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Isolation and Characterisation of Bioactive Compounds from Methanolic Extract of *Thuidium delicatulum* (Hedw.)

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Abstract

Background: Medicinal plants have played a critical role in treating various diseases, with studies indicating that approximately 80% of the global population relies, either directly or indirectly, on herbal medicines. However, bryophytes remain significantly understudied, comprising only 6% of phytochemical research despite their widespread occurrence and chemical richness. *Thuidium* species of bryophytes have been utilised in traditional medicinal systems such as Native American, Chinese, and Indian medicine for their therapeutic benefits. **Aim:** To isolate and identify volatile bioactive compounds from *Thuidium delicatulum*, with a focus on elucidating the structure of a newly isolated compound. **Methods:** Methanolic extract were analysed through qualitative phytochemical screening, FTIR spectroscopy, HPLC, GC-MS, and NMR (^{1}H and ^{13}C) for structural elucidation and confirmed *via* CSEARCH ^{13}C-NMR spectral similarity search tool. **Results:** The extract revealed a rich presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics. FTIR confirmed functional groups such as hydroxyl, carbonyl, ether, and aromatic moieties. HPLC showed high levels of gallic acid (61.05 µg/mL), ellagic acid (40 µg/mL), and other flavonoids like rutin and quercetin. GC-MS identified 32 volatile compounds, with Methyl 8,9-octadecadienoate (25.33%) being the most abundant. NMR analysis led to the identification of a novel compound, 2-(2-hydroxyethylamino)-1,7-dihydro-6H-purin-6-one ($C_8H_{17}N_3O$), validated with 100% spectral similarity. **Conclusion:** These findings suggest that *T. delicatulum* is a promising source of natural bioactive molecules with potential antioxidant and therapeutic applications. Further studies are recommended to evaluate the antimicrobial, antioxidant, and cytotoxic potential of the extract and the newly identified compound.

Major Findings: The study presents the first detailed chemical profiling of *T. delicatulum*, revealing rich phytochemical diversity and identifying 50 compounds through GC-MS. A novel bioactive compound, 2-(2-hydroxyethylamino)-1,7-dihydro-6H-purin-6-one, was identified.

Keywords: Bioactive Compound, GC-MS, Methanol Extract, NMR, *T. delicatulum*

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1. Introduction

Phytochemicals, the bioactive compounds present in plants, have long been recognised for their potential in modern medicine. Despite significant advancements in Western medicine, herbal remedies continue to remain popular due to their affordability, accessibility, and broad safety margins, hence, their global demand is rapidly increasing. According to updated national pharmacovigilance surveys covering 2020-2023, the estimated proportion ranges from 65 % to 88 % across income, underscoring the continuing relevance of

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natural-product discovery[1]. Since prehistoric times, plants have served as a rich source of diverse secondary metabolites with therapeutic properties[2]. Among the vast plant kingdom, bryophytes; amphibious, non-vascular plants found across a wide range of habitats including tropical forests, deserts, arctic zones, and mountain peaks; have gained increasing attention. Notably, these plants are often avoided by insects and herbivores due to their unique secondary metabolites[3]. However, bryophytes remain significantly understudied, comprising only 6% of phytochemical research despite their widespread occurrence and chemical richness[4]. To date, more than 2,200 organic compounds have been identified from these plants, and this number continues to grow with ongoing phytochemical investigations[5]. Given the global interest in natural products with minimal side effects, bryophytes represent a promising and underexplored source of novel bioactive compounds for modern medicine and preventive healthcare[6]. Secondary metabolites such as flavonoids, terpenoids, alkaloids, and phenolics play essential roles in plant self-defence and possess a wide range of medicinal, agricultural, and industrial applications. These compounds are known to exhibit significant antibacterial, antifungal, antiviral, antioxidant, anticancer, and other bioactive properties[7,8]. Among lower plants, various bryophyte genera including *Targionia*, *Plagiochasma*, *Marchantia*, and *Riccia* have long been used in traditional medicine for the treatment of liver disorders, yellow fever, inflammation, and wound healing[9].

Thuidium delicatulum (Hedw.), commonly referred to as the delicate fern moss, belongs to the family *Thuidiaceae* within the order *Hypnales*. It is a medium-sized to moderately large, robust moss, typically found in large, loose mats or wefts with a distinctive colour ranging from yellowish-green to yellowish-brown or reddish-brown. Its stem leaves are ovate-triangular and gradually or abruptly taper into a broad acumen, but do not form a capillary point[10]. Historically, bryophytes; including species of *Thuidium*; have been utilised in traditional medicinal systems such as Native American, Chinese, and Indian medicine for their therapeutic benefits[11]. The antimicrobial activity observed in extracts of *T. delicatulum* supports its ethnopharmacological relevance. Despite its wide distribution, *T. delicatulum* remains chemically unexplored: No chromatographic fingerprint, volatile profile, or individual secondary metabolite has been reported, and its antioxidant, antimicrobial or cytotoxic potential has never been tested experimentally. In particular, the bioactive compounds present in the methanolic extract of *T. delicatulum* have not been previously characterised. This study provides the first integrated GC-MS, HPLC and NMR chemical profile of *T. delicatulum*, leading to the identification of a previously unreported purine-type scaffold and establishing a reference metabolome for future bioactivity-guided investigations.

2. Materials and Methods

2.1 Sample Collection and Identification

At an elevation of 29.624657° (Lat.) and 79.830827° (Long.), the bryophyte sample was gathered in November 2024 from the Jageshwar range in Uttarakhand, India. To obtain the ideal concentration of phytoconstituents, samples were taken in the afternoon from natural habitats. During the collection, a 1 Km radius was covered using the random sampling technique. The herbarium accession number for *T. delicatulum* (Hedw.) is ITM-BRY-006a.

2.2 Extract Preparation

To get rid of surface impurities and debris, the moss samples were thoroughly cleaned with water couple of times. To avoid the susceptible compounds from degrading, the cleaned moss was spread out in single layer on clean and dry filter paper and protected from the sunlight. To enhance the surface area for extraction, the dried moss was ground into a fine powder. A thimble of filter paper filled with 10 g of powdered moss and put into a Soxhlet extractor. The extraction process was run continuously for 8 hours using 100 mL of methanol as solvent. The following extract was concentrated using a rotatory evaporator and it was then kept at 4°C for additional analysis[12].

2.3 Qualitative Analysis of Phytoconstituents

The phytochemical analysis of the methanolic extract was carried out to detect active secondary metabolites and various constituents, including tannins, alkaloids,

flavonoids, terpenoids, steroids, carbohydrates, proteins and saponins following the method described by Trease and Evans[13,14].

2.4 Quantification of Compounds

An Agilent 1220 HPLC system with a binary pump, autosampler, and Diode Array Detector (DAD) was used to analyse the methanolic extract. A reversed-phase Agilent TC-C18(2) column (250 mm × 4.6 mm, 5 µm) was used to achieve separation. The mobile phase contained 0.1 % formic acid in 60 % methanol: 40 % acetonitrile, pH 3.0, and was ultrasonically degassed for 15 min. The injection volume was 10 µL and the flow rate was 1.0 mL min^{-1}. Detection was carried out between 200 and 600 nm. Retention times and UV spectra were compared with those of authentic standards for phenolic identification. Samples were prepared at 100 µg mL^{-1}, sonicated for 10 min and filtered through a 0.22 µm nylon membrane prior to injection[15].

2.5 Volatile Compound Profiling

The methanolic extract was analysed using a GC-MS system (Agilent 5977A MSD) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature program was as follows: Initial temperature of 50° C held for 2 min; increased to 150° C at a rate of 10° C/min (held for 2 min); further increased to 250° C at 5° C/min (held for 5 min); and finally ramped to 280° C at 10° C/min (held for 5 min). The injector temperature was maintained at 250° C and operated in split mode with a 10:1 ratio. Helium served as the carrier gas at a constant flow rate of 1 mL min^{-1}. Electron Ionisation (EI) was performed at 70 eV, and mass spectra were recorded over the m/z range of 35-500. Compound identification was carried out by comparing the obtained mass spectra and retention times with those in the NIST mass spectral library[16].

2.6 Structural Elucidation

NMR analysis (^{1}H and ^{13}C) was used to elucidate the structure of isolated pure compound 1 obtained from column chromatographic separation was performed using Merck 7734 silica gel (60-120 mesh), and TLC experiment was carried out with precoated Merck silica gel 60 PF254 aluminium sheets; the spots were visualised under UV light. The compound 1 was dissolved in deuterated solvents ($CDCl_3$/DMSO-d6), and spectra were recorded on a Bruker 400 MHz spectrometer[17].

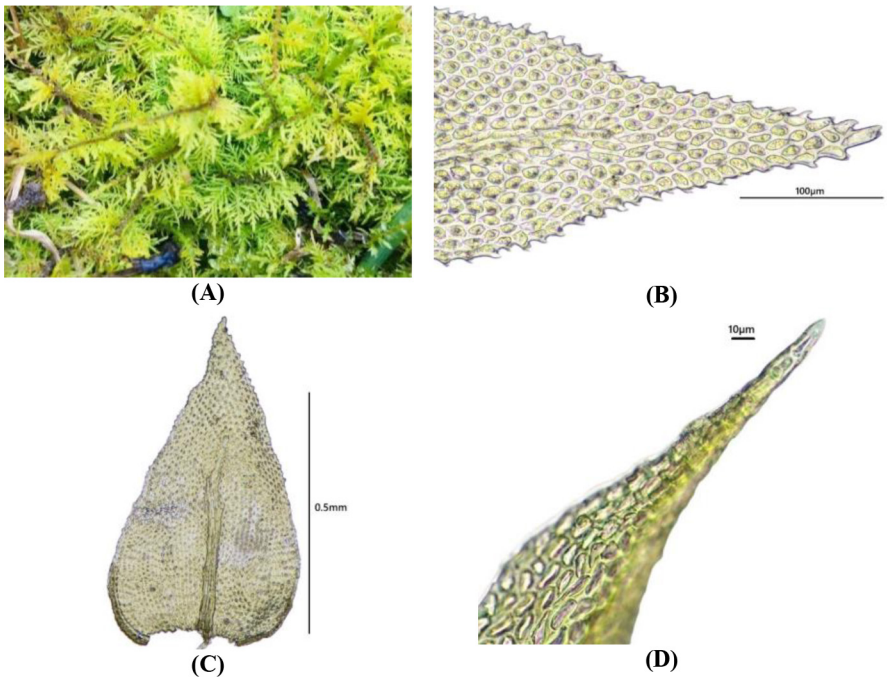


Figure 1. *T. delicatulum* **(A)** moss in natural habitat **(B)** Microscopic cells of leaf apex **(C)** Whole leaf microscopic view **(D)** Stem leaf apex.

2.7 Determining and Validating the Chemical Structure

To determine and validate the chemical structure of the isolated compound, a spectral similarity search was conducted using the CSEARCH ^{13}C-NMR spectral similarity search tool. This platform enables the comparison of experimental ^{13}C-NMR data against an extensive database comprising 615,365,172 reference spectra. The analysis involved the input of chemical shift values (in ppm) along with their corresponding multiplicities into the search engine in a standardised peak list format: Shift value (multiplicity). A deviation tolerance of ±3 ppm was allowed for the chemical shift values to ensure accurate matching[18].

3. Results

3.1 Sample Identification

Morphological, anatomical, microscopic evaluation revealed that the identified moss belonged to *T. delicatulum* (Hedw.) shown in Figure 1 with authentic literature[10]. Under microscopic analysis the stem leaves were 1.5-2 mm long and longitudinally ridged and branch leaves were up to 0.5 mm long.

3.2 Qualitative Analysis of Phytoconstituents

The preliminary phytochemical analysis of the methanolic extract of *T. delicatulum* (Table 1) revealed the presence of diverse classes of secondary metabolites, including alkaloids, flavonoids, triterpenoids, tannins, phenols, saponins, and glycosides. The detection of these bioactive constituents suggests that *T. delicatulum* harbours a rich and complex phytochemical profile. This chemical diversity provides a strong basis for its selection for further investigation aimed at identifying and characterising specific bioactive compounds through advanced analytical techniques.

Table 1. Phytochemical screening of *T. delicatulum* methanolic extract

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Triterpenoids	+
Phlobatannins	-
Tannins	+
Anthraquinones	-
Reducing sugar	+

- Not detected; + present

3.3 HPLC Analysis of Compounds

HPLC analysis of the methanolic extract of *T. delicatulum* confirmed the presence of several phenolic and flavonoid compounds, as detailed in Table 2. Among the identified constituents, gallic acid was found in the highest concentration, followed by ellagic acid. Other notable compounds included rutin and quercetin, whereas p-coumaric acid was not detected in the extract.

These findings highlight the abundance of antioxidant phenolic compounds, particularly gallic and ellagic acids, in *T. delicatulum*, suggesting its potential as a source of natural antioxidants. The corresponding HPLC chromatograms supporting these results are presented in Figures 2 to 5.

3.5 GC-MS Analysis

The Figure 6 displayed the chromatographic characteristics of detected compounds. The methanolic extract of *T. delicatulum* revealed the presence of 32 volatile compounds, as identified through GC-MS chromatographic analysis as summarised in Table 3, the major constituents, identified based on their peak intensities, include Methyl 8,9-octadecadienoate, 13-Hexyloxacyclotridec-10-en-2-one, n-Hexadecanoic acid (Palmitic acid), 2,2,3,3-Tetraethyloxirane, 2,3,5,6-Bis(1,5-octadenyl)-2,5-dibora-1,4-dioxane, and Gorlic acid. The last entry and other heterocycles

Table 2. Quantitative analysis of standard compounds in the methanolic extract of *T. delicatulum* by HPLC

S. No.	Standard Compounds	Retention Time	Area	Concentration (µg/mL)
1	Ellagic acid	6.283	21629372	40
2	Gallic Acid	3.250	6739739	61.05
3	P-Coumaric acid	-	-	-
4	Quercetin	3.107	10914	2.305
5	Rutin	10.113	84061	9.827

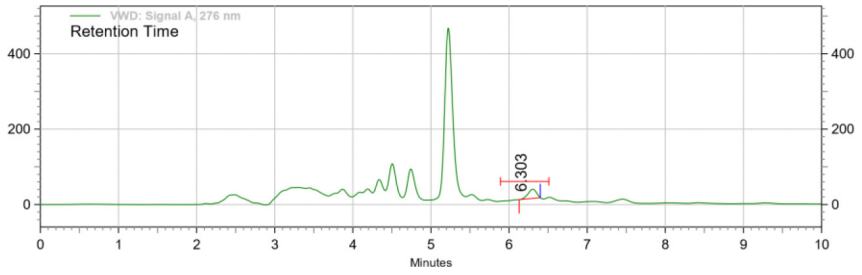


Figure 2. HPLC chromatogram of the methanolic extract of *T. delicatulum*, showing peaks corresponding to ellagic acid.

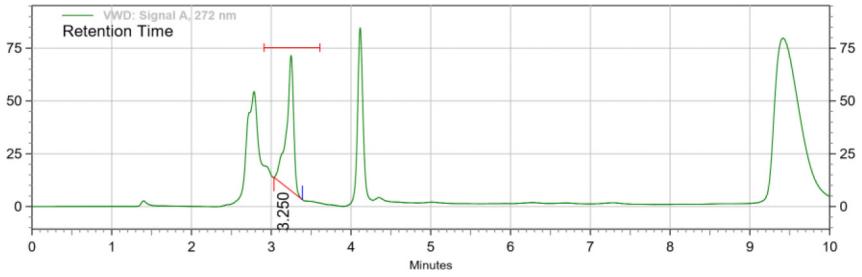


Figure 3. HPLC chromatogram of the methanolic extract of *T. delicatulum*, showing peaks corresponding to gallic acid.

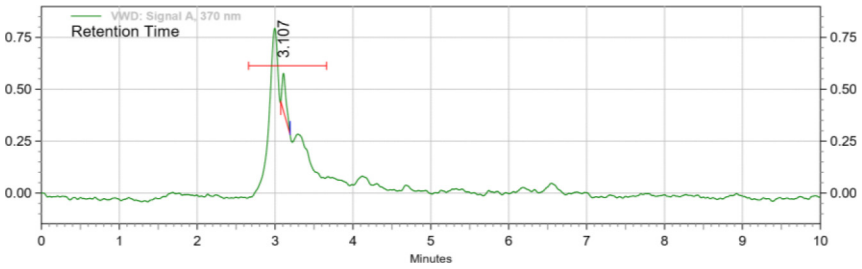


Figure 4. HPLC chromatogram of the methanolic extract of *T. delicatulum*, showing peaks corresponding to quercetin.

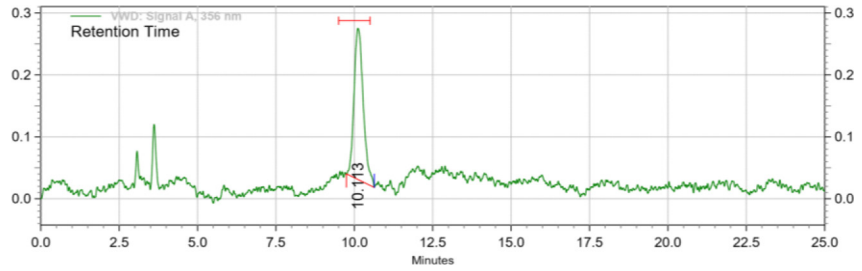


Figure 5. HPLC chromatogram of the methanolic extract of *T. delicatulum*, showing peaks corresponding to rutin.

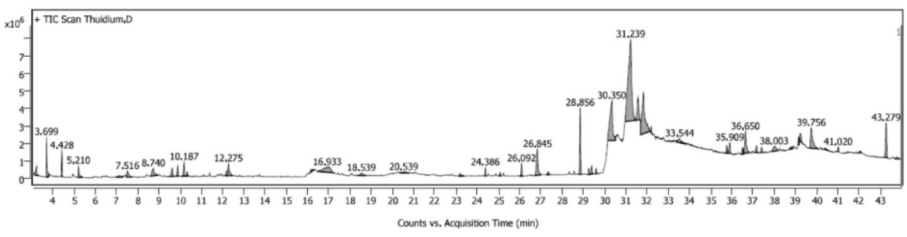


Figure 6. GC-MS spectrum of *T. delicatulum* methanolic extract.

Table 3. Identified major constituents in *T. delicatulum* methanolic extract

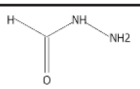
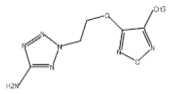
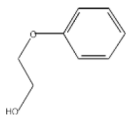
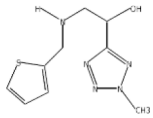
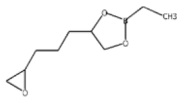
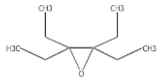
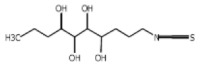
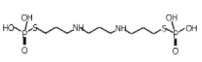
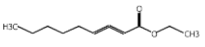
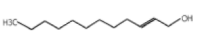
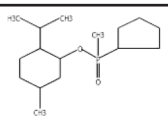
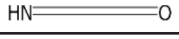
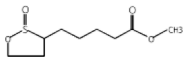
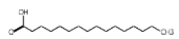
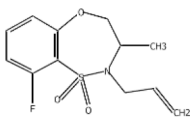
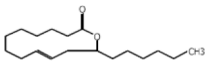
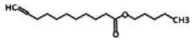
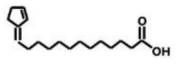
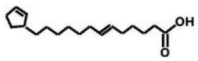
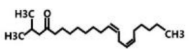
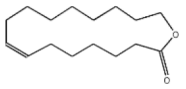
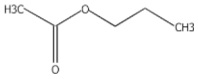
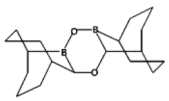
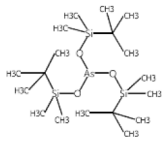
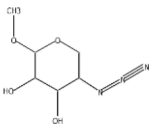
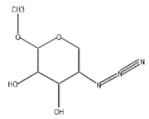
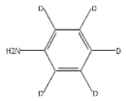
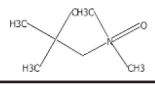
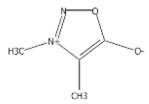
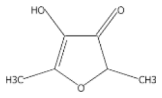
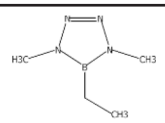
S. No.	RT (min)	Compound Name	Molecular Formula	MW g/mol	Abundance (%)	Structure
1	3.234	Formic acid hydrazide	CH_4N_2O	60.05	1.06	
2	3.699	Hydrogen isocyanate	CHNO	43.03	2.25	
3	4.428	n-Propyl acetate	$C_5H_{10}O_2$	102.14	1.22	
4	5.210	Methyl-2-oxopropanoate	$C_4H_6O_3$	102.09	0.90	
5	7.146	Methyl 4-azido-4 deoxy-β-L xylopranoside	$C_6H_{11}N_3O_4$	189.17	0.58	
6	7.516	Benzene-d5-amine	$C_6H_2D_5N$	98.16	0.91	
7	8.740	N,N,2,2-Tetramethylpropylamine N-oxide	$C_7H_{17}NO$	131.22	1.56	
8	9.628	Sydnone, 3,4-dimethyl-	$C_4H_6N_2O_2$	114.10	0.91	
9	9.887	Furaneol	$C_6H_8O_3$	128.13	0.73	
10	10.187	5-Ethyl-1,4-dimethyl-Delta.2-tetrazaboroline	$C_4H_{11}BN_4$	125.97	1.11	
11	10.328	2-[2-(4-Methyl-1,2-oxadiozole-3-yloxy) ethyl]- 2H-tetrazol-5-amine	$C_6H_9N_7O_2$	211.19	0.35	
12	12.181	Ethanol, 2-phenoxy	$C_8H_{10}O_2$	138.17	0.26	
13	12.275	2-[(2-Methyl-2H-tetrazol-5-yl) amino]-1-(thiophen-2-ylmethyl) ethanol	$C_9H_{13}N_5OS$	239.30	1.47	
14	16.222	2-Ethyl-4-(3-oxyranylpropyl)-1,3,2-dioxaborolane	$C_9H_{17}BO_3$	184.04	0.45	
15	16.933	2,2,3,3-Tetraethyloxirane	$C_{10}H_{20}O$	156.27	3.43	
16	18.539	4,5,6,7-Tetrahydroxydecyl isothiocyanate	$C_{11}H_{21}NO_4S$	263.36	0.58	
17	20.539	S-[3-(Bis(3-mercaptopropyl) amino) propyl] phosphorothioate	$C_9H_{24}N_2O_6P_2S_2$	382.38	0.51	
18	23.186	Ethyl nona-2,3-dienoate	$C_{11}H_{18}O_2$	182.26	0.31	

Table 3. Continued...

S. No.	RT (min)	Compound Name	Molecular Formula	MW g/mol	Abundance (%)	Structure
19	24.386	Trans-Dodec-2-en -1-ol	$C_{12}H_{24}O$	184.32	0.52	
20	25.086	2-Isopropyl-5-methylcyclohexyl cyclopentyl (methyl) phosphinate	$C_{16}H_{31}O_2P$	286.40	0.28	
21	26.092	Methyl 5-[2-oxo-1,2-oxathiolan-3yl] pentanoate	$C_9H_{16}O_4$	220.29	0.87	
22	26.845	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	3.71	
23	27.356	(R)-2-Allyl-9-fluoro-3-methyl-3,4-dihydro-1,4,5-benzoxathiazepine-1,1-dioxide	$C_{12}H_{14}FNO_3S$	271.31	0.34	
24	28.856	13-Hexyloxacyclotridec-10 en-2-one	$C_{18}H_{32}O_2$	280.45	4.78	
25	29.609	Undec-10-ynoic acid, pentyl ester	$C_{16}H_{28}O_2$	252.40	0.38	
26	31.239	Methyl 8,9-octadecadienoate	$C_{19}H_{34}O_2$	294.48	25.33	
27	36.515	2-Cyclopentene-1tridecanoic acid, (S)-	$C_{18}H_{32}O_2$	280.44	0.72	
28	36.650	Gorlic acid	$C_{18}H_{30}O_2$	278.44	3.11	
29	37.174	Isopropyl linoleate	$C_{21}H_{38}O_2$	322.53	0.66	
30	37.409	Oxacycloheptadec-8-en2-one, (8Z)-	$C_{16}H_{28}O_2$	252.40	0.46	
31	43.279	2,3,5,6-Bis (1,5-octadenyl)-2,5-dibora-1,4-dioxane	$C_{18}H_{30}B_2O_2$	300.06	3.40	
32	43.773	Tris (tert-butyldimethylsilyloxy) arsane	$C_{18}H_45AsO_3Si_3$	468.73	0.30	

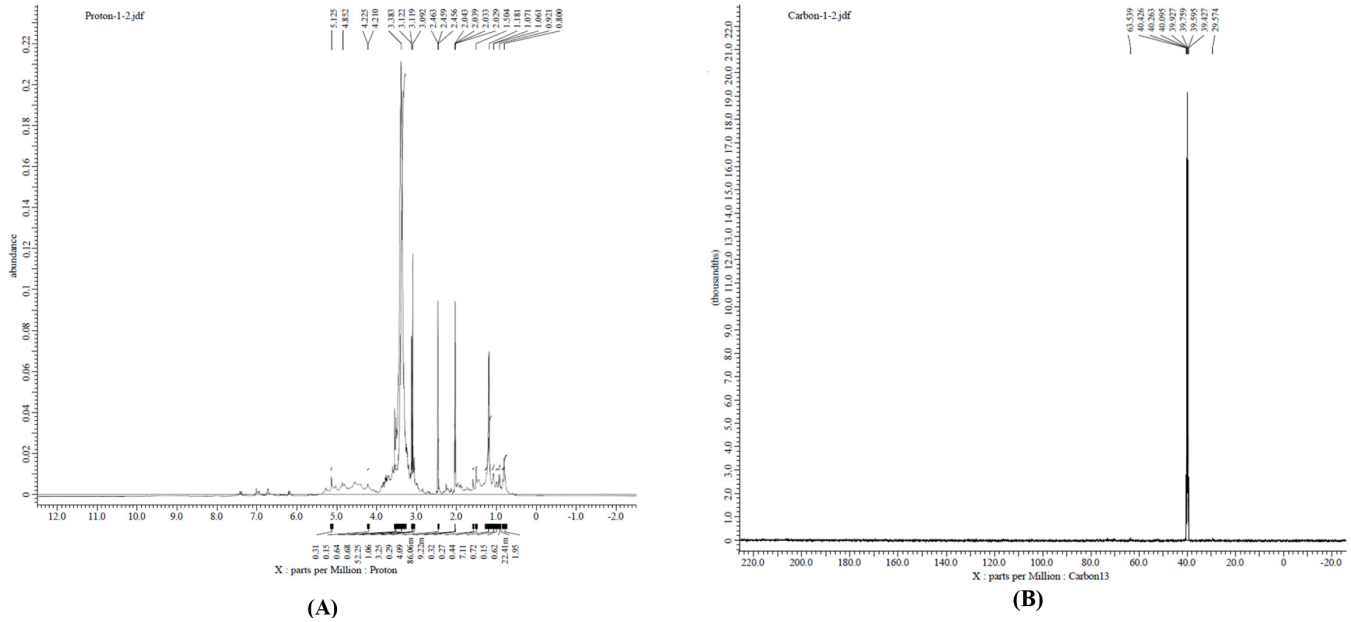


Figure 7. The spectra of the newly isolated compound from *T. delicatulum* **(A)** ^{1}H NMR **(B)** ^{13}C NMR.

Table 4. ^{1}H NMR in table format

S. No.	Chemical Shift (δ, ppm)	Multiplicity	Integration	Tentative Proton
1	5.125	d	1H	Vinylic or -CH adjacent to electronegative atom (*e.g.*, -OH, -OR)
2	4.825	d	1H	-CH near ester or alcohol group
3	4.225	t	2H	-CH_2 next to -OH or ester (*e.g.*, -CH_2-O-)
4	3.920	t	2H	-CH_2 near oxygen or nitrogen
5	3.330	m	1H	Possibly methine proton in a crowded environment
6	3.112	m	2H	-CH_2-N or -CH_2-O
7	2.582	t	2H	-CH_2- adjacent to carbonyl or aromatic ring
8	2.469	dd	2H	Benzylic -CH_2- near π-system or carbonyl
9	2.363	m	2H	CH_2 near -CO or aromatic system
10	2.293	m	2H	Aliphatic –CH_2 near polar group
11	2.039	m	2H	CH_2 in long chain or near weak deshielding group
12	1.920	m	2H	-CH_2 in saturated aliphatic chain
13	1.204	d	3H	Methyl group near -CH
14	1.181	d	3H	Methyl on secondary carbon
15	1.071	t	3H	Terminal methyl (-CH_2CH_3)
16	1.041	t	3H	Similar terminal methyl
17	0.921	t	3H	Terminal CH_2 in alkyl chain
18	0.800	d	3H	CH_2 adjacent to CH (isopropyl-type)

flagged by the library likely arise from co-eluting contaminants or spectral similarity to common esters and lactones rather than authentic boron-containing metabolites, while several additional unidentified peaks suggest the presence of further minor or novel secondary metabolites.

Table 5. ^{13}C NMR in table format

S. No.	Chemical Shift (δ, ppm)	Tentative Carbon
1	63.539	-CH_2-O or -CH-O (*e.g.*, alcohol, ester, or ether group)
2	40.626	Tertiary carbon or –CH_2-N
3	40.206	Similar -CH_2 near N or quaternary carbon
4	40.057	Likely -CH_2- in aliphatic chain
5	39.950	Same region, possibly overlapping signals
6	39.915	-CH_2 or -CH group (saturated hydrocarbon)
7	39.559	Internal aliphatic carbon
8	39.499	Possibly symmetrical carbon or overlapping peak
9	25.474	-CH_2- in aliphatic region, likely methylene in long chain
10	23.574	Terminal methyl group (-CH_2) or secondary alkyl

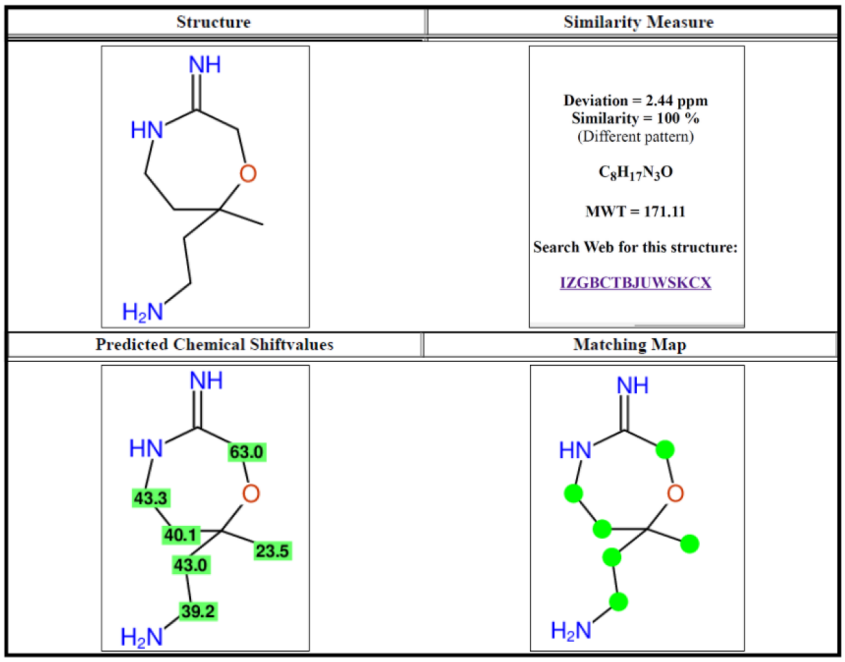


Figure 8. Tentative structure of newly isolated compound from *T. delicatulum*.

3.6 NMR

Based on the detailed interpretation of the ^1H and ^{13}C NMR data, the molecular formula can be deduced systematically (Figure 7, Tables 4 and 5). The ^1H NMR integration values sum to 17 protons, indicating a moderately saturated compound with multiple aliphatic protons. The spectrum includes signals for methyl (-CH_3), methylene (-CH_2), and methine (-CH) groups, along with deshielded protons likely adjacent to electronegative atoms such as oxygen or nitrogen. In the ^{13}C NMR spectrum (0-220 ppm), symmetry-equivalent C-2 and C-8 appear as one resonance, accidentally isochronous C-4 and C-6 merge into a second, and the six remaining carbons (1, 3, 5, 7, 9, 10) each furnish a distinct signal, collapsing the expected ten resonances to eight observed lines. Whereas, two quaternary purine carbons at δ 159.4 (C-6, carbonyl) and δ 154.8 (C-4a), four methane or methylene ring carbons between δ 39-43, one oxygen-bearing CH at δ 63.0, and one terminal methyl at δ 23.5. This gives the molecular formula $C_8H_{17}N_3O$, corresponding to DBE = 3 (one ring plus two π-bonds), consistent with a 6-oxo-purine core and the stick structure with a 2-(2-hydroxyethyl) substituent on the exocyclic amino group.

3.7 Structure Elucidation and Validation

The molecular structure of the target compound was determined and validated through spectral analysis and database comparison. The elucidated compound, assigned the molecular formula $C_8H_{17}N_3O$, possesses a Molecular Weight (MWT) of 171.11. Structural analysis revealed a heterocyclic ring system incorporating oxygen and nitrogen atoms, as well as alkyl and amine functional groups (Figure 7A and B). The IUPAC name of the identified compound is 2-(2-hydroxyethylamino)-1,7-dihydro-6H-purin-6-one. The compound achieved a similarity score of 100% when compared with reference spectral patterns, with a deviation of 2.44 ppm, signifying a high degree of accuracy in structure prediction and validation. The match was categorised under a "different pattern", indicating that while the spectral features matched closely, the pattern represents a less common or novel spectral arrangement.

Chemical shift values for the carbon atoms were as follows:

1. 63.0 ppm (oxygen-linked carbon).
2. 43.3 ppm, 43.0 ppm, 40.1 ppm, 39.2 ppm, and 23.5 ppm for remaining carbons in aliphatic chains and heterocyclic rings.

The matching map (Figure 8A and B) confirmed complete structural validation, with all key positions showing successful spectral correspondence, as indicated by green nodes.

4. Discussion

The current study represents a novel and comprehensive attempt to evaluate the chemical diversity of *T. delicatulum* (Hedw.) Schimp. using an integrative approach involving FT-IR fingerprinting, GC-MS, HPLC, and NMR spectroscopy. While over 22 published investigations have explored various biological, ecological, and chemical aspects of *T. delicatulum*, this study advances previous work by generating high-resolution metabolite profiles, presence of 32 volatile compounds and identifying a tentative structure of novel bioactive compound.

FT-IR spectra have been effectively used to characterise the chemical part of moss. *T. delicatulum* consistently contains flavonoids, tannins, and terpenoids, supporting its therapeutic potential, as also shown by Karaoglu *et al.* through its antibacterial activity[19]. In another study, Adebiyi *et al.* detected the presence of steroids, saponins, flavonoids, and alkaloids in the aqueous extracts of *Thidium gratum* and *Barbula indica*. Mohandas and Kumaraswamy identified various bioactive compounds; lipids, proteins, carbohydrates, terpenoids, phenols, organic acids, fatty acids; in *Thuidium tamariscellum* extracts[20,21]. Deora and Guhil found cardiac glycosides, terpenoids, flavonoids, and sterols in *Bryum argenteum*, *B. capillare*, and *B. cellulare*[15]. Similarly, Valeeva *et al.,* identified alkaloids, flavonoids, saponins, steroids, and phenols in *Physcomitrium patens* and *Sphagnum fallax*[22]. Alves *et al.,* reported flavonoids and terpenoids in *Octoblepharum albidum* whereas, Commisso *et al.,* highlighted the antioxidant, antimicrobial, and anti-inflammatory roles of such compounds in bryophytes[23,24]. Compared to these, *T. delicatulum* shows a broader phytochemical diversity. Studies by Klegin *et al.* on essential oils and cytotoxicity in related mosses further illustrate the functional significance of moss-derived metabolites[25,26].

Chromatography results further revealed that *T. delicatulum* contain large amounts of chemical compounds ellagic acid and gallic acid. Ellagic acid, a natural polyphenol found in fruits like pomegranates, berries, and walnuts, exhibits strong antioxidant, anti-carcinogenic, and anti-inflammatory properties. It reduces oxidative stress, inhibits tumour growth, and downregulates pro-inflammatory mediators such as TNF-α and IL-6, highlighting its therapeutic potential in cancer prevention[27-29]. Gallic acid, though structurally related to ellagic acid, has distinct antioxidant activity, protecting LDL from oxidation and showing anti-proliferative effects on cancer cells[30]. It also enhances the antioxidant capacity of polyphenol-rich foods like pomegranate juice[31]. Combined with other phenolics, gallic acid exhibits synergistic biological effects, as noted by Vattem and Shetty, further validate the medicinal relevance of *T. delicatulum*[32].

Aslanbaba *et al.*, also identified 31 compounds from the water extract of *T. tamariscinum*, which exhibited potent antioxidant activity. GC-MS analysis of *T. delicatulum* has not been extensively documented in the current literature specifically targeting this bryophyte[33]. On the other hand, in higher plants some similar compounds have been found, Prabha and Bushra reported that 9, 12-Octadecadienoic acid

(Z,Z)-2,3-dihydroxypropyl ester was a major bioactive compound in *Andrographis paniculata*. Whereas Anandan *et al.* and Priya *et al.* reported the identification of n Hexadecanoic acid in *Aerva lanata* and *Diospyros virginiana*, respectively[34,35]. Further identification of E-8-methyl 9 tetradecen-1-ol acetate was made by Jebaseelan in the flower extract of *Millingtonia hortensis* and was found to be present in higher quantities than other bioactive compounds[36]. From *Phyllanthus niruri*, Uparkar and Ganatra reported the identification of a methyl ester, (Z, Z, Z)[37]. Moreover, Sharmila *et al.* also isolated several compounds from *Nasika churnam* such as cyclobutene derivatives, aromatic ketones, cinnamate esters, saturated and unsaturated fatty acids, phenyl esters, phytosterols like stigmasterol and γ-sitosterol, as well as terpenoid esters of succinic acid[38].

One of the primary challenges in this research is obtaining sufficient quantities of pure samples due to their small size. However, NMR is a key advantage in this context; its non-destructive nature and ability to provide detailed structural insights into organic compounds; making it highly valuable for elucidating the chemical composition and biological functions of these non-vascular land plants. For instance, Wei *et al.* describe the isolation of bioactive compounds, including erucamide and β-sitosterol, from *Bacillus megaterium*, employing NMR techniques such as ^{1}H and ^{13}C NMR for structural characterisation. The identified compound 2-(2-hydroxyethylamino)-1,7-dihydro-6H-purin-6-one contains functional groups; aminoethyl and pyrimidinyl; supporting its potential as a drug candidate through interactions with biological targets and nucleic acids. Structurally similar compounds have shown notable biological activities; for example, a related piperazine derivative exhibited dopamine uptake inhibition, suggesting neurological relevance[39]. Pyrimidine-based compounds are known for their diverse pharmaceutical properties[14].

Bryophytes are emerging as sources of bioactive molecules with antibacterial, antifungal, and therapeutic effects. Klavina *et al.*, emphasised the rich phytochemical diversity in mosses, supporting their pharmaceutical potential[14]. Structural studies using NMR have further aided the synthesis and functional evaluation of such compounds, reinforcing the promise of developing therapeutics inspired by bryophyte-derived structures like 2-(2-hydroxyethylamino)-1,7-dihydro-6H-purin-6-one[40]. As these studies have confirmed that *T. delicatulum* methanolic extract contains higher amount of bioactive compound which have different kind of medicinal properties. As bryophytes have been used successfully in *Ayurvedic* medicines from ancient times, more clinical trials should be conducted to support its therapeutic use.

5. Conclusion

The present study represents a pioneering effort to explore the phytochemical composition of *T. delicatulum* collected from the Kumaon region of Uttarakhand, India; an area previously underexplored for the bioactive potential of bryophytes. Through a comprehensive analytical approach employing GC-MS, HPLC, and NMR techniques, the methanolic extract of *T. delicatulum* was found to contain a diverse range of bioactive compounds. Notably, a novel compound was also detected for the first time in this species, highlighting the potential of *T. delicatulum* as a source of previously uncharacterised natural products. Despite limitations related to the small biomass of bryophytes, this study demonstrates that modern analytical techniques can successfully uncover their chemical richness. Follow-up work could therefore aim to isolate and fully characterise the newly detected constituent, and, once sufficient quantities are available, to subject both the pure compound and the crude extract to routine antimicrobial, antioxidant, and cytotoxicity screens; such assays would provide the experimental evidence needed for future biomedical relevance or agrochemical relevance.

6. Acknowledgement

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Author Contribution

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