IJP (2025), Vol. 12, Issue 9 **(Research Article)**

International Journal of Pharmacognosy

Received on 29 August 2025; received in revised form, 27 September 2025; accepted, 29 September 2025; published 30 September 2025

THIN-LAYER CHROMATOGRAPHY AND GC-MS PROFILING OF BIOACTIVE COMPOUNDS FROM MOSS *BRYUM CAPILLARE* HEDW

S. Sarswati, G. S. Deora [*] and M. K. Shekhawat

Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur - 313001, Rajasthan, India.

Keywords:

Bryum capillare Hedw., Bioactive compounds, Non-polarsolvent, UV-Visible spectroscopy, GC-MS

Correspondence to Author:
G. S. Deora

Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur - 313001, Rajasthan, India.

E-mail: gsdeora0802@gmail.com

ABSTRACT: Bryophytes are known for chemically diverse group of non-vascular plants, and secondary metabolite of these plants, particularly in their lipophilic fractions yet remain unexplored in most of the plants. The present study deals with the analysis and investigation of the non-polar chemical composition of hexane extract of *Bryum capillare* using Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) technologies. TLC revealed the different bands of presence of non-polar compounds and GC-MS analysis identified a range of hydrocarbons, fatty acids esters, terpenoids including diterpenes, triterpenes. These findings contribute to the scant phytochemical literature available on mosses and suggest the existence of non-polar bioactive compounds that may have therapeutic potentialities.

INTRODUCTION: Bryophytes are non-vascular plants characterized by their simple body structure and the absence of differentiated vascular structures. With an estimated 25,000 species, taxonomically distributed across three phyla: mosses (Bryophyta), liverworts (Marchantiophyta), and hornworts (Anthocerotophyta) [1-3]. Several bryophytes, especially moss-derived compounds exhibit medicinally relevant activities, including the ability to inhibit microbial growth, suppress tumor-development, modulate immune responses, and support cardiac function. Such bioactivities underline their potential as source of novel therapeutic agents [4-8]. Bryophytes feature biological substances that shield them against insects, bacteria, and fungus [9].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.12(9).735-39

Article can be accessed online on:
www.ijpjournal.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.12(9).735-39

In phytochemical investigations, the selection of an appropriate solvent is essential for efficient extraction of target compounds. Hexane, being non-polar, is widely used to extract lipophilic-secondary metabolites, including hydrocarbons, terpenoids, long-chain fatty acids, sterols, and hydrocarbons [10-11]. Thin layer chromatography is a convenient and affordable technique for compound-class profiling and preliminary separation [12]. However, using their mass spectrum fingerprints, GC-MS offers comprehensive qualitative and quantitative information on individual elements [13].

GC-MS analysis of methanolic extract of whole plant of moss *Semibarbula orientalis* revealed the presence of 49 bioactive phytochemicals which included mainly n-hexadecanoicacid, cis-vaccenic acid, azulen, hexadecanoicacid methyl ester, 1,3 propanediol, 2-methyl-2 nitro, 9, 12 octa-dedienolchloride and octadecanoic acid *etc* which have various important medicinal properties [14]. 33 semi volatile secondary metabolites were analysed and evaluated from *Bryum argenteum* using GC-

MS, UV, FT-IR techniques. These uncovered chemicals displayed different pharmacological values like anti-bacterial anti-cancerous, allelopathic hypocholesterolemic, anti-oxidant and anti-infalmmatary [15]. The current research aims to investigate the bioactive phytochemical constituents from hexane extract of moss *Bryum capillare* through the combined application of Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) techniques. The present study significantly advances our understanding of the chemical composition and potential bioactive properties of moss *B. capillare*, highlighting their significance in both scientific pharmacological point of view.

MATERIAL AND METHODS:

Plant Material Collection: Field collection of *Bryum capillare* Hedw. was conducted from moist-wall habitat at Mount Abu during rainy season of 2022-2024. Morphological examination aided by standard taxonomic keys and relevant moss flora- 'Moss Flora of Rajasthan (India) [16]. The identification was later verified and authenticated by certain expert bryologist.

***Bryum capillare* Hexane Extraction Preparation:** Fresh moss *B. capillare* samples were initially cleaned in running tap water to remove debris, followed by rinsing with double-distilled water. The moss samples were dried under shaded conditions at room temperature, and finely powdered for experimental use. Soxhlet extraction was employed to obtain the crude hexane extract using sample powder and solvent in a *1:10* w/v ratio [17]. The extraction resulted in a yield of 21%, and the non-polar extract was subsequently stored at 4 °C for further analysis.

Thin Layer Chromatographic (TLC) Analysis: The standard Silica gel 60 F254 TLC plates (Merck, Germany) were used to separate the compounds in Thin Layer Chromatographic analysis. As the mobile phase, a best solvent system with a ratio of 4:1 for hexane and ethyl acetate was employed to the separation of a maximum number of components with high resolution from hexane extract of *Bryum capillare*. After the solvent system was saturated in TLC chamber, the marked TLC plates with the extract in it and allowed to rise. After this, different color spots were observed on TLC-plate of non-polar solvent extract at short wavelength Uv254 nm and long wavelength Uv365 nm in UV chamber and retention values were calculated using following formula:

Rf = Distance travelled by the solute (cm) / Distance travelled by the solvent (cm)

Gas Chromatography-Mass Spectroscopy Experimental conditions: The chemical profiling of the sample was performed using a "GC-MS-QP2010 Ultra Shimadzu, Japan". The temperature of oven gradient ranged from 70 to 300 °C at 10°C/min. The separation was employed with *helium* as carrier-gas at 16.3 mL/min constant flow rate. The injection was carried out at 260 °C with a hexane sample volume of one micro litre. Structural elucidation, molecular weight determination, and identification of the unknown bioactive compounds were based on their mass spectral and chromatographic data, matching against the *NISTM1* library for confirmation.

RUSULTS:

Characterization by Thin Layer Chromatogram (TLC): The solvent system, n-hexane: ethyl acetate (4:1) showed good resolution under UV light. The results of TLC with this solvent system showed that hexane extract contained ninecolors spots with *Rf* values 0.10, 0.19, 0.27, 0.35, 0.47, 0.54, 0.63, 0.80, and 0.83. These colored spots on the TLC plates under UV light indicate the presence of chemical compounds separated from extract samples.

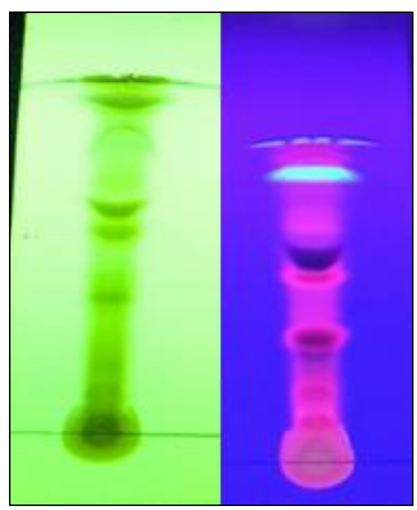

FIG. 1: HEXANE EXTRACT'S TLC PLATE OF *B. CAPILLARE* UNDER SHORT AND LONG UV WAVELENGTH

Characterization by GC-MS Profiling: Hexane extraction of *B. capillare* was analysed by gas chromatography-mass spectroscopy, revealing their chemical-diversity among non-polar bioactive compounds, as indicated by relative-peak areas. The outcomes of the study showed that the selected moss species had twenty-onediverse non-polar chemical compounds. These compounds ascertained by spectral verification utilizing reference databases, such as *Wiley* and *NISTM1* spectral libraries.

The major compound in hexane extract was '2,3-Dimethyl-pentane' which dominated the GC-MS profile with an 49.63% peak area. Some other major compounds were hexane (18.53%), 5-Methy l-2-(2-methyl-2-tetrahydrofuryl) tetrahydrofuran (15.70%), 2-methyl-pentane (4.46%) etc. as shown in **Table 1** with identified compounds' name, molecular weight and formulas, retention-time and peak areas. Further compounds such as heneicosane, o-xylene and Eicosane have been identified in lesser quantities, 0.01%.

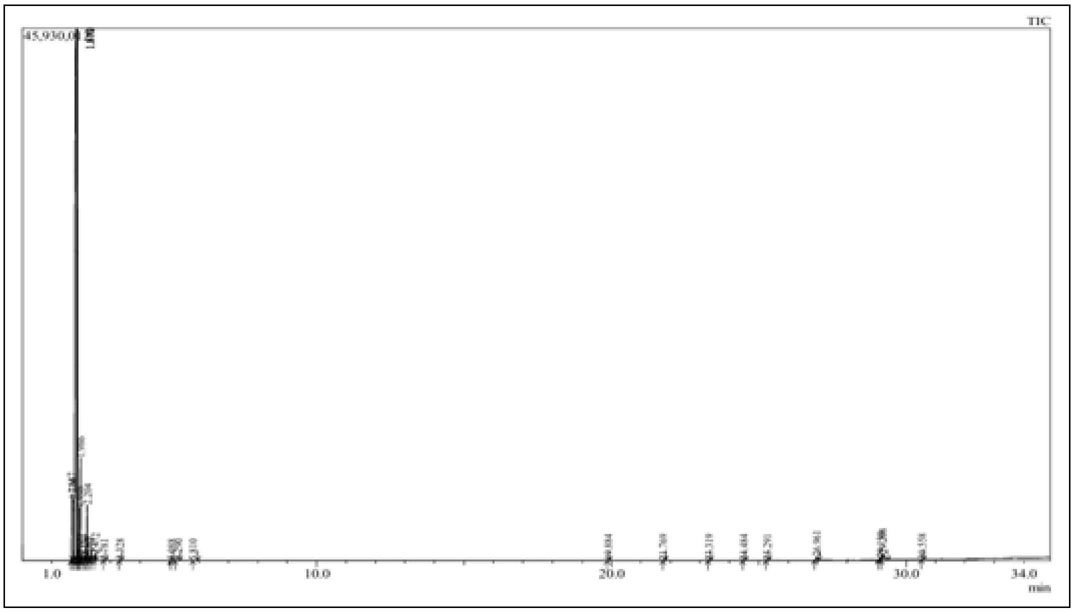

FIG. 2: GC-MS CHROMATOGRAM OF HEXANE EXTRACTOF *BRYUM CAPILLARE*

TABLE 1: LIST OF PHYTOCHEMICAL COMPOUNDS IDENTIFIED BY GC-MS ANALYSIS IN HEXANE EXTRACT OF THE MOSS *BRYUM CAPILLARE* HEDW

Peak no.	RT (min)	Peak area (%)	Molecular weight	Name of bio-active compounds	Molecular formula
1	1.832	49.63	86	2,3-Dimethyl-pentane	C_7H_{16}
2	1.870	18.53	86	n-Hexane	$C_5H_{10}O$
3	1.849	15.71	170	5-Methyl-2-(2-methyl-2-tetrahydrofuryl) tetrahydrofuran	$C_{10}H_{18}O_2$
4	1.687	4.46	86	2-methyl-pentane	C_6H_{14}
5	1.986	3.94	84	Methyl-cyclopentane	C_6H_{12}
6	1.726	2.87	157	Butylisocyanatoacetate	$C_7H_{11}NO_3$
7	2.204	1.82	84	Cyclohexane	C_6H_{12}
8	1.946	1.33	100	2,2-Dimethyl-pentane	C_7H_{16}
9	29.208	0.57	278	(Z,Z,Z)-9,12,15-Octadecatrienoicacid	$C_{18}H_{30}O_2$
10	26.961	0.25	256	n-Hexadecanoicacid	$C_{16}H_{32}O_2$
11	2.021	0.21	128	2,2,3,4-Tetramethyl-pentane	C_9H_{20}
12	2.472	0.18	128	3,4-Dimethyl-heptane	C_9H_{20}
13	29.130	0.15	280	(Z,Z)-9,12-Octadecadienoicacid	$C_{18}H_{32}O_2$
14	2.136	0.12	100	3,3-Dimethyl-pentane	C_7H_{16}
15	2.265	0.07	100	3-Methyl-hexane	C_7H_{16}
16	5.290	0.03	106	1,3-Dimethyl-benzene	C_8H_{10}
17	25.291	0.02	278	Neophytadiene	$C_{20}H_{38}$
18	30.558	0.02	410	Squalene	$C_{30}H_{50}$
19	5.810	0.01	106	o-Xylene	C_8H_{10}
20	19.884	0.01	296	Heneicosane	$C_{21}H_{44}$
21	23.319	0.01	282	Eicosane	$C_{20}H_{42}$

DISCUSSION: Previous researches have characterized several phytochemicals in bryophytes using GC-MS analysis [18-20]. The use of Gas Chromatography –Mass Spectrometry (GC-MS) is well-established technique for identification of some bioactive compounds in medicinal plants. Most of the phytochemicals identified in the present study have been reported to possess different pharmacological similarities. 9, 12 Octadecadioeonoic acid, methyl ester having anti-inflamatory, anti-arthritic, anti-histominic, hepatoprotective, anti-coronary, anti-aczemic and insectifuge [21]. Cis vaccenic acid is an omega -7 fatty acid known for its antibacterial activities and hyplipidemic effects [22]. 9-Octadecenoic acid (Z) – methyl ester has been found effective against Fungi *Aspergillus flavus* [23].

Distinct bands of TLC plates emerge under UV light, indicating chemical variety in the extracts and the detected TLC profiles serve as a foundation for additional focused isolation and identification and offer an initial sign of chemical richness. Hexane extract's GC-MS analysis provided a more precisely identification of the volatile and semi-volatile substances. With many conspicuous peaks that corresponded to fatty acids, triterpenes, sesquiterpenes, diterpenes and their methyl esters, the GC-MS chromatogram showed an intricate mixture. The TLC findings allowed for quick screening of chemical classes, whereas GC-MS allowed for more thorough identification and quantification of non-polar compounds.

Numerous non-polar metabolites have been thoroughly studied in the literature for their pharmacological nature, indicating that hexane extracts studied bryophyte may have a variety of medicinal benefits. The triterpene compound 'squalene' was known for antioxidant, antitumor, lipoxygenase inhibitor, cancer preventive, and pesticide [24]. n-Hexadecanoic acid (fatty acid) has been linked to potent mosquito-larvicide, hemo-lytic, hypo-cholesterolemic, nematicide [25, 26] while diterpene 'neophytadiene' has anti-microbial activity [27]. The polyunsaturated fatty acid, 9,12,15 Octadecatrienoic acid, methyl ester, (Z,Z,Z) showed many activities such as anti-bacterial, anti-candidal, anti-inflammatory [28]. Heneicosane was known for anti-microbial and anti-bacterial activities [29, 30] (Abdul *et al.*, 2024; and eicosane has larvicidal, anti-tumor, antifungal and cytotoxic activities [31]. Nevertheless, this investigation was restricted to a single species and solvent system. To profile the entire spectrum of metabolites, comparative extraction using solvents of different polarity should be a part of future studies.

CONCLUSION: The chemical profiling of *B. capillare* through TLC and GC-MS analysis of hexane extract revealed inert compounds. The current study reinforces the notion that the mosses possess significant metabolic diversity, particularly in their non-polar extractable constituents. The identification of pharmacologically active compounds implies their potential therapeutic value. Future research should also investigate the bioactivity of individual compounds and assess their potential for pharmaceutical or industrial use.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST Nil

REFERENCES:

1. Asakawa Y, Ludwiczuk A and Nagashima F: Phytochemical and biological studies of bryophytes. Phytochemistry 2013; 91: 52-80.
2. Vanderpoorten A and Goffinet B: Introduction to bryophytes. Cambridge University Press 2009.
3. Gradstein SR, Churchill SP and Salazar-Allen N: Guide to the bryophytes of tropical America. Memoirs-New York Botanical Garden 2001.
4. Pejin B, Bianco A, Newmaster S, Sabovljevic M, Vujisic LJ, Tesevic V, Vajs V and De Rosa S: Fatty acids of Rhodobryumontariense (Bryaceae). Natural Product Research 2012; 26(8): 696-702.
5. Pejin B, Bogdanovic-Pristov J, Pejin I and Sabovljevic M: Potential antioxidant activity of the moss Bryum moravicum. Natural Product Res 2013; 27(10): 900-902.
6. Cheng X, Xiao Y, Wang X, Wang P Li, H, Yan H and Liu Q: Anti-tumor and pro-apoptic activity of ethanolic extract and its various fractions from *Polytrichum commune* L. Ex Hedw in L1210 cells. J Ethnopharmacol 2012; 431: 49–56.
7. Singh M, Rawat AKS and Govindarajan R: Antimicrobial activity of some Indian mosses. Fitoterapia 2007; 78(2): 156-158.
8. Ilhan S, Savaroğlu F, Çolak Ferda Ğ, İscen CF and Erdemgil FZ: Antimicrobial activity of *Palustriella commutata* (Hedw.) ochyra extracts (Bryophyta). Turkish Journal of Biology 2006; 30(3): 149-152.
9. Kumar P and Chaudhary BL: Antibacterial activity of moss *Endotodonmyurus* (Hook) Hamp. against some pathogenic bacteria. Bioscan 2010; 5(4): 605-608.
10. Adebiyi AO and Tedela PO: Phytochemical Profiling and GC-MS Analysis of Extracts of Two Tropical Moss Species. Scholars Academic Journal of Biosciences 2023; 5: 181-190.
11. Novaković M, Ludwiczuk A, Bukvicki D and Asakawa Y: Phytochemicals from bryophytes: Structures and

biological activity. Journal of the Serbian Chemical Society 2021; 86(12): 1139-1175.

12. Wagner H and Bladt S: Plant drug analysis: a thin layer chromatography atlas. Berlin, Heidelberg: Springer Berlin Heidelberg 1996.
13. Sparkman OD, Penton Z and Kitson FG: Gas chromatography and mass spectrometry: a practical guide 2011: Academic press.
14. Deora V and Deora GS: *Semibarbula orientalis* (Web.) Wijk. & Marg. A potential source of bioactive and high value of phytochemicals. Journal of Pharmaceutical Research International 2012; 33(31): 34-43.
15. Sarswati S, Deora GS and Shekhawat MK: UV, FT-IR and GC-MS profiling of bioactive compounds from moss *Bryum argenteum* Hedw. EC Pharmacology and Toxicology 2025; 13(8): 1-8.
16. Chaudhary BL and Deora GS: Moss Flora of Rajasthan, India. Himanshu Publications 1993; 80-88.
17. Harborne AJ: Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media 1998.
18. Stoclet JC and Schini- Kerth: Dietary flavonoids and human health. Annals Pharma Franc 2011; 69(2): 78-90.
19. Cao J, Zhang Y, Chen W and Zhao Z: The relationship between fasting plasma concentrations of selected flavonoids and their ordinary dietary intake. British Journal of Nutrition 2010; 103(2): 249-253.
20. Sofowora A: medicinal plants and traditional medicine in Africa. John Willy & Sons USA 1984; 256-257.
21. Nishanthini A, Mohan VR and Jeeva S: Phytochemical FT-IR and GC-MS analysis of stem and leaf of *Filicora acuminate* (Linn.) Hook.F.8Thomas (Menispermaceae). International Journal of Pharmaceutical and Research 2014; 5(9): 3977- 3986.
22. Hamazaki K, Suzuki N, Kitamura KI, Hattori A, Nagasawa T, Itomura M and Hamazaki T: Is vaccenic acid (18: It t -7) associated with an increased incidence of hip fracture? An explanation for the calcium paradox. Prostaglandins Leukotrienses and Essential Fatty Acids 2016; 109: 8-12.
23. Marimuthu K, Nagaraj N and Ravi D: GC-MS analysis of phytochemicals, fatty acids and antimicrobial potency of dry *Chritmas lima* beans. International Journal of Pharmaceutical Sciences Review and Research 2014; 27(2): 63-69.
24. Kalaivani CS, Sathish SS, Janakiraman N and Johnson M: GC-MS studies on *Andrographis paniculata* (Burm. f.) Wall. ex Nees-a medicinally important plant. International Journal of Medicinal and Aromatic Plants 2012; 2(1): 69-74.
25. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, and Haridas M: Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. Chemical Biology Drug & Design 2012; 80: 434–439.
26. Kumar PP, Kumaravel S and Lalitha C: Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. African Journal of Biochemistry Research 2010; 4: 191–195.
27. Valarmathi R, Natarajan D, Suryadevara N, Maziz NHM, Appalaraju VVSS, Ragavan, ND, Vairavan CAS and Neevashini C: GC-MS Analysis and Antibacterial Activity of *Dryopteris hirtipes (*Blumze) Kuntze Linn. Journal of Survey in Fisheries Sciences 2023; 10(1S): 3718-3726.
28. Baba H, Kashimawo AJ and Ibe AC: Phytochemical evaluation and GC-MS profiling of the dichloromethane and ethanol extracts of *Ocimum gratissimum* L. and *Lasianthera africana*. BEAUV. Journal of Phytomedicine and Therapeutics 2022; 20(2): 640-655.
29. Abdul U, Manikandan DB, Arumugam M, Alomar SY, Manoharadas S and Ramasamy T: GC–MS based metabolomic profiling of *Aporosa cardiosperma* (Gaertn.) Merr. leaf extracts and evaluating its therapeutic potential. Scientific Reports 2024; 14(1): 16010-16026.
30. Vanitha V, Vijayakumar S, Nilavukkarasi M, Punitha VN, Vidhya E and Praseetha PK: Heneicosane A novel microbicidal bioactive alkane identified from *Plumbago zeylanica* L. Industrial Crops and Products 2020; 154: 112748-112756.
31. Arora S, Kumar G and Meena S: GC-MS analysis of bioactive compounds from the whole plant hexane extract of *Cenchrus setigerus. Vahl.* Pharma Sci Moni 2017; 8(4): 137-46.

How to cite this article:
Sarswati S, Deora GS and Shekhawat MK: Thin-layer chromatography and GC-MS profiling of bioactive compounds from moss *Bryum capillare* HEDW. Int J Pharmacognosy 2025; 12(9): 735-39. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(9).735-39.