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Insights into the fatty acid profile and taxonomy of the extremophilic moss *Hedwigia emodica* (Bryophyta, Hedwigiales, Hedwigiaceae)

Estudios del perfil de ácidos grasos y la taxonomía del musgo extremófilo *Hedwigia emodica* (Bryophyta, Hedwigiales, Hedwigiaceae)

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Abstract

Extremophilic mosses are known to produce a variety of long-chain polyunsaturated fatty acids in response to various abiotic stresses. These fatty acids facilitate in membrane fluidity, enabling their survival in extreme conditions. The present study investigates the fatty acid profile and taxonomy of the extremophilic moss *Hedwigia emodica*. The species was found in the Northwestern Indian Himalayan region and is characterized by certain identifying features such as straight leaves, 0.6–0.8 mm wide; long hyaline hair-pointed tip ca. 20-40% of leaf length; leaf margins weakly recurved or plane; median leaf cells with simple to minimally branched adaxial papillae, and obscuring cell walls. Gas Chromatography-Mass Spectrometry analysis revealed a total of 20 different fatty acids, including saturated, monounsaturated, and polyunsaturated fatty acids. Notably, α-linolenic acid is found to be present in the highest amounts,accounting for 35.44% of the total fatty acids, followed by arachidonic acid with 15.05% of the total fatty acids. We also quantified these fatty acids per gram of moss tissue. The content of α-linolenic acid (ω-3 essential fatty acid) was 5059.86±0.66 μg/g, whereas α-linoleic acid (ω-6 essential fatty acid) was 1785.24±0.00 μg/g. Furthermore,

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the amounts of long-chain polyunsaturated fatty acids such as eicosapentaenoic and arachidonic acid were found to be 1026.37±0.06 and 2137.95±0.05 μg/g, respectively. Our findings revealed a significant proportion of nutritionally, medicinally, and biologically important fatty acids that can be used for industrial purposes. The present study is the first-ever quantitative estimation of fatty acid content in this taxon, paving the way for further research into the field of moss lipid biochemistry and the environmental influence on moss fatty acids.

Keywords: GC-MS; LC-PUFA; mosses; ω-3-Fatty acid; quantification.

Resumen

Se sabe que los musgos extremófilos producen una variedad de ácidos grasos poliinsaturados de cadena larga en respuesta a diversos tipos de estrés abiótico. Estos ácidos grasos ayudan a la fluidez de la membrana, lo que permite su supervivencia en condiciones extremas. Por lo tanto, el presente estudio investiga el perfil de ácidos grasos y la taxonomía de un musgo extremófilo, *Hedwigia emodica*. La especie se encontró en las zonas de la región del Himalaya del noroeste de la India y se caracteriza por ciertos caracteres identificativos, como hojas rectas de 0,6 a 0,8 mm de ancho; punta larga con forma de pelo hialino de aproximadamente el 20 al 40 % de la longitud de la hoja; márgenes de las hojas débilmente curvados o planos; células medianas de las hojas con papilas adaxiales simples o mínimamente ramificadas; paredes celulares oscuras. El análisis de cromatografía de gases y espectrometría de masas reveló un total de 20 ácidos grasos diferentes, que incluyen ácidos grasos saturados, monoinsaturados y poliinsaturados. Se encontró que el ácido α-linolénico se encuentra en las cantidades más altas con un 35,44% de los ácidos grasos totales, seguido del ácido araquidónico con un 15,05% de los ácidos grasos totales. También cuantificamos estos ácidos grasos por gramo de tejido de musgo. El contenido de ácido linolénico (ácido graso esencial ω-3) fue el de mayor valor con 5059,86 ± 0,66 μg/g entre todos los ácidos grasos, mientras que el ácido α-linoleico (ácido graso esencial ω-6) fue de 1785,24 ± 0,00 μg/g. Se encontró que la cantidad de ácidos grasos poliinsaturados de cadena larga como el ácido eicosapentaenoico y el ácido araquidónico fue de 1026,37 ± 0,06 y 2137,95 ± 0,05 μg/g, respectivamente. Nuestros hallazgos revelaron una proporción significativa de ácidos grasos importantes desde el punto de vista nutricional, medicinal y biológico que pueden utilizarse con fines industriales. El presente estudio es la primera cuantificación de la estimación de ácidos grasos en este taxón. Estos hallazgos abrirán caminos para realizar más investigaciones en el campo de la bioquímica de lípidos del musgo y la influencia ambiental en los ácidos grasos del musgo.

Keywords: GC-MS; LC-PUFA; musgos; ácido graso ω-3; cuantificación.

INTRODUCTION

In nature, plants are exposed to various biotic and abiotic stresses. To overcome these stresses, plants have adopted a variety of defence mechanisms, one of which is the production of secondary metabolites (Ramakrishna & Ravishankar, 2011; Yeshi *et al.*, 2022). Among different secondary metabolites, fatty acids are crucial biomolecules that perform several important functions in plants such as maintaining membrane fluidity, acting as energy reservoirs, and cell signalling (Choudhary *et al.*, 2017). Several fatty acids in plants have pharmaceutical and nutritional properties (Pereira *et al.*, 2012). There is a diverse array of fatty acids among the group of land plants that includes usual and unusual fatty acids (Cahoon & Beisson, 2020; Kazaz *et al.*, 2022).

The mosses are known to possess an arsenal of secondary metabolites which are involved in the treatment of several diseases such as cardiovascular, cystitis, bronchitis, skin infections, etc. (Canli *et al*., 2014). Moreover, mosses are rich sources of nutritionally important fatty acids and long-chain polyunsaturated fatty acids (LC-PUFAs), crucial for flourishing in extreme climates. Lipids in these plants play vital roles in energy storage and cellular adaptation, with very long-chain polyunsaturated fatty acids (VL-PUFAs) being abundant (Beike *et al.*, 2014). Eicosapentaenoic acid ($20:5\Delta^{5,8,11,14,17}$) (EPA) and arachidonic acid ($C20:4\Delta^{5,8,11,14}$) (AA) are among the predominant fatty acids found in mosses, which are also essential constituents of the human diet and medicine. Various nutritionally important fatty acids, such as linolenic acid ($C18:3\Delta^{9,12,15}$), linoleic acid ($C18:2\Delta^{9,12}$), and palmitic acid (C16:0) have been documented in previous studies on many different moss taxa (Beike *et al.*, 2014; Poddar-Sarkar *et al.*, 2022; Filippova *et al.*, 2023).

Presently, the rare taxon *Hedwigia emodica* Hampe ex Müll. Hal. was found growing on exposed dry rocks in Pangi Valley, Himachal Pradesh (Fig. 1A-E). Several different extracts of the moss species *Hedwigia* P. Beauv. are known to acquire a variety of secondary metabolites such as flavonoids, polyphenols, terpenoids, and fatty acids in response to extreme conditions which are of critical importance as they possess various antibacterial, antifungal, anti-inflammatory, and anticancerous properties (Beike *et al.*, 2014). Extracts of *H. ciliata* (Hedw.) Boucher is known to possess immunomodulatory effects (Poddar-Sarkar *et al.*, 2022). In *H. emodica* extract, antibacterial activity was found to be significant against *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus carnosus*, and *S. epidermidis* (Canli *et al.*, 2014). Therefore, a comprehensive analysis of fatty acid profiles in mosses thriving in extreme climates, exemplified in our study on *H. emodica* is paramount for advancing our understanding of their adaptive mechanisms and potential applications.

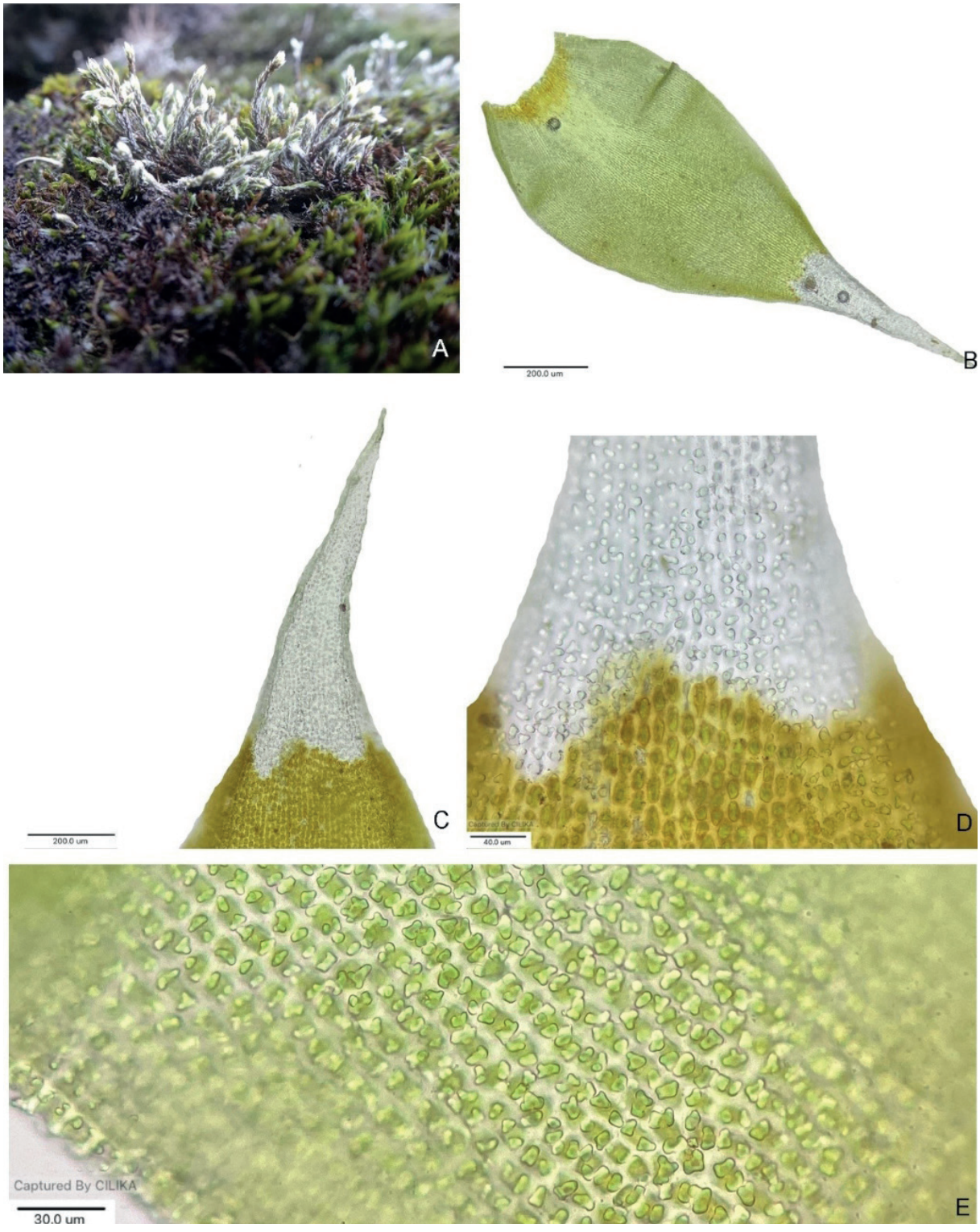

Fig. 1. *Hedwigia emodica.* Taxonomic characters. A) Aspect of the moss. B) Whole mount of the leaf. C) Apical portion of the leaf showing hyaline tip. D) Hyaline tip with branched papillations. E) Median leaf cells with papillations.

Fig. 1. *Hedwigia emodica*. Caracteres taxonómicos. A) Aspecto del musgo. B) Preparación completa de la hoja. C) Porción apical de la hoja con ápice hialino. D) Ápice hialino con papilas ramificadas. E) Células medianas de la hoja con papilas.

MATERIALS AND METHODS

Sample collection and Identification.— The samples were collected in June 2024 from Purthi village in Chamba district of Himachal Pradesh, India, which is located at an average elevation of 2287 masl and lies between 32°52'78"N, 76°25'16"E and 32°55'36"N, 76°27'43"E. The area remains dry for most of the year with extreme winters and cold summers. The samples were collected from the rocks and placed in the sampling bags. They were then brought to the laboratory where they were cleaned, sorted, and identified based on micromorphological characteristics using standard literature (Lunić *et al.*, 2022; Filippova *et al.*, 2023). The voucher specimen is submitted to Delhi University Herbarium (DUH).

Sample preparation.— Since the population size of *H. emodica* was too less in the area, only a few samples were taken, and the study was done in biological duplicates. The moss extract was prepared following Choudhary *et al.* (2017). Briefly, 20 mg of tissue was rehydrated for four hours at 25°C, ground, and then put in glass vials (Pyrex). Then, one ml of 2 % (v/v) methanolic HCl was added along with 10 μl heptadecanoic (C17:0) acid as an internal standard with a concentration of 10 μg/ml. The samples were incubated at 80°C and then allowed to cool for five minutes. Thereafter, one ml of 0.9% sodium chloride (NaCl) and one ml n-hexane of HPLC grade were added followed by a vortex for 45 seconds and a centrifugation for phase separation. After phase separation, the upper layer was dried using nitrogen gas. Finally, the samples were then resuspended in 100 μl of n-hexane for GC-MS analysis.

GC-MS analysis and interpretation.— The analysis of fatty acid methyl esters (FAMEs) was conducted using a Gas Chromatography-Mass Spectrometry (GC-MS) system manufactured by Agilent Technologies, consisting of a 7890B GC series and a 5977A Mass Selective Detector (MSD). A DB-wax capillary column with dimensions of 30 m $\times$ 0.25 mm $\times$ 0.25 mm (Agilent Technologies) was employed for separation. Helium gas was used as a carrier gas at a pressure of 10 psi. The temperature of the oven was programmed to increase gradually from 50°C to 180°C at a rate of 25°C per minute and then from 180°C to 230°C at a rate of 5°C per minute, while the carrier gas flowed at a rate of 1.8 ml/min. A consistent injection volume of 1 μl was maintained with a split ratio of 10:1. Identification of FAMEs was accomplished by comparing their mass spectral values with the spectral data entries in the National Institute of Standards and Technology (NIST) library, ensuring accurate identification of the compounds.

RESULTS

Taxonomic treatment

Hedwigia emodica Hampe ex Müll. Hal.,
Flora, 61: 82. 1878. ≡ *Hedwigia ciliata* var. *leucophaea*
Bruch & Schimp., Bryol. Eur., 3: 153. 273b. 1846.
Type citation: 'Himalayae montes: S. Kurz'.

Plant medium size, brownish below and yellowish-green at top with a hyaline tip; plant slender, somewhat straight, form dense erect tuft; leaves arranged spirally, imbricate, erect to erectopatent when dry and spreading when moist; leaf shape long-ovate to wide-lanceolate, ± 2.5 mm long, slightly concave, flat mostly, not plicate; leaf margins slightly recurved or sometimes plane in some leaves in lower part; leaf apex long acuminate, with a long hyaline tip which comprises up to 20-40 % of leaf length; leaf median cells short rounded to rectangular, papillose with 2 or more branched papillation per cell on adaxial and abaxial surface; leaf upper cells also short rounded to rectangular, with 2 branched papillae, papillae branched more on abaxial surface; leaf topmost apical cells with tall papillae; perichaetial leaves long lanceolate in shape with ciliate margins, cells narrow- oblong, consisting of median row of simple papillae; sporophyte not seen in the specimen.

Habitat and Ecology.— Specimen found growing on exposed dry rocks.

Distribution.— Brazil, China, India, Kenya, Mongolia, Nepal, Tanzania, Uganda, and United Kingdom.

Specimen Examined.— INDIA. Himachal Pradesh, Chamba district, Pangi valley, Purthi Village, 2268 m, 32°52'78"N, 76°27'48"E, 08-VI-2024, *Anshul Dhyani 15340* (DUH).

KEY TO THE INDIAN SPECIES

1 Leaf margins weakly recurved (1/4th to 2/3rd of leaf length) or plane; median leaf cells with two or more simple or sometimes branched adaxial papillae; leaf apices not reflexed when dry 2
1' Leaf margins prominently recurved throughout; median leaf cells with one or two highly branched adaxial papillae; papillae stellate; leaf apices reflexed when dry ..... *H. stellata*
2 Leaf hyaline tip long, ca. 20-40% of the leaf length; papillae on abaxial side branched or stellate; leaf margins recurved (1/4th to 1/3rd of leaf length) or plane ..... *H. emodica*
2' Leaf hyaline tip comparatively short, ca. 5-20% of the leaf length; papillae on abaxial side simple or branched weakly, never stellate; leaf margins recurved (1/3rd to 2/3rd of the leaf length) or plane ..... *H. ciliate*

GC-MS analysis

The GC-MS analysis revealed a total of 20 different fatty acids, most of which have nutritional significance (Fig. 2). A total of six saturated fatty acids (SFA), three monounsaturated fatty acids (MUFA), and 11 polyunsaturated fatty acids (PUFA) have been identified. *H. emodica* has been found to contain SFA, which include pelargonic acid (C9:0), lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid, and stearic acid (C18:0) acids. However, MUFA such as palmitoleic acid (C16:1Δ^{9}), oleic acid (C18:1Δ^{9}), and cis-vaccenic acid (C18:1Δ^{11}) acids have also been detected. Among the SFA (16.25%), palmitic acid is the most abundant (1750.34±0.21 μg/g), while oleic acid (431.91±0.12 μg/g) is the highest among MUFA (5.70%). Moreover, PUFAs found in *H. emodica* are *cis*-7,10-hexadecadienoic acid (C16:2$\Delta^{7,10}$), *cis*-7,10,13-hexadecatrienoic acid (C16:3$\Delta^{7,10,13}$), linoleic acid, γ-linoleic acid (C18:3$\Delta^{6,9,12}$), α-linolenic acid, stearidonic acid (C18:4$\Delta^{6,9,12,15}$), dihomo-γ-linolenic acid (C20:3$\Delta^{8,11,14}$), AA, dihomo-linolenic acid (C20:3$\Delta^{11,14,17}$), methyl-8,11,14,17-eicosatetraenoic acid (C20:4$\Delta^{8,11,14,17}$), and EPA (Table 1).

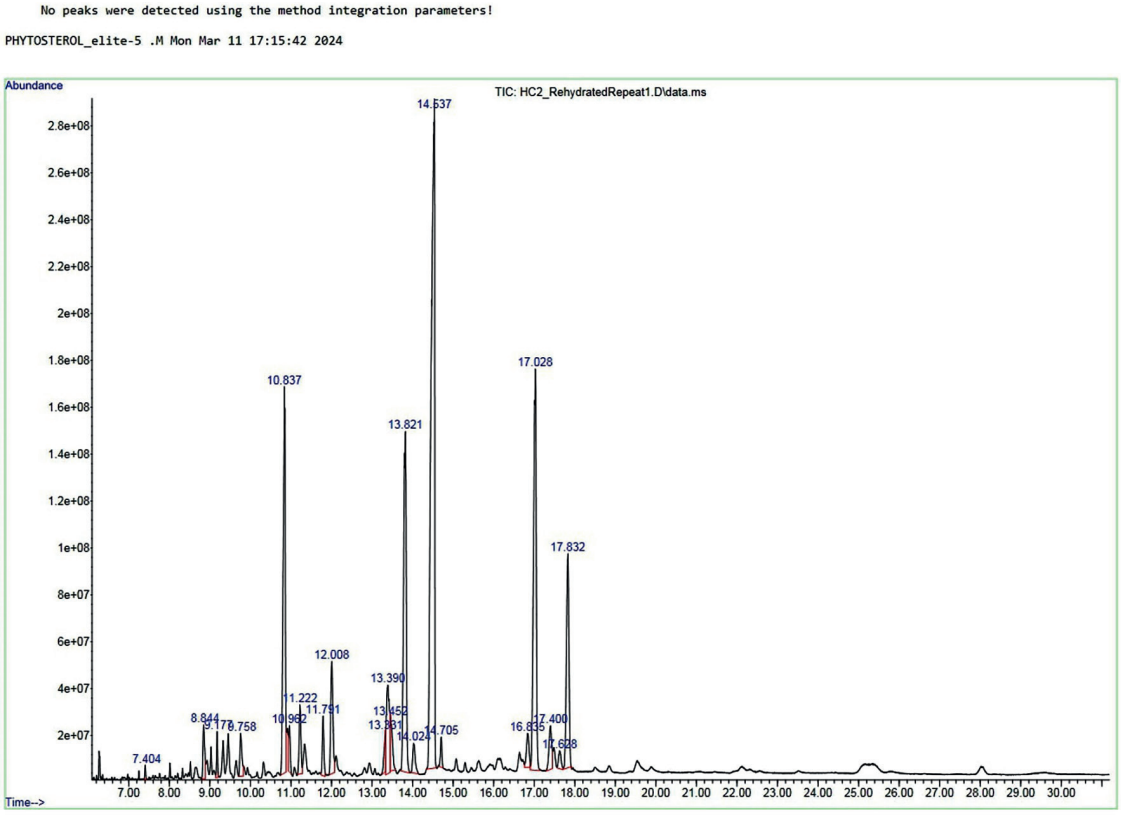

Fig. 2. Gas Chromatography-Mass Spectrometry of all reported fatty acids along with their retention time. The Y-axis shows the abundance value and the X-axis shows the retention time of the reported fatty acids.

Fig. 2. Cromatografía de gases-espectrometría de masa de todos los ácidos grasos reportados, junto con su tiempo de retención. El eje Y muestra el valor de abundancia y el eje X, el tiempo de retención de los ácidos grasos reportados.

Table 1. Fatty acid composition and quantification in *Hedwigia emodica* moss. Values are presented as percentages and ug/g of dry weight of mosses.

Tabla 1. Composición y cuantificación de ácidos grasos en el musgo *Hedwigia emodica.* Los valores son presentados como porcentaje y como ug/g de peso seco de musgos.

Fatty Acid	Common Name	Fatty Acid Percentage	Fatty acid (μg/g)
Dodecanoic acid (C12:0)	Lauric acid	0.16±0.01	23.23±0.00
Tetradecanoic acid (C14:0)	Myristic acid	1.24±0.03	177.22±0.00
Nonanoic acid (C9:0)	Pelargonic acid	0.62±0.02	89.52±0.00
Pentadecanoic acid (C15:0)	Pentadecylic acid	1.11±0.06	158.74±0.00
Hexadecanoic acid (C16:0)	Palmitic acid	12.26±0.70	1750.34±0.21
9-cis-Hexadecenoic acid (C16:1)	Palmitoleic acid	0.97±0.00	138.62±0.00
cis-7,10-Hexadecadienoic acid (C16:2)	–	1.67±0.07	238.77±0.00
7,10,13-Hexadecatrienoic acid (C16:3)	Roughanic acid	1.27±0.09	181.72±0.01
Octadecanoic acid (C18:0)	Stearic acid	0.84±0.03	120.12±0.01
9-Octadecenoic acid (C18:1)	Oleic acid	3.05±0.71	431.91±0.12
11-Octadecenoic acid (C18:1)	Cis-vaccenic acid	1.67±0.21	239.91±0.05
9,12-Octadecadienoic acid (C18:2)	Linoleic acid	12.54±0.38	1785.24±0.00
6,9,12-Octadecatrienoic acid (C18:3)	γ-Linoleic acid	0.95±0.13	134.82±0.02
9,12,15- Octadecatrienoic acid (C18:3)	α-Linolenic acid	35.44±2.28	5059.86±0.66
6,9,12,15-Octadecatetraenoic acid (C18:4)	Stearidonic acid	0.60±0.02	86.24±0.00
8,11,14-Eicosatrienoic acid (C20:3)	Dihomo- γ-Linolenic acid	1.13±0.14	161.17±-0.02
5,8,11,14-Eicosatetraenoic acid (C20:4)	ω-6-Arachidonic acid	15.02±0.72	2137.95±0.05
11,14,17-Eicosatrienoic acid (C20:3)	Dihomo linolenic acid	1.49±0.23	212.30±0.03
Methyl-8,11,14,17 Eicosatetraenoic acid (C20:4)	ω-3-Arachidonic acid	0.65±0.02	93.08±0.00
5,8,11,14,17- Eicosapentaenoic acid (C20:5)	Eicosapentaenoic acid	7.21±0.53	1026.37±0.06
Nutritional Index (ΣPUFA/ΣSFA)			5.15

We reported α-linolenic acid as the major fatty acid followed by AA. Furthermore, we also reported a significant percentage of palmitic acid, linoleic acid, and EPA.

DISCUSSION

The genus *Hedwigia* is characterized by ecostate leaves, branched leaf papillae, hyaline leaf apices, and perichaetial leaves ciliate. The name is conserved against an earlier homonym named *Hedwigia* Sw. from the family of flowering plant Burseraceae (Dalton *et al.*, 2013; Ignatova *et al.*, 2016). *Hedwigia* is represented by three species in the Himalayan region– *H. ciliata*, *H. Stellata* Hedenäs, and *H. emodica* respectively (Dalton *et al.*, 2013). The species *H. emodica* was earlier placed as synonymous with *H. ciliata* var. *leucophaea* (Dalton *et al.*, 2013) and has been found in India, China, and Nepal. From India, the species has previously been reported from Kashmir, but the type locality remains unclear (Dalton *et al.*, 2013; Ignatova *et al.*, 2016). The species is known to grow on exposed dry acidic rocks (Hedenäs, 1994) and we also found the moss growing on exposed dry rocks.

H. emodica possesses 20 different fatty acids which include several fatty acids of medicinal and nutritional value. *H. emodica* contains both ω-3 (α-linolenic acid) and ω-6 (linoleic acid) essential fatty acids in significant amounts, with n3:n6 ratio of 2.82:1. Humans do not synthesize these essential fatty acids because our body lacks the FAD2 and FAD3 enzymes necessary for essential fatty acids (Choudhary *et al.*, 2021). *H. emodica* also contains nutritionally significant LC-PUFAs such as AA and EPA. The PUFAs ranged from 2-5% and the highest percentage was α-linolenic acid. In humans, it exerts a pivotal influence on cardiovascular health by disrupting the synthesis of proinflammatory eicosanoids, thereby impacting the progression of cardiovascular disease (Din *et al.*, 2004; Wilczynska-Kwiatek *et al.*, 2010; Sharma, 2012). It is also a crucial component of the thylakoid membranes of chloroplast in plants (Calder, 2021). We recorded the significant presence of LC-PUFAs such as AA and EPA in the current study which is comparatively higher (15.02% AA and 7.21% EPA) in our study than previous studies on mosses (Beike *et al.*, 2014; Ludwiczuk, 2019; Poddar-Sarkar *et al.*, 2022; Lu *et al.*, 2023; Filippova *et al.*, 2023). Furthermore, various microalgae are used for the isolation of ω-3-fatty acids such as EPA and docosahexaenoic acid (DHA) for dietary purposes. These fatty acids are important fatty acids for performing several functions in human physiology (Doughman *et al.*, 2007; Boelen *et al.*, 2013; Mühlroth *et al.*, 2013). In the present study, we found a significant amount of EPA which is comparatively higher than studied by Boelen *et al.* (2013) in various microalgae. The medical significance of AA is well-known in several studies (Moncada & Vane, 1979; Lausanne *et al.*, 1983; Brash, 2001; Lv *et al.*, 2024). Furthermore, the ratio of EPA to AA is found to have a significant effect on cardiovascular diseases (Nelson & Raskin, 2019). Our sample has an EPA:AA ratio of 2.08:1. Various other fatty acids such as oleic acid, myristic acid, lauric acid, stearic acid, etc. were also reported and frequently found in the plant kingdom. Other fatty acids such as palmitic acid, γ-linolenic acid, and linoleic acid were also reported in significant proportions. Palmitic acid also has industrial uses worldwide (Mba *et al.*, 2015). Similarly, γ-linolenic acid and linoleic acid have been linked to nutritional and medicinal benefits such as dietary supplements and are effective against rheumatoid arthritis (Horrobrn, 1992; Zurier *et al.*, 1996).

The quantification of these fatty acids revealed that *H. emodica* contains a significant amount of α-linolenic acid (5059.86±0.66 μg/g of dry weight), AA (2137.95±0.05 μg/g of dry weight), and EPA (1026.37±0.06 μg/g of dry weight). α-linolenic acid is known to be a precursor of jasmonic acid (C12H18O3) which plays a crucial role in resistance against different biotic stress in plants (Mata-Pérez *et al.*, 2015). AA and EPA are predominantly found in green algae and bryophytes but are completely absent in higher plants which can be seen as the retention of a shared character depicting the evolutionary relationship between algae and bryophytes (Lu *et al.*, 2019). Biotechnological tools should be utilized to extract more EPA

and AA from the mosses so that they can be useful for industrial purposes and be used as a substitute for marine algae.

The bryophytes are well-known to accumulate a variety of LC-PUFAs and VL-PUFAs in response to different stress conditions. *H. emodica* is found to accumulate a variety of LC-PUFAs and VL-PUFAs. This can be explained on account of extreme climatic conditions such as harsh winters and cold summers in the present study area which is primarily crucial for plants. Several studies have supported this notion that plants accumulate a variety of LC-PUFAs and VL-PUFAs by adjusting their membrane fluidity and lipid composition to tolerate these harsh winters (Dembitsky *et al.*, 1993; Lu *et al.*, 2023). To gain deeper insights into the underlying mechanisms of these adaptations, it is imperative to undertake molecular studies, thereby facilitating a comprehensive understanding of the adaptive strategies employed by these plants.

CONCLUSION

In conclusion, our study provides a comprehensive investigation into the fatty acid profile and taxonomy of the moss species *H. emodica* found in the Northwestern Indian Himalayan region. Through detailed morphological examination, we have differentiated this species from other known *Hedwigia* species in the region, highlighting distinct characteristics such as leaf morphology and papillae structure. Additionally, our GC-MS analysis of moss extracts revealed the presence of 20 different fatty acids, with α-linolenic acid and AA being the most abundant, and other important fatty acids including EPA, palmitic acid, and linoleic acid. Moreover, this study represents the first-ever quantification of fatty acid content in *H. emodica*, providing valuable insights into its nutritional composition. These findings not only contribute to our understanding of the species but also pave the way for future research endeavours to explore the ecological and physiological significance of fatty acid accumulation in bryophytes.

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AUTHOR'S CONTRIBUTIONS

Conceptualization, AD, KS, PLU; Investigation, AD, AKC, KS; Visualization, AD, AKC; Writing - original draft, AD; Writing - review and editing, AD, AKC, KS, PLU; All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY

Data will be made available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

The authors confirm that this work is original and has not been published elsewhere.

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