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Phytochemical Characterization of the lichen *Parmotrema* sp. Using GC–MS Analysis

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Abstract

The lichen genus *Parmotrema* is recognized as a rich source of structurally diverse secondary metabolites with considerable pharmaceutical potential. The present study investigated the phytochemical composition of *Parmotrema* sp. using Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS analysis of the methanolic extract identified eight phytochemical constituents, with orcinol (76.59%) as the predominant compound, followed by benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester (12.10%) and chloroatranorin (4.04%). The GC–MS findings provide a comprehensive phytochemical profile of *Parmotrema* sp. and demonstrate its richness in phenolic and aromatic secondary metabolites. These findings provide a scientific basis for future isolation and pharmacological evaluation of bioactive compounds from *Parmotrema* sp.

INTRODUCTION

Lichens are unique poikilohydric symbiotic cryptogamic flora between alga (phytobiont) and a fungus (mycobiont) (Singh *et al.,* 2013; Parrot *et al.,* 2016), that adapt to a wide range of environments, with their population influenced by factors like humidity, temperature, air quality and nutrient levels (Geiser *et al.,* 2021). They covered approximately 8% of the Earth's land surface, even though they are among the slowest-growing organisms (Malhotra *et al.,* 2008). In lichen, the fungal partner possesses a thick polysaccharide cell wall and produces wide variety of secondary metabolites that are not found in other organisms (Asahina *et al.,* 1954; Culberson *et al.,* 1969; Huneck *et al.,* 1996). Lichens produce a wide range of natural compounds, including depsides, depsidones, dibenzofurans, quinones, chromones, carotenoids, polysaccharides, monosaccharides, and aliphatic acids (Millot *et al.*, 2016; Tatipamula *et al.,* 2021; Macedo *et al.,* 2021; Urena-Vacas *et al.,* 2021; Badiali *et al.,* 2023). These compounds protect lichens from

environmental stress and have significant pharmacological potential, including anti-HIV, anti-oxidative, cytotoxic, antimicrobial (antibacterial, antifungal, and antiviral), anti-inflammatory, and antiproliferative activities (Nguyen *et al.,* 2014; Dandapat and Paul 2019; Maulidiyah *et al.,* 2021; Ozturk *et al.,* 2021; Mendili *et al.,* 2022; Kello *et al.,* 2023; Boustie *et al.,* 2005; Muller *et al.,* 2001; Ramya *et al.,* 2017).

Figure 1. Field photograph of *Parmotrema* sp.

Parmotrema A. Massal. (previously known as *Parmelia* S. lat.) is one of the largest genera of parmelioid core in the family Parmeliaceae (Blanco *et al.,* 2005). The name *Parmotrema* originates from the Greek terms *parmos* (cup) and *trema* (perforation), alluding to the perforate apothecia characteristic of members of this genus (Feige,1998). Among lichen-forming fungi, Parmeliaceae is one of the largest, most common, and well-known families of the phylum Ascomycota (Hawksworth *et al.,* 1995, Blanco *et al.,* 2004 a, b), comprising more than 2,765 species about 85 genera all over the world (Lucking *et al.,* 2016). Species of *Parmotrema* produce various secondary metabolites, including atranorin, usnic acid, orsellinates, gyrophoric acid, salazinic acid, and lobaric acid. These compounds are well known for their significant pharmacological properties and phytochemical significance (Gómez Serranillos *et al.,* 2014). Extracts and isolated compounds from various *Parmotrema* species have demonstrated antibacterial and antifungal activities (Tuan *et al.,* 2020; Shiromi *et al.,* 2021; Dwarakanath *et al.,* 2024; Chakarwarti *et al.,* 2024).

GC-MS (Gas Chromatography – Mass Spectrometry) is a powerful technique for the separation, identification and characterization of volatile and semi-volatile compounds in complex mixtures. It plays vital role in the bioactive profiling. GC-MS enables accurate identification of chemical compounds and widely employed in natural product research (Chandrasekar *et al.,* 2024). Therefore, the present study aimed to characterize the phytochemical profile of *Parmotrema* sp. using GC–MS analyses, with the objective of chemical constituents that may contribute to its biological and pharmacological potential.

MATERIALS AND METHODS

Study Area

The study was conducted at Kenthorai, located in the Nilgiris Hills of the Western Ghats, Tamil Nadu, India (11.438884° N, 76.745264° E). The region is characterized by a cool and humid climate, providing favorable conditions for the growth of corticolous (bark) lichens (Pascal, 1988).

Figure 2. Geographic location of the study area at Kenthorai, Nilgiris, Tamil Nadu.

Figure 3. *Parmotrema* sp. growing on tree bark.

Sample Collection

Healthy thalli of *Parmotrema* sp. were collected from tree bark at the study site in October 2025. The specimens were carefully detached from the substrate by hand, placed in clean paper bags, and transported to the laboratory. Morphological identification of the collected specimens was carried out using standard taxonomic keys (Awasthi, 2007).

Sample preparation

Freshly collected lichen samples were gently rinsed with distilled water to remove adhering dust and extraneous materials without prolonged washing. The samples were cut into small pieces and shade-dried at ambient temperature until a constant weight was attained. The dried material was pulverized into a fine powder using a laboratory mixer grinder. The powdered samples were wrapped in butter paper, transferred to airtight zip-lock bags, and stored under dry conditions until further phytochemical analysis.

Sample extraction

1 gram of the powdered lichen sample was transferred into a 25 mL test tube and extracted with 5 mL of analytical-grade methanol. The mixture was vortexed for 2 min and subsequently shaken using a mini rotary flask shaker at 150 rpm for 14–16 h at room temperature. The extract was filtered through Whatman No. 1 filter paper, transferred into GC–MS vials, and stored at 4 °C until GC–MS analysis.

Gas chromatography - Mass spectrometry (GC-MS) analysis

GC–MS analysis was performed using an Agilent Gas Chromatography–Mass Spectrometry system equipped with an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. A 2 µL aliquot of the methanol extract was injected in split mode (10:1), with the injector temperature

maintained at 280 °C. The oven temperature was initially held at 110 °C for 3.5 min, increased to 200 °C at a rate of 10 °C min^{-1}, and then to 280 °C at 5 °C min^{-1}, followed by a final hold of 12 min. Mass spectra were acquired in electron ionization (EI) mode at 70 eV over a scan range of 50–550 m/z. The phytochemical constituents were identified by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Library and by referring to standard spectral interpretation methods (Silverstein *et al.,* 2005; Pavia *et al.,* 2009; Sasidharan *et al.,* 2011).

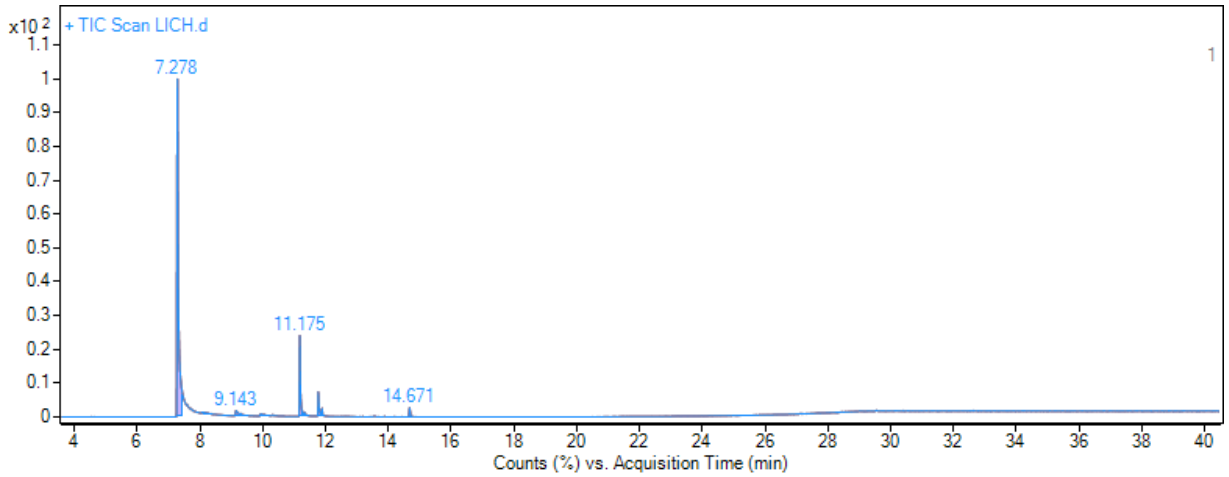
RESULTS AND DISCUSSION:

GC MS analysis of the methanolic extract of the *Parmotrema* sample revealed the presence of eight phytochemical compounds (Figure 4; Table 1). The compounds were identified based on their retention time and mass spectral comparison with the NIST library. Orcinol was the dominant compound with a peak area of 76.59%, followed by a Benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester, (12.10%) and chloroatranorin (4.04%). The remaining compounds, including dopamine, Pyrazine, 2-methoxy-3-(1-methylpropyl)-, 1,3-Benzenediol, 5-pentyl-, Propyl 2,4-dihydroxy-6-methylbenzoate, and 4-methylesculetin, were detected in smaller amounts.

Table: 1. GC-MS profile of *Parmotrema* sp

R.T	Compound Name	Molecular Weight	FORMULA	Peak Area %
7.27	Orcinol	124	$C_7H_8O_2$	76.59
8.08	Dopamine	153	$C_8H_{11}NO_2$	0.91
9.14	Pyrazine, 2-methoxy-3-(1-methylpropyl)-	166	$C_9H_{14}N_2O$	2.88
9.94	1,3-Benzenediol, 5-pentyl-	180	$C_{11}H_{16}O_2$	1.46
11.17	Benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester	182	$C_9H_{10}NO_4$	12.10
11.33	Propyl 2,4-dihydroxy-6-methylbenzoate	210	$C_{11}H_{14}O_4$	0.86
11.76	Chloroatranorin	408	$C_{19}H_{17}ClO_8$	4.04
11.88	4-Methylesculetin	192	$C_{10}H_8O_4$	1.16

Figure 4. GC-MS chromatogram of *Parmotrema* sp



Lichens represent more than 20% of the known fungal diversity, and their symbiotic relationship results in the production of unique secondary metabolites (Zambare and Christopher, 2012). They are recognized for their remarkable chemo-diversity, producing a wide range of bioactive compounds, nearly 80% of which are unique when compared with those of other organisms (Leela *et al.,* 2017). These metabolites are chemically distinct from those produced by fungi, algae, and higher plants (Lokajova *et al.,* 2014). More than 1,050 secondary metabolites have been identified from lichen species (Letwin, 2017), many of which have shown promising pharmaceutical potential by exhibiting potent anti-bacterial, anti-fungal, anti-septic, anti-inflammatory, anti-oxidant, anti-cancer and anti-proliferative activities (Vivek *et al.,* 2014; Rajan *et al.,* 2016; Kumar and Khurana, 2019).

In the present study, GC-MS analysis of the methanolic extract of *Parmotrema* sp. identified 8 phytochemical compounds. In previous GC-MS studies reported 13 major compounds in *P. rampoddense* and 12 major compounds were reported in *P. tinctorium* respectively, using hexane, chloroform and acetonitrile extracts (Shiromi *et al.,* 2021). The observed variation in the number and composition of compounds may be attributed to differences in species, geographical location, environmental conditions, extraction solvent, and GC–MS analytical conditions (Shrestha and St. Clair, 2014; Thadhania *et al.,* 2021).

Among the identified phytochemical constituents, Orcinol was the predominant compound, accounting for 76.59% of the total peak area. For instance, methanolic extract of *Parmotrema perlatum* has similarly been reported to contain Orcinol as a major constituent with a peak area (63.26%) (Dwarakanath *et al.,* 2024), while GC-MS analysis of *Parmotrema tinctorum* also identified Orcinol among the major phenolic groups (Sahoo *et al.,* 2025). Furthermore, mass spectral analysis confirmed the identification of Orcinol as 5-methyl-1,3-benzenediol in lichen extracts (Bhaktavalsala *et al.,* 2021). The consistent detection of orcinol is a major phytochemical constituent that may contribute significantly to the biological activities of *Parmotrema* sp. Previous studies have reported that orcinol and structurally related derivatives possess diverse biological activities, including antioxidant, cytotoxic, antidepressant, and

immunomodulatory effects (Tang *et al.,* 2004; Wu *et al.,* 2005; Bafna *et al.,* 2006). In addition, orcinol has been reported to induce dose-dependent cytotoxic and apoptotic effects against human colorectal cancer cells (Yanik *et al.,* 2023), further supporting its importance as a bioactive phenolic compound in the methanolic extract of *Parmotrema* sp.

Lichens are known to produce a wide range of secondary metabolites, many of which belong to the phenolic class and exhibit significant biological activities (Molnar and Farkas, 2010). Among these metabolites, benzoic acid derivatives have been reported from several lichen species, including *Parmotrema* species. In the present study, benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester was identified with a relative abundance of 12.10%. Similar benzoic acid-related compounds have also been reported in *Parmotrema perlatum* by (Dwarakanath *et al.,* 2024) suggesting that these metabolites are commonly associated with the genus. Khader *et al.,* 2024 demonstrated the anti-inflammatory potential of a benzoic methyl ester isolated from *Parmotrema reticulatum*, highlighting the biological significance of benzoic acid-derived metabolites in the genus *Parmotrema*. The occurrence of this benzoic acid derivative further supports the presence of bioactive phenolic constituents in *Parmotrema* sp., which may contribute to its biological activities.

Followed by Chloroatranorin was identified as the third major constituent of the methanolic extract, accounting for 4.04% of the total peak area. Chloroatranorin is a well-known lichen depside commonly reported in several lichen species. Previous studies have demonstrated that chloroatranorin possesses moderate antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* (Turk *et al.,* 2006). Rankovic *et al.,* 2014, also reported that chloroatranorin exhibited antibacterial activity comparable to atranorin, although its antioxidant activity was relatively low in the DPPH assay. Therefore, the presence of chloroatranorin in the methanolic extract may contribute to the antimicrobial potential of *Parmotrema* sp.

Some compounds with less composition such as, dopamine (0.91%), pyrazine, 2-methoxy-3-(1-methylpropyl)- (2.88%), 1,3-benzenediol, 5-pentyl- (1.46%), propyl 2,4-dihydroxy-6-methylbenzoate (0.86%), and 4-methylesculetin (1.16%), were identified. Overall, the GC-MS profile of the methanolic extract of *Parmotrema* sp. demonstrated the presence of several bioactive phytochemicals. The identified compounds, particularly phenolic metabolites such as orcinol, benzoic acid derivatives, and chloroatranorin, may contribute to the antioxidant, antimicrobial, and other biological activities reported for *Parmotrema* species. However, further isolation and biological evaluation are required to confirm the pharmacological properties of the individual compounds.

Conclusion

In conclusion, the present study demonstrated that *Parmotrema* sp. possesses a diverse phytochemical profile, as evidenced by GC–MS analysis. The GC–MS findings confirmed the presence of phenolic and aromatic metabolites, with orcinol identified as the predominant

constituent. These results provide valuable insights into the chemical composition of *Parmotrema* sp. and support its potential as a promising source of bioactive natural products. Further studies are required to isolate the active constituents and validate their pharmacological properties.

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