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GC-MS Analysis of Volatile Phytochemical compounds from the Whole plant Methanolic extract of *Entodon rubicundus* (Mitt.) A. Jaeger. and *Thyridium fasciculatum* (Hook. & Grev.) Mitten

Shiwani Latwal[1*], Meenal Sharma[1] and Anju Rao[2]
[1]Ph.D. Scholar, Department of Botany, Panjab University (Chandigarh), India.
[2]Associate Professor, Department of Botany, Panjab University (Chandigarh), India.

(Corresponding author: Shiwani Latwal)*
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ABSTRACT: Medicinal plants are the most formidable chemical laboratory of bioactive compounds attributed with healing properties against various ailments. The phytochemistry of the plants has been exploited to satisfy the growing demand for therapeutic needs. When it comes to potential medical use, bryophytes are among the most neglected land plants. Using the gas chromatography-mass spectrometry (GC-MS) technology, the study sought to assess potential phytochemical components and their medicinal importance in the aqueous methanol fraction of plants. *Entodon rubicundus* (Mitt.) A. Jaeger and *Thridium fasciculatum* (Hook. & Grev.) Mitten are two mosses from the Western Himalayan region belonging to the family Entodontaceae and Calymperaceae respectively. The samples were collected during their favourable season, washed, air- dried and then macerated to produce the methanolic extract. GC- MS analysis was carried out followed by the identification of volatile compounds using mass spectra library search (NIST 2.0). Five volatile constituents were detected and identified in the plant extract of *Entodon rubicundus* out of which Trans-13-Octadecenoic acid, methyl ester showed the highest peak area (46.58%) and the lowest peak area was shown by Methyl stearate (4.10%). In *Thridium fasciculatum* seven compounds were identified out of which Cyclopropaneoctanoic acid showed the highest peak area (44.95%) and 6,9 Octadecadienoic acid, methyl ester showed lowest the peak area (1.68%). To the best of our knowledge, no literature has previously been published on GC–MS and phytochemical investigations of these moss species, making it the first description of the phytochemical elements that demonstrate their pharmacological importance. The GC- MS profiling of both the mosses indicated therapeutically promising bioactive compounds with a potent anti-bacterial, anti-microbial, anti-tumor, anti-inflammatory and anti-oxidant activity which can be used in pharmaceutical industries for the development of novel drugs.

Keywords: *Entodon rubicundus, Thyridium fasciculatum,* Mosses, GC-MS, Phytochemicals.

INTRODUCTION

Bryophytes being the earliest and less evolved than vascular plants still managed to successfully dominate the land as the second largest group of plants having three subgroups: Bryophyta (includes mosses), Marchantiophyta (includes both leafy and thalloid liverworts) and Anthocerophyta (Harris *et al*., 2020). Bryophytes are usually found in moist environments and form thick mats and cushion-like structures on rock, soil and tree trunks. These tiny plants have also developed defense mechanisms in terms of both biotic and abiotic factors through their unique constitutive chemistry. The chemical metabolites not only equipped these small non-vascular plants with defense mechanisms but also granted these plants great medicinal properties which can be further exploited to develop new drugs and medicines (Mishra *et al*., 2014; Moti *et al*., 2023). Various primary as well as secondary metabolites such as tannins, terpeniods, flavonoids, phenols and other vital compounds that are known to be biologically active are reportedly produced by bryophytes (Manoj *et al*., 2022; Commisso *et al*., 2021; Naumova *et al*., 2013; Soriano *et al*., 2019; Peters *et al*., 2019; Chen *et al*., 2018). Furthermore, these plant genera are the most unexplored in terms of their applicability to the pharmaceutical and medical industries. The demand for the herbal industry, which uses plants as a source of medicine, is also rising quickly as a result of the widespread acceptance and appeal of plant-based medications. The herbal plants are widely regarded as safer, more affordable, and more natural than contemporary synthetic medications.

Entodon rubicundus is a pleurocarpic moss belongs to family Entodontaceae. The plant is dioicous, forming broad mats of yellow-green to golden-green (sometimes with a reddish tinge) colour. The plant is creeping and shows branching. Leaves present on the stem are wide ovate, apiculate, ± 1.6mm long and 1mm wide. Leaf cells are rhomboid in shape, size of cells becomes

slightly smaller at the basal end. Branch leaves are smaller, dense, concave, and ovate to lanceolate in shape. Costa is absent in stem leaves while branch leaves occasionally have two short veins. Sporophytes are abundantly present on the main stem having a reddish-brown cylindrical capsule. Capsules are ± 4.4mm long and ± 0.77 mm in diameter. The peristome is long and has exostome teeth. Microscopic fine papillose spores are present inside the capsule.

Thyridium fasciculatum is an acrocarpic moss that belongs to the family Calymperaceae having a dioicous, robust epiphytic creeping stem with a 2 cm long erect shoot. Leaves are erectopatent and are ±3.1mm long and 1.5 mm broad with serrulate tip. 9 rows of long hyaline cells are prominent in the leaves of *T. fasciculatum.* Cells filled with chlorophyll are oval and smaller in size. A narrow, rough and per current costa is present on the back near the tip of a leaf. The sporophytic plant body was not found.

MATERIAL AND METHOD

All the chemicals and reagents used for the phytochemical research were of analytical grade. The fresh moss samples were collected in ziplock bags from Himachal Pradesh, a state situated in the Western Himalayan region. The damp, humid and relatively low temperature (20°C to 25°C) of this state is ultimate for the colonization of mosses as shown in Table 1.

A. Preparation of plant extract

The plant material was brought to the laboratory, it was thoroughly cleaned with distilled water to get rid of any remaining dirt particles. It was then allowed to air dry at room temperature and ground into a powder using an electric blender. The 500 mg of ground plant samples were macerated in 10 millilitres of methanolic solvent and left for 72 hours at room temperature in an orbital shaker set at 150 rpm. Whatman filter paper 1 was used to filter the mixture. The same solvent was used for multiple extractions until a clear, colourless solvent was produced. The resulting extract was dried by evaporation and kept for later use at 4°C in an airtight container.

B. Gas chromatography-Mass spectrometry (GC-MS) analysis

The hybrid analytical method known as gas chromatography-mass spectrometry (GC-MS) combines the detection capabilities of MS with the separation powers of GC to increase sample analysis efficiency. The volatile components in a sample can be separated by GC, however, MS aids in fragmenting the components and identifying them based on mass. The present experiment used this technique to analyze the phytochemical constituents present in *E. rubicundus* and *T. fasciculatum*, using Thermo- Trace 1300 GC coupled with Thermo TSQ 8000 Triple Quadrupole Mass Spectrometer (for GC- Thermo Trace 1300 GC; for MS- Thermo TSQ 8000). As a carrier gas, pure helium (99.999%) was passed with a constant flow rate of 1 mL/min45ml/min split flow. The injector volume used was 10 µl with a 29.08 run time at a temperature of 250°C. TG 5MS (30m × 0.25 mm, 0.25µm) column with 5% diphenyl and 95% dimethyl polysiloxane column was used for the analysis. The mass spectrum was taken in the range 50-700 at ionization voltage 70eV. The ion source temperature was set at 230°C. The chromatogram has indicated the presence of compounds with separate retention times. The contents of phytochemicals present in the test samples were tentatively detected by comparing their retention indices and mass spectra using the database present in the NIST 2.0 library (National Institute of Standard and Technology, Gaithersburg, MD, USA)., the final results were recorded. The obtained retention indices and mass spectra of the compounds were analyzed along with their molecular formula and molecular weight. Data pertaining to the medicinal and therapeutic uses of these compounds were analyzed and documented.

RESULTS AND DISCUSSION

The GC- MS chromatogram of methanolic leaf extract of *E. rubicundus* and *T. fasciculatum* recorded a total of 5 and 7 peaks as shown in Fig. 1 and 3 respectively. The phytochemicals were recognized by relating their peak area (%), height (%), retention time, molecular formula, molecular weight and mass spectral fragmentation patterns. The major phytoconstituents in the methanolic fraction of *E. rubicundus* leaf extract were Hexadecanoic acid, methyl ester (27.32%), 9,12-Octadecadienoic acid (Z,Z), methyl ester (15.85%), Trans-13- Octadecenoic acid, methyl ester (46.58%), Methyl stearate (4.10%), Arachidonic acid (6.14%) as shown in Table 2.The major chemical compounds obtained from methanolic leaf extract of *T. fasciculatum* were Hexadecanoic acid, methyl ester (18.52%), 6,9- Octadecadienoic acid, methyl ester (1.68%), 9,12- Octadecadienoic acid (Z,Z), methyl ester (20.14%), Cyclopropaneoctanoic acid (44.95%), Methyl stearate (2.68%), 10,12- Tricosadiynoic acid (10.14%), Methyl 8,11,14 heptadecatrienoate (2.27%) as shown in Table 3. The structures of compounds obtained from *E. rubicundus* and *T. fasciculatum* are indicated in Fig. 2 and 4 respectively. Among all chemical compounds identified, Hexadecanoic acid was reported to have anti-inflammatory, anti-oxidant, anti-microbial, anti-bacterial anti-fibrinolytic, lubricant, hemolytic, anti-androgenic, nematicide, hemolytic, anti-cancerous as well as anti-hyperlipidemic activities (Aparna *et al*., 2012; Abubakar *et al*., 2016; Olivia *et al*., 2021). 9,12- Octadecadienoic acid (Z,Z)-, methyl ester documented to have anti-tumor, anti-inflammatory, anti-oxidant, anti-arthritic, antifungal and anticancer activity against prostate cancer cell lines (Bharath *et al*., 2021; Mizushina *et al*., 1996). Trans-13-Octadecenoic acids are linoleic acid esters having anti-inflammatory, cancer-preventive, anti-androgenic, dermatitigenic, anti-arthritic, anti-leukotriene- D-4, hypocholesterolemic, 5- alpha-reductase inhibitor, anti-acne, anemiagenic properties (Krishnamoorthy and Subramaniam 2014; Abdullah *et al*., 2020). Methyl stearate along with other molecules that act synergistically with rifampicin shows anti-tuberculosis activity (Nirmal *et al*., 2022). The bioactive components of methyl stearate are also used to reduce

root gall disease and egg masses of nematodes in the soil (Lu *et al*., 2020). Arachidonic acid provides cell membrane fluidity and flexibility. ARA was found to play a vital role during embryogenesis, cell-apoptosis and necrosis. It also possesses potent schistosomicidal activity. Studies conducted *in-vivo* and *in-vitro* in human cervical carcinoma (HELA) cells reported ARA as a potent anti-cancer drug (Tallima and El Ridi 2018; Sagar and Das 1995; Brash, 2001; Pompeia *et al*., 2000). Methyl 8, 11,14- heptadecatrienoate has anti-inflammatory, anti-bacterial activity and antioxidant activity (Vivekraj *et al*., 2017; Rani and Kapoor 2019). Cyclopropaneoctanic acid is an important compound for upregulation of genes responsible for hepatocyte lipid synthesis and release (Czumaj *et al*., 2018) 10, 12-Tricosadiynoic acid is an orally active inhibitor of acyl-CoA oxidase-1 (ACOX-1). Both *in-vivo* and *in-vitro* studies have proved that obesity-induced metabolic diseases in male wister rats can be treated by 10,12-Tricosadiynoic acid treatment which significantly decreases hepatic lipid and ROS contents, by improving mitochondrial lipid and ROS metabolism (Zeng *et al*., 2017). 6, 9 Octadecadienoic acid is an anti-inflammatory fatty acid (Kapoor and Huang 2006).

Table 1: Time, place of collection, altitude, latitude and longitude of collected samples.

Sr. No.	Name of the moss species / Family/ Voucher No.	Collection time	Place of Collection	Altitude, Latitude & Longitude
1.	*Entodon rubicundus* (Mitt.) A. Jaeger. (Entodontaceae), V/N. 6414	July 2021	Solan (Moist and shady wall)	Alt-1927 m, N 30° 22′-33° 12′ and E 75° 45′-79° 04′
2.	*Thyridium fasiculatum* (Hook. & Grev.) Mitten. (Calymperaceae), V/N. 6415	July 2021	Kasauli (Bark of tree trunk)	Alt-1927 m, N 30° 22’-33° 12′ and E 75° 45′-79° 04′

V/N- Voucher no, Alt- Altitude, N- North, E- East

Table 2: GC-MS chromatogram phytoconstituents in aqueous methanolic fraction of *Entodon rubicundus.*

Compound Name	Retention Time	Molecular Formula	Molecular Weight	Compound Nature	% Peak Area Percentage
Hexadecanoic acid, methyl ester	18.52	$C_{17}H_{34}O_2$	270.45	Palmitic acid	27.32
9,12- Octadecadienoic acid (Z,Z)-, methyl ester	20.16	$C_{19}H_{34}O_2$	294.5	Methyl ester	15.85
Trans-13-Octadecenoic acid, methyl ester	20.22	$C_{18}H_{34}O_2$	282.5	Methyl ester	46.58
Methyl stearate	20.47	$C_{19}H_{38}O_2$	298.5	Strearic acid, methyl ester	4.10
Arachidonic acid	21.73	$C_{20}H_{32}O_2$	304.5	Fatty acid	6.14

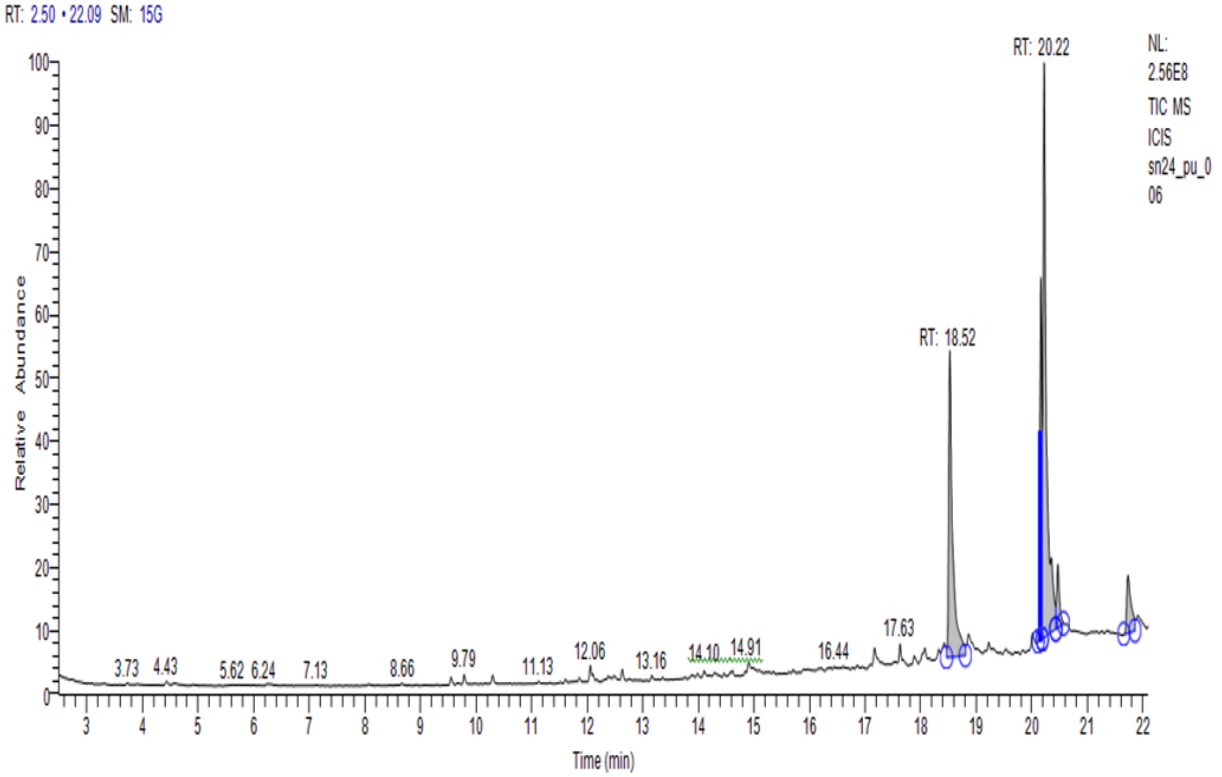
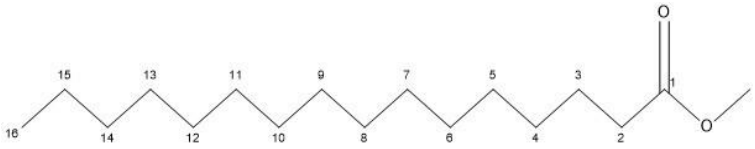


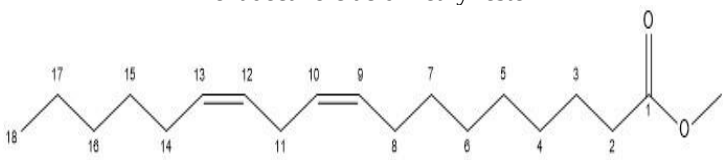
Fig. 1. GC-MS chromatogram of phytoconstituents in aqueous methanolic fraction of *E. rubicundus*.

Table 3: GC-MS chromatogram phytoconstituents in aqueous methanolic fraction of *Thyridium fasciculatum.*

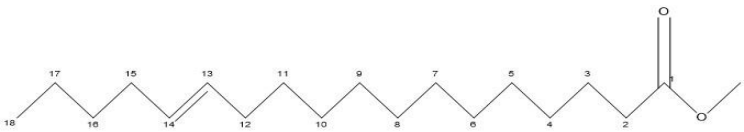
Compound Name	Retention Time	Molecular Formula	Molecular Weight	Compound Nature	% Peak Area Percentage
Hexadecanoic acid, methyl ester	18.52	$C_{17}H_{34}O_2$	270.5	Fatty acid compound	18.14
Methyl 8,11,14-heptadecatrienoate	21.91	$C_{18}H_{30}O_2$	278.4	Fatty acid compound	2.27
9,12- Octadecadienoic acid (Z,Z)-, methyl ester	20.15	$C_{19}H_{34}O_2$	294.5	Linoleic acid	20.14
Cyclopropaneoctanoic acid	20.22	$C_{22}H_{38}O_2$	334.5	Fatty acid compound	44.95
Methyl stearate	20.46	$C_{19}H_{38}O_2$	298.5	Fatty acid methyl ester	2.68
10,12- Tricosadiynoic acid, methyl ester	20.58	$C_{24}H_{40}O_2$	360.6	Fatty acid methyl ester	10.14
6,9 Octadecadienoic acid, methyl ester	20.08	$C_{19}H_{34}O_2$	294.5	Fatty acid compound	1.68



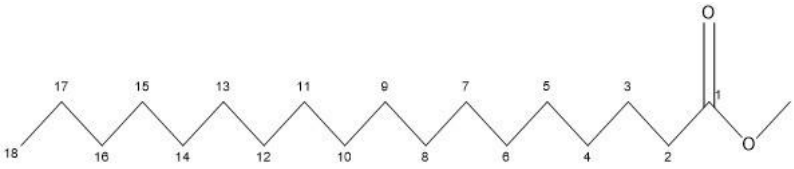
Hexadecanoic acid methyl ester



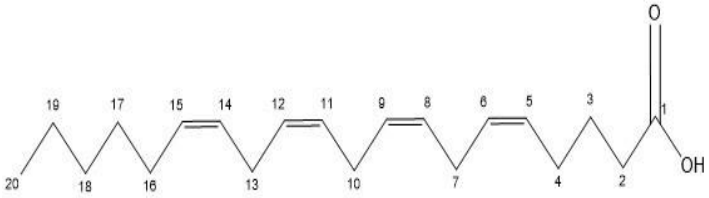
9, 12- Octadecadienoic acid (Z,Z) methyl ester



Trans-13- Octadecenoic acid



Methyl stearate



Arachidonic acid

Fig. 2. Chemical structure of compounds present in aqueous methanolic fraction of *E. rubicundus.*

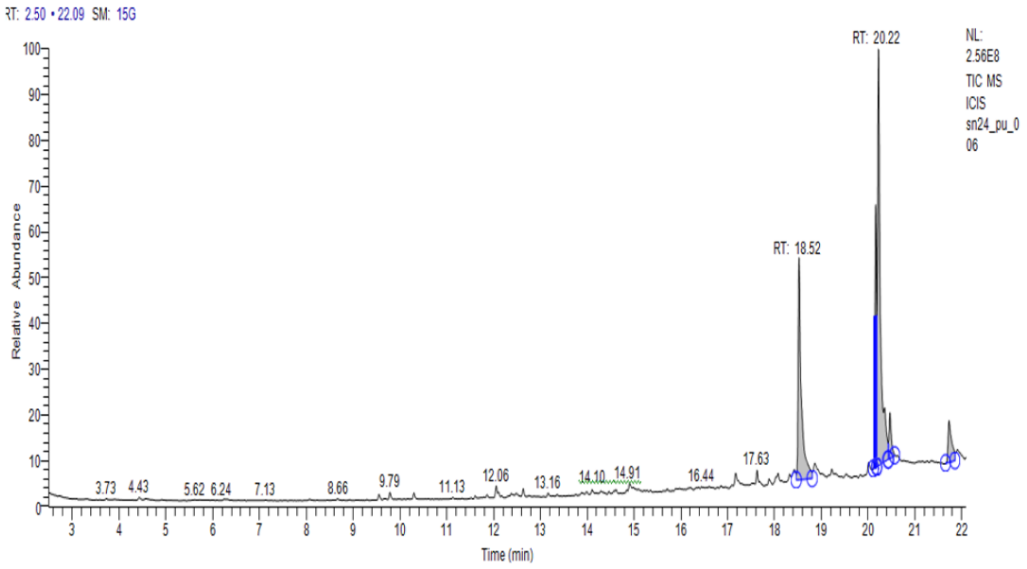
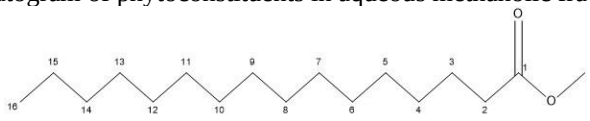
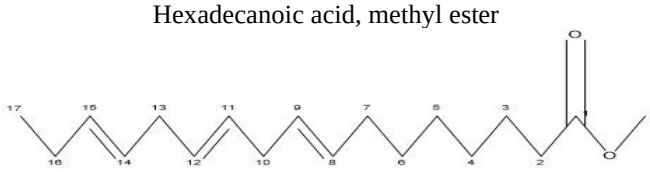


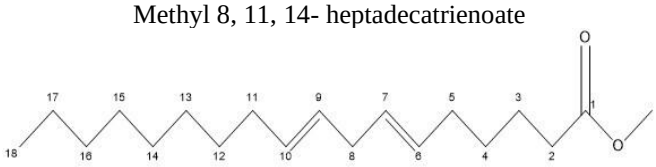
Fig. 3. GC- MS chromatogram of phytoconstituents in aqueous methanolic fraction of *T. fasciculatum.*



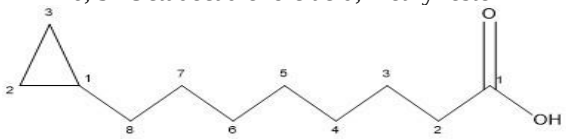
Hexadecanoic acid, methyl ester



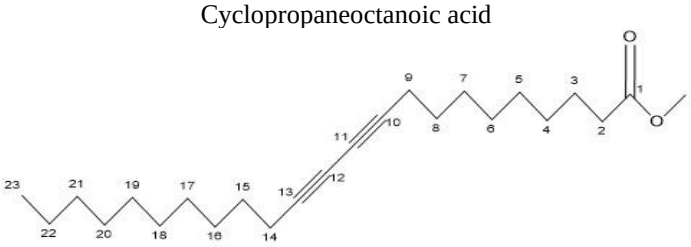
Methyl 8, 11, 14- heptadecatrienoate



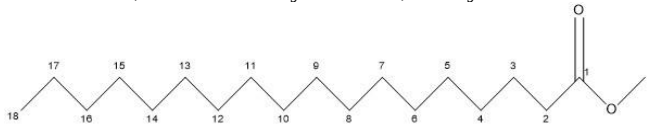
6, 9- Octadecadienoic acid, methyl ester



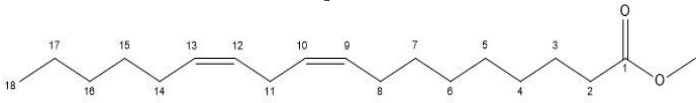
Cyclopropaneoctanoic acid



10, 12- Tricosadiynoic acid, methyl ester



Methyl stearate



9,12- Octadecadienoic acid (Z,Z)- methyl ester

Fig. 4. Chemical structure of compounds present in aqueous methanolic fraction of *T. fasciculatum.*

CONCLUSIONS

The pharmacological use of plants as a source for the development of innovative drugs has gained widespread recognition in the last few years. To gain a thorough understanding of the phytochemical profile of the mosses found in the Western Himalayan region, a GC-MS analysis of the mosses in a methanolic solvent was conducted. The resulting compounds displayed a wide range of biological characteristics, including anti-inflammatory, anti-bacterial, anti-acne, anti-tumor, anti-cancerous, anti-oxidant, and anti-arthritic capabilities. Based on the conducted research, it is possible to conclude that both *E. rubicundus* and *T. fasciculatum* could be recommended as a plant of pharmaceutical importance.

FUTURE SCOPE

As the literature on the biological activities of compounds in bryophytes is limited, further research on the isolation and identification of new compounds using different solvents and evaluating their medicinal importance along with pre-clinical and clinical studies is recommended.

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Conflict of Interest. None.

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