

UV-VIS, FT-IR and GC-MS Profiling of Bioactive Compounds from Moss *Bryum argenteum* Hedw.

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Abstract

The main aim of the present study was to analyze and evaluate the bioactive compounds from hexanic extract of moss *Bryum argenteum* Hedw.'s non-polar (hexane) extract using UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy (FT-IR) and Gas Chromatography Mass Spectroscopy (GC-MS) techniques. Spectral data of UV-Visible analysis revealed the presence of flavonoids, phenolics compounds and terpenoids, and FTIR spectra's absorption peaks displayed distinct vibrational stretching including 'C-H, C-O, C-N, C=C and O-H bending' and supporting the presence of lipophilic substances, including alkyne, alkane, aromatic compounds, alcohol, alkene, phenols and amine. Hexane extract's GC-MS investigation resulted a complex profile of thirty-three semi-volatile and volatile bio-active compounds. With an 84.96% of peak-area, the non-polar molecule, 2,4-dimethyl-pentane was the principal compound of moss's hexane extract. These uncovered secondary-metabolites displayed differ pharmaceutical values like anti-bacterial, anti-cancerous, allelopathic, hypocholesterolemic, anti-candidal anti-oxidant, nematocide, and anti-inflammatory. This research's combined analytical analysis, highlights the chemical diversity of *Bryum argenteum*'s hexane extract and suggests that its non-polar metabolites may have pharmacological significance in drug-discovery. Further investigations must be concentrated on detailed bioassay and structural clarifications of individual moss-derived natural compounds.

Keywords: *Bryum argenteum* Hedw.; Moss; Hexane Extract; UV-Visible Spectroscopy; FTIR; GC-MS

Introduction

Mosses belongs to first plants group that grow on land, "bryophytes" and include roughly 10000 species of mosses [1-3]. Owing to taxonomic complexity and their minute size, mosses' phytochemistry have long been marginalized and leading to the perception that they hold limited substantial bioactive potential [4]. While, research on mosses have some attention, predominantly limited to specific chemical classes-such as lipids, fatty-acids and volatile oils- rather than exploring their practical or industrial applicability [5,6]. The beginning of an arising body of research looking into fat-soluble metabolites in mosses species, exemplified by a 'GC-MS investigation' of *Hypnum cupressiforme* that unveiled hydrocarbons, fatty esters, and terpenoids with possible pharmacological potential [1].

Traditional medicinal practices have included the use of several mosses for a range of health conditions. For instance, *Cratoneuron filicinum* has been utilized to manage cardiac conditions [7,8], whereas *Philonotis fontana* and *Mnium* species serve in the treatment of burn, wounds and as antipyretic [2,9]. *Rhodobryum roseum* has been employed as a sedative in cardio-vascular diseases [2,10]. In

addition to these ethno-medicinal applications, mosses have demonstrated 'antimicrobial, cytotoxicity, anti-bacterial, anti-oxidant' activities in recent studies [11-14]. Mosses have been historically used by North-American's indigenous communities as remedies for various conditions, particularly in the cure of skin injuries and illnesses, as well as treating neurological and respiratory conditions, including tuberculosis, pneumonia, neurasthenia, among others [15,16]. The integration of phytochemical profiling of moss extracts has been achieved through conjunction with classical screening methods and sophisticated chromatographic and spectroscopic approaches. Gas chromatography-mass spectrometry (GCMS) is especially suited for detecting lipid-soluble metabolites like sterols, fatty-acid methyl esters, and terpenoids [17,18]. Despite mosses are becoming growing in popularity as prospective providers for emerging bioactive metabolites, insufficient is understood about their chemical diversity in juxtaposition with vascular plants.

This research seeks to characterize the non-polar (hexane) extract of a moss species, *B. argenteum* and to profile lipid-soluble bioactive metabolites by employing an integrated analytical strategy (UV-Visible spectroscopy, FT-IR, GC-MS), the finding are intended to enhance the expanding knowledge of moss-based products for prospective therapeutic benefits.

Material and Methods

Sample collection and identification

Bryum argenteum Hedw. was collected from moist wall habitat at Mount Abu (Rajasthan) during rainy season of 2022. The collected plant sample was identified based on morphological characteristics with the aid of relevant moss floras; 'Mosses of Eastern India and adjacent Regions' [19]; 'Moss Flora of Rajasthan (India)' [20] and subsequently authenticated by a moss expert Dr. G.S. Deora (Mohanlal Sukhadia University, Udaipur, Rajasthan).

Non-polar (n-Hexane) extraction

Collected fresh moss samples were cleaned using tap water and double distilled water, air-dried under shade at room temperature and subsequently powdered. Preparation of crude non-polar extract of selected moss plant was done by Soxhlet extraction method and in this process, hexane solvent (100 ml) and sample powder (10g) were employed in 1:10 w/v ratio. The yield of hexane extraction (%) was calculated as 17% and stored at 4°C until further analysis.

UV-visible and FTIR analysis

After diluting the non-polar crude extract with hexane solvent (1 mg/mL), it was scanned with 'UV-visible true double-beam spectrophotometer (Motras Scientific, India)' to recognize an array of chemical substances in *Bryum argenteum*'s hexane extract that appear as absorption bands. The qualitative UV-Visible Spectrum and its peak values were captured at 200 - 700 nm wavelength ranges.

FTIR-spectra was obtained by using 'FTIR spectroscopy (Bruker alpha Eco-ATR)' in the scan range of 4000 - 600 cm^{-1} and the functional groups were interpretate from FTIR-spectra comparing with standard reference spectra [21].

GC-MS conditions

The chemical composition was analysed using 'GC-MS-QP2010 Ultra Shimadzu, Japan'. Helium was the carrier gas at a constant flow-rate of 16.3 mL/min. The oven's temperature had been configured from 70°C to 300°C at a rate of 10°C per minute. The injector temperature was set at 260°C and injector volume was 1 μL . The structure, molecular- weight and name of unknown bio-active substances were documenting based on their mass-spectra and chromatograms and comparing findings with NISTM1 library.

Results

UV-visible spectral analysis

Thirteen peaks of non-polar metabolites were uncovered in UV-Visible profiles of selected moss's hexane extract at 203, 211, 259.5, 271, 307.5, 332.5, 344, 356.5, 358, 360, 400, 473, 669 nm with absorption-ranging from 0.3 to 4.0. The spectrum (Figure 1) exhibited

peaks from 211 to 344 nm, which were associated with flavonoids and phenolic constituents. The occurrence of terpenoids revealed by absorbance at 400 and 473 nm while a band at 669 nm corresponds to chlorophyll content in the extract. Characteristic absorption ranges for flavonoids and phenolic compounds have been reported in earlier studies at 230-290 nm (band I), 300-350 nm (band II) and broadly from 234-676 nm. Peaks within 400-550 nm have been correlated with terpenoids compounds in prior studies [22-24].

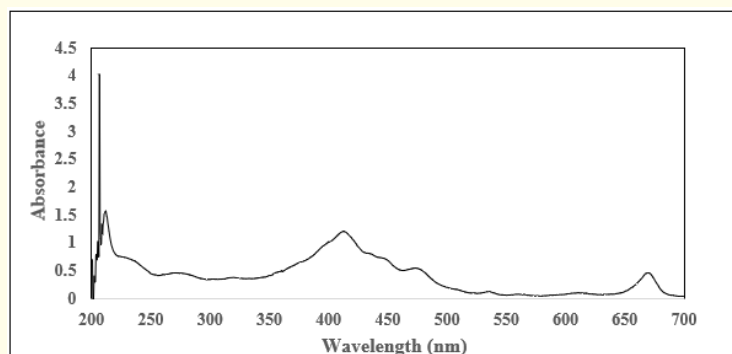


Figure 1: UV-visible spectrum of hexane extract of *Bryum argenteum*.

FT-IR spectroscopic characterization

Ten different frequency ranges showed the presence of functional group in hexane extract's FT-IR spectra of *B. argenteum* (Figure 2 and table 1). A prominent sharp peak at 2954.43 cm^{-1} corresponds to 'C-H stretching of alkyne', while signals at 2922.86 cm^{-1} and 2863.25 cm^{-1} were characteristic of 'C-H vibrations', indicative of alkane group. The 'C-H bending' were detected around 1714.71 cm^{-1} and 1460.45 cm^{-1} associated with aromatic-compound and alkane respectively. An O-H bending at 1378.78 cm^{-1} suggesting the presence of phenol. Stretching vibrations at 1140.28 cm^{-1} and 1057.28 cm^{-1} were assigned to 'C-O stretching and C-N stretching' groups respectively. Additionally, 'C=C stretching' of alkene appeared at 890.18 cm^{-1} and 724.24 cm^{-1} .

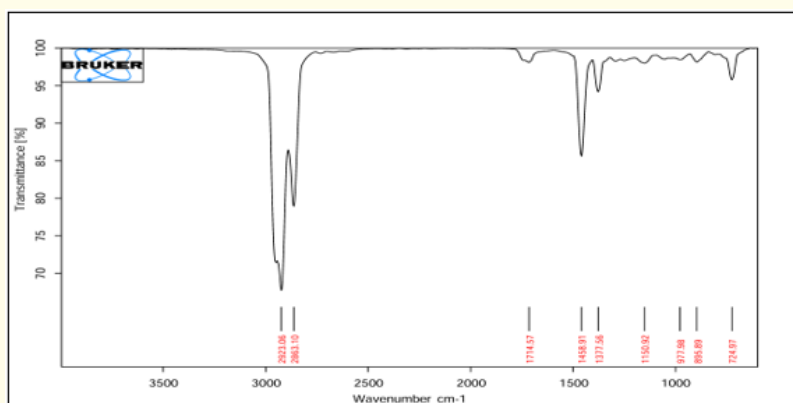


Figure 2: FTIR spectrum of *Bryum argenteum*'s hexane extract.

S. No.	Frequency ranges (cm ⁻¹)	Frequency- peaks (cm ⁻¹)	Stretching	Functional group
1	3000-2500	2954.43	C-H stretching	Alkyne
2	3000-2840	2922.86	C-H stretching	Alkane
3	3000-2840	2863.25	C-H stretching	Alkane
4	2000-1650	1714.71	C-H bending	Aromatic compound
5	1600-1300	1460.45	C-H bending	Alkane (methylene group)
6	1390-1310	1378.78	O-H bending	Phenol
7	1400-1000	1140.28	C-O stretching	Alcohol
8	1250-1020	1057.28	C-N stretching	Amine
9	1000-650	890.18	C=C bending	Alkene (vinylidene)
10	730-665	724.24	C=C bending	Alkene

Table 1: The results of non-polar (Hexane) extract’s FTIR spectrum of *B. argenteum*.

GC-MS analysis of hexane extract

Non-polar bio-active chemical diversity with relative-peak area were obtained from hexane extract of selected moss species *Bryum argenteum* Hedw. by employing GC-MS analysis. The analysis resulted in the detection of thirty-three distinct non-polar constituents in moss *B. argenteum*. Their identities were achieved through spectral matching with reference databases, including spectral library of NIST and Willey. ‘GC-MS detailed data with retention-time (RT), relative-abundance, peak area, compound’s name, molecular-weight and formulas are shown in table 2. Hexane extract’s GC-MS chromatogram is showed by figure 3.

The non-polar compound, 2,4-dimethyl-pentane dominated the GC-MS profile with 84.96% peak-area, indicating its abundance in hexane extract. Conversely, minor constituents, including 6,10,14-Trimethyl-2-pentadecanone, Phthalic acid, methyl 2-hydroxy-octadeca-9,12,15-trienoate among others were present in minimal quantities, each contributing approximately 0.01%.

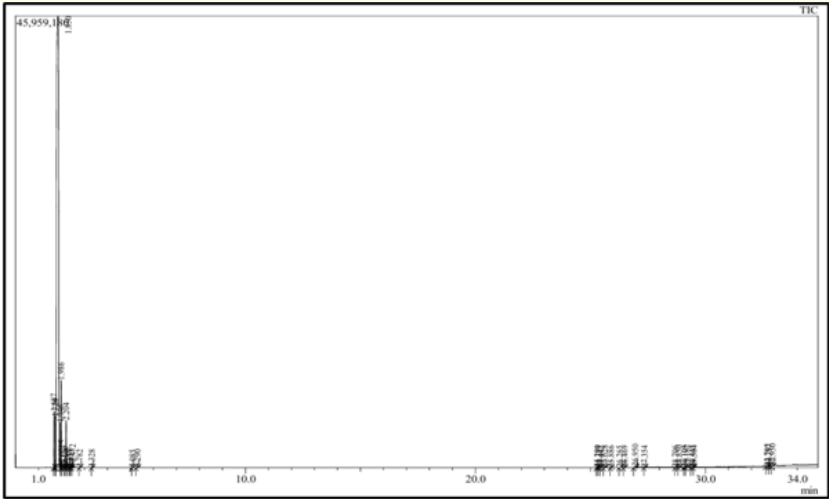


Figure 3: Hexane extract’s GC-MS chromatogram of *Bryum argenteum*.

Peak no.	RT (min)	Peak area (%)	Identified compounds	Molecular weight	Molecular formula
1	1.687	4.46	2-Methyl-pentane	86	C ₆ H ₁₄
2	1.726	2.87	3-Methyl-pentane	86	C ₆ H ₁₄
3	1.850	84.96	2,4-Dimethyl-pentane	100	C ₇ H ₁₆
4	1.946	0.71	2,2-Dimethyl-pentane	100	C ₇ H ₁₆
5	1.986	3.98	Methyl-cyclopentane	84	C ₆ H ₁₂
6	2.021	0.22	2,2,3,4-Tetramethyl-pentane	128	C ₉ H ₂₀
7	2.136	0.13	3,3-Dimethyl-pentane	100	C ₇ H ₁₆
8	2.204	1.85	Cyclohexane	84	C ₆ H ₁₂
9	2.265	0.09	3-Methyl-hexane	100	C ₇ H ₁₆
10	2.357	0.02	2,3-Dimethyl-hexane	114	C ₈ H ₁₈
11	2.413	0.01	Trans-1,2-Dimethyl-cyclopentane	98	C ₇ H ₁₄
12	2.472	0.19	3,4-Dimethyl-heptane	128	C ₉ H ₂₀
13	2.782	0.01	Methyl-cyclohexane	98	C ₇ H ₁₄
14	3.328	0.02	Toluene	92	C ₇ H ₈
15	5.085	0.01	Ethylbenzene	106	C ₈ H ₁₀
16	5.290	0.03	o-Xylene	106	C ₈ H ₁₀
17	25.290	0.02	Neophytadiene	278	C ₂₀ H ₃₈
18	25.357	0.01	6,10,14-Trimethyl-2-pentadecanone	268	C ₁₈ H ₃₆ O
19	25.462	0.01	Acetic acid 3,7,11,15-tetramethyl-hexadecyle ester	340	C ₂₂ H ₄₄ O ₂
20	25.628	0.04	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	C ₁₆ H ₂₂ O ₄
21	25.886	0.01	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	C ₂₀ H ₄₀ O
22	26.265	0.01	Phthalic acid, cyclohexylmethyl butyl ester	318	C ₁₉ H ₂₆ O ₄
23	26.469	0.01	Methyl-hexadecanoic acid	270	C ₁₇ H ₃₄ O ₂
24	26.950	0.12	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂
25	27.354	0.01	Ethyl-hexadecanoic acid	284	C ₁₈ H ₃₆ O ₂
26	28.706	0.01	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	292	C ₁₉ H ₃₂ O ₂
27	28.850	0.02	Phytol	296	C ₂₀ H ₄₀ O
28	29.108	0.02	(Z,Z)-9,12-Octadecadienoic acid	280	C ₁₈ H ₃₂ O ₂
29	29.182	0.09	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	278	C ₁₈ H ₃₀ O ₂
30	29.445	0.02	Octadecanoic acid	284	C ₁₈ H ₃₆ O ₂
31	29.504	0.01	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	308	C ₁₉ H ₃₂ O ₃
32	32.757	0.01	(Z)-7-Hexadecenal	238	C ₁₆ H ₃₀ O
33	32.930	0.03	Hexanoic acid, undecyl ester	270	C ₁₇ H ₃₄ O ₂

Table 2: Bio-active compounds identified by GC-MS analysis in *Bryum argenteum*'s hexane extract.

Discussion

Analytical techniques (UV-Visible, FT-IR and GC-MS) were employed to investigate the identity and presence of bioactive compounds in selected moss plant's hexane extracts. The hexane extract of *B. argenteum*'s UV-Visible spectrum demonstrated that it contained specific

secondary metabolites including phenolic compounds, terpenoids, flavonoids and relative-derivatives, as evidenced by distinct absorption patterns. Complementary FT-IR results supported these findings by detecting vibrational bands associated with functional groups, including alkene, phenols, amine, secondary alcohol, terpenes, flavonoids, alkanes and aromatic compounds, affirming the non-polar chemical complexity of *Bryum argenteum*'s extract. GC-MS profiling identified several pharmacological relevance bio-active compounds, including diterpenes, sesquiterpenes, flavonoids, fatty esters among others. Many of these non-polar metabolites have been extensively documented in the literature for their pharmacological properties, suggesting potential of n-hexane extracts of *Bryum argenteum* to have a range of therapeutic effects. The diterpene neophytadiene is known for its antimicrobial activity [25] while n-hexadecanoic acid methyl ester and ethyl-hexadecanoic acid display anti-fungal, anti-oxidant, hemolytic, nematocide effects [26-28]. The Sesquiterpene compound, 6,10,14-Trimethyl-2-pentadecanone along with phthalic acid, cyclohexylmethyl butyl ester has demonstrated anti-inflammatory, allelopathic and antibacterial activities [28,29]. Additionally, anti-cancer, anti-candidal and hypocholesterolemic effects associated with polyunsaturated fatty acid compound, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) [30]. Hexanoic acid, undecyl ester has been linked to anticancer and antioxidant properties [31]. Another diterpene compound 'phytol' has many pharmacological properties like anti-cancer, anti-viral, anti-inflammatory etc. [32]. The extraction profile obtained with hexane reflects its efficiency in isolating non-polar phytochemicals such as lipid-soluble metabolites which are typically less accessible through polar solvents. Current outcomes that are consistent with prior research imply that the selected moss species (bryophyte) may have profound therapeutic benefits.

Conclusion

The phytochemical profiling of *B. argenteum* through UV-Visible and FT-IR spectroscopy with GC-MS analysis discloses non-polar molecules. Hexane extraction has been employed in this study to determine the chemical-complexity of studied moss plant. The analytical approach's combined output offers a thorough chemical fingerprint of hexane extract, emphasizing the mosses' potential as substitutes sources of natural products. An initial phytochemical landscape of selected *Bryum argenteum*'s non-polar extract under consideration is offered by the current findings. These finding warrant future drug discovery endeavours based on moss-derived therapeutic agents imperative. Follow-up research should concentrate on detailed bioassay and structural elucidation of individual compounds to thoroughly examine and harness this moss species' medicinal potential.

Competing Interest

On the behalf of all authors of this submission, we declare no competing financial interests on any personal or other relationship.

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