is calculated using the following formula;
Yield=W1/W2*100

W1- The weight of extract residue after solvent removal.

W2- The weight of the powdered sample taken for extraction.

Physico-chemical parameters.

Standard procedures were used to examine physico-chemical characteristics such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive, and volatile oils.[15]

Preliminary qualitative analysis

Qualitative phytochemical analysis of a moss *B. argenteum* and *D. hirsuta* extract was done by the standard protocol to determine whether certain bioactive chemicals are present or not.[16,17]

QUANTITATIVE ESTIMATION

Total Phenolic Content (TPC)

The analysis of the total phenolic content in the extract was conducted by employing the Folin-Ciocalteu reagent.[18] An aliquot of 0.5 mL of the extract was treated with 2.5 mL of Folin–Ciocalteu reagent. After 5 min, add 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution. The reaction mixture was incubated for 15 min at 45°C. The blue color developed was detected at 765 nm. Gallic acid served as the reference standard. The results were represented as milligrams of gallic acid equivalents (mg GAE) per gram of sample dry weight.

Total Flavonoid Content

The total flavonoid content of the extract was ascertained using the aluminum chloride colorimetric method, according to Woisky and Salatino.[19] 0.5 mL of extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate (CH_3COOK), and 2.8 mL of distilled water. The blend was left to incubate for 30 min at ambient temperature. At 415 nm, the absorbance was determined. The standard was maintained to be quercetin. The unit of measurement for TFC is milligrams of Quercetin Equivalent (mg QE)/g of dry sample weight.

Total Tannin Content

The tannins were determined by the Folin-Ciocalteu method with gallic acid as the standard. Using a UV/Visible spectrophotometer, the absorbance of the test and standard solutions was determined to the blank at 725 nm. In terms of mg of GAE/g of extract, the tannin concentration was stated.

Thin layer chromatography (TLC)

The TLC analysis was done with Silica gel 60 F_{254} TLC (Merck, Germany) plate, hexane: ethyl acetate: chloroform: formic acid (3:1:0.5:0.5 v/v/v/v) as the mobile phase. Each solvent extracts were spotted on a silica plate with glass capillaries. Pour the solvent system into the TLC chamber and close with the lid for 15 min to make the system saturated. Then place the marked plate within the chamber and allow rising. Mark the solvent front immediately after taking the plate from the chamber. After being exposed to UV 254 and UV 365nm light without any chemical treatment, the chromatograms were examined again with the spray reagents. R_f value was calculated.

R_f=distance travelled by the solute front/ distance travelled by the solvent front.

RESULTS

Physico-chemical parameters

Physicochemical parameter measurement plays a vital role in detecting adulterants and assessing improper drug handling. Ash values serve as essential quantitative

benchmarks and criteria for evaluating the origin and quality of illicit substances, especially in powder form.[20] The analysed physico-chemical parameters of *B. argenteum* and *D. hirsuta* are given in Table 1.

Table 1: Physico-chemical parameters.

Parameters	*B. argenteum*	*D. hirsuta*
Total ash	32.835	32.13
Acid insoluble ash	20.268	20.61
Water soluble ash	29.38	30.15
Alcohol soluble extractive	1.94	1.11
Water soluble extractive	3.86	3.13
Volatile oil	Nil	Traces

The percentage yield of extracts

The yields of the extract are displayed in Table 2. The obtained crude extracts were further lyophilized and stored at -20ºC.

Preliminary Qualitative analysis

In addition to primary metabolites such as proteins and carbohydrates, plants also possess various secondary metabolites, as indicated by an initial phytochemical analysis of five solvent extracts (Table 3). This suggests that because of their strong polarity, these solvents are useful for isolating biologically active molecules.[21] The thallus of *B. argenteum* contains primary polyphenols, including phenolics, flavonoids, and tannins, along with the presence of terpenoids and alkaloids. Similar metabolites are also identified in the solvent extracts of *D. hirsuta*. The phytochemical investigation of *Dicranum scoparium* and *Plagiochasma appendiculatum* also shows the presence of phenols, flavonoids, terpenoids and alkaloids.[22]

Table 2: Percentage Yield of various solvent extracts.

Solvent extracts		Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Distilled water
Percentage yield (%)	*B. argenteum*	0.22	1.09	0.65	0.34	0.16
	D. hirsuta	0.41	1.12	1.51	0.57	0.24

Table 3: Preliminary phytochemical analysis of various solvent extracts.

Metabolites	PE		CH		EA		ETH		DW	
	B	D	B	D	B	D	B	D	B	D
Saponins	-	-	-	-	-	-	-	-	-	+
Tannins	+	+	+	+	+	+	+	+	+	+
Terpenoids	-	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	-
Alkaloids	+	+	-	-	+	+	+	+	+	+
Carbohydrates	-	+	-	-	+	+	+	+	+	+
Proteins	-	-	+	+	+	+	+	+	+	+

(**PE**- Petroleum ether, **CH**- Chloroform, **EA**- Ethyl acetate, **ETH**- Ethanol, **DW**- Distilled water, + Presence, - Absence/not detected, **B**- *B. argenteum*, **D**- *D. hirsuta*)

Quantitative estimation

The phenol concentration was highest in the ethyl acetate extract of both plants *B. argenteum* and *D. hirsuta* i.e., 24.90±0.12 mgGAE/g and 26.70±0.25 mgGAE/g and minimum in aqueous extract. The situation is the same in the case of the concentration of flavonoids (Table 4). The results of Vidisha Kandpal *et al*., provided support for this outcome.[23, 24]

Polyphenols are high-potential molecules with antioxidant property that scavenges the oxy-free radical that causes much damage to cells.[25] The therapeutic potential of plant-based preparations is related to the content of polyphenols within it.[26] This finding demonstrates the levels of polyphenolic components in the two bryophyte plant groups, indicating the biological functions of the plant extract.

Thin Layer Chromatography (TLC)

The TLC visualization of crude extract of *B. argenteum* and *D. hirsuta* revealed the presence of different compounds with different R_f values (Table 5). Among various solvent extracts of *B. argenteum,* ethyl acetate

Table 4: Quantitative estimation of various solvent extracts.

Concentration (mg/g)	*D. hirsuta*					*B. argenteum*				
	PE	CH	EA	ETH	AQ	PE	CH	EA	ETH	AQ
PHENOLS	13.43±0.29	23.04±0.30	26.70±0.25	25.97±0.07	9.29±0.07	15.15±0.49	16.60±0.40	24.90±0.12	24.18±0.02	16.44±0.33
FLAVONOIDS	10.01±0.00	13.94±0.18	23.38±0.04	12.80±0.05	7.34±0.17	10.35±0.0.33	12.97±0.62	20.84±0.56	16.44±0.01	2.82±0.00
TANNINS	6.65±0.01	11.01±0.02	12.23±0.05	13.92±0.01	7.82±0.23	9.50±0.25	13.67±0.04	14.03±0.49	12.62±0.30	10.90±0.30

(Values= Mean±SE).

shows the presence of 8 compounds having R_f of 0.16, 0.56, 0.70, 0.73, 0.76, 0.80, 0.83, 1.66. With the ethanol and chloroform extract 4 compounds having R_f 0.83, 0.76, 0.70, 0.56 (Figure 1A). Compared to the liverwort *D. hirsuta*, the moss *B. argenteum* shows a higher number of bands on TLC. There are 7 differentiated bands obtained from ethyl acetate and chloroform extract of *D. hirsuta* with R_f values 0.73, 0.76, 0.63, 0.8, 0.46, 0.43 and 0.03 (Figure 1B). Each banding visualized on TLC represents various metabolites in plant extracts.

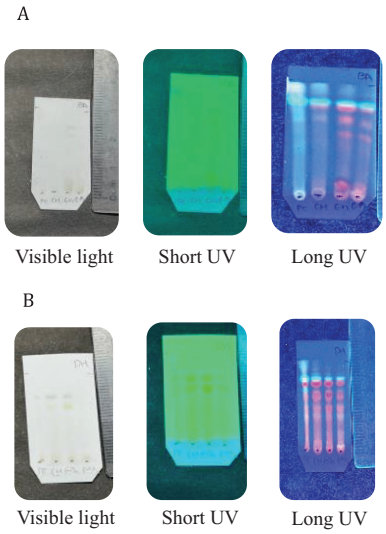

Figure 1: A: TLC profile of extracts of *B. argenteum* in visible light, short UV, Long UV, B: TLC profile of extracts of *D. hirsuta* in visible light, short UV, Long UV.

Table 5: Number of compounds with R_f values.

Visualizing lights	R_f values							
	B. argenteum				*D. hirsuta*			
	PE	CH	EA	ETH	PE	CH	EA	ETH
Visible light	-	-	0.73 0.80	-	0.73	0.73 0.63	0.73 0.63	-
254 nm	-	0.76 0.73	0.76 0.73 0.16	-	0.76	0.76 0.63	0.76 0.63	-
366 nm	0.83	0.83 0.76	0.83 0.76 0.70 0.56 0.16	0.83 0.76 0.70 0.56	0.8 0.76 0.73 0.63 0.46 0.43	0.8 0.73 0.63 0.46 0.43 0.03	0.8 0.73 0.63 0.46 0.43 0.03	0.8 0.73 0.63 0.46 0.43 0.03

(**PE**- Petroleum ether, **CH**- Chloroform, **EA**- Ethyl acetate, **ETH**- Ethanol, - Absence/ not detected)

DISCUSSION

The amphibians of the plant kingdom, which thrive near the soil, may appear simple in their external structure, but their internal chemistry is anything but complicated.The phytochemical screening of crude solvent extracts from the thallus of *D. hirsuta* and *B. argenteum* showed a wide range of naturally occurring compounds such as phenols, flavonoids, terpenoids, tannins, and alkaloids with high physiological activity. According to Kessler A and Kalske A, a wide range of metabolites produced by plants interact with both their biotic and abiotic environments.[6] Likewise, the thallus of bryophytes contains powerful compounds to develop immune resistance to natural predators.[27] The studies done by Haines W P and Renwick support that the insect deterrent property of these plants is due to the chemical barriers.[28]

The quantitative estimation of all crude extracts reveals an elevated level of polyphenols. Compared to the alcohol extract of *Brachythecium buchananii*, phenols and flavonoids are the most abundant in these two plants and they provide significant health advantages.[29] Di Lorenzo C *et al*., documented the health benefits of phenolic compounds. Regular consumption of phenolic compounds has been linked to a lower risk of cancer and cardiovascular disease, because of their antioxidant capabilities.[30] Similar studies conducted on flavonoids by Karak P point out the anti-inflammatory and anti-metastatic properties of flavonoids, which contribute to overall health improvement.[31] These findings are consistent with previous studies done on *Hyophila involuta, Dicranum scoparium* and *Plagiochasma appendiculatum* by Ramya *et al*., (2015) and Bhadauriya G. *et al*., (2018).[32-34] These data highlight the abundance of bioactive phytochemicals in bryophytes that support their biological functions and establish them as a prospective natural source of polyphenols.

According to the TLC profiling of both the plant maximum number of compounds obtained in the ethyl acetate extract. There are only a few R_f values common to both plants. That denotes the difference in the chemical composition of mosses and liverworts. It is essential to separate the active compound from the crude extract for the biological purpose.[35] Waksmundzka- Hajnos M points out that Thin-layer chromatography is the simplest and fastest way of separation technique to analyze the chemical profiling of metabolites.[36] A clear primary understanding of the number of compounds present in the plant extract and their pattern of separation in the solvent systems was provided by the TLC profiling carried under various light wavelengths. This information will support and improve purification in advanced research. These chemical compounds derived from plants are increasingly being used in a variety of industries, specifically targeting the metabolites generated within their plant structures.

Native cultures in North America, India, and China have long used bryophytes in traditional

medicine.[13,37] Chandra S *et al*., documented the medicinal property of *B. argenteum* and *D. hirsuta*.[10] But there is no authentic report of the principle component that lead the biological potentialities of these plants. Determining the precise functionality and structure-activity correlations of the effects of phytochemicals on biological systems is difficult based on the data found in this study. Its difficulty mainly arises from the multitude of phytochemicals with similar chemical compositions and the complexity of physiological reactions. Whereas these findings provide scientific support for the in-depth research on the chemical components that contribute to the plant's potential for medicinal use.

SUMMARY

A comprehensive analysis of the phytochemical composition of D. *hirsuta* and *B. argenteum* revealed their richness in various secondary metabolites. Preliminary examination identified a range of major phytochemicals, with quantitative assessment indicating a phenolic concentration of approximately 26.70±0.25 mgGAE/g in *D. hirsuta* and 24.90±0.12 mgGAE/g in *B. argenteum*. Through TLC analysis, the presence of up to eight compounds was identified. This preliminary screening sheds light on the chemical constituents harbored within these diminutive plants.

CONCLUSION

The outcomes of the current study highlight that the thallus of *B. argenteum* and *D. hirsuta* serve as a significant reservoir of secondary metabolites. These results propose that both plants hold promise as potential sources of natural compounds with considerable therapeutic value for treating infections caused by various organisms. The analysis conducted indicates that amphibians in the plant kingdom can be harnessed for the environmentally friendly synthesis of pharmaceuticals and nutraceuticals. Our future research endeavors will prioritize further purification, identification, and characterization of the active compounds present in these plants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

mgGAE/g: Milligram Gallic acid equivalent per gram; **R_f:** Retention factor; **TLC:** Thin Layer Chromatography.

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