

GC–MS Profiling of the Lichen *Roccella montagnei* Bel. from Thengapattanam, Kanyakumari District, Tamil Nadu, India

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Abstract:

The coastal distribution and distinctive secondary-metabolite of *Roccella montagnei* Bel. provide a relevant basis for investigating its chemical diversity within the Roccellaceae, with the species frequently associated with coastal substrates and mangrove vegetation in India. The present study investigated the chemical profile of *R. montagnei* Bel. collected from the Thengapattanam coastal region of Kanyakumari District, Tamil Nadu, India, with particular emphasis on its GC–MS-detectable secondary metabolites. The lichen material was subjected to solvent extraction and GC–MS analysis, and the detected constituents were identified based on their mass spectral characteristics. The resulting profile was dominated by orcinol (49.92%) and methyl 2,6-dihydroxy-4-methylbenzoate (45.16%), while erythritol (1.10%), norfenefrine (1.35%), palmitoleic acid (0.77%) and 1,1'-biphenyl, 5-hydroxy-3,4-dimethoxy- (1.69%) occurred as minor constituents. The predominance of phenolic metabolites, particularly orcinol and methyl 2,6-dihydroxy-4-methylbenzoate, highlights the characteristic secondary-metabolite composition of the analyzed specimen. This study provides a GC–MS-based chemical profile of *R. montagnei* from the Kanyakumari coastal region and contributes to the chemical characterization of this ecologically specialized lichen, providing a basis for subsequent targeted isolation and structural and biological evaluation of its major metabolites.

Keywords: *R. montagnei*, coastal distribution, orcinol, GC-MS, secondary metabolites.

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INTRODUCTION

Lichens constitute a widely distributed group of slow-growing symbiotic organisms capable of persisting under diverse and often extreme environmental conditions (Sipman & Aptroot, 2001; Nayaka *et al.*, 2003; Loppi *et al.*, 2003). They represent complex biological systems arising from an association between a heterotrophic, lichen-forming fungus and an autotrophic photosynthetic partner (Juriado *et al.*, 2019; Elkhateeb *et al.*, 2020). The fungal component predominantly belongs to the phylum Ascomycota, although members of Basidiomycota are also known to occur, whereas the photosynthetic partner is generally represented by green algae or cyanobacteria (Wang *et al.*, 2014; Lucking *et al.*, 2017; Bates *et al.*, 2011). Lichens produce a diverse array of secondary metabolites with distinct chemical structures, including phenolic compounds, dibenzofurans, depsides, depsidones, aliphatic acids, quinones, pulvinic acid derivatives, and anthraquinone-related compounds (Muller, 2001; Boustie *et al.*, 2005).

The genus *Roccella* comprises 24 recognized species, which are predominantly restricted to coastal habitats (Tehler *et al.*, 2010). The lichen species *Roccella montagnei* Bel. belongs to the family Roccellaceae and exhibits a fruticose growth form (Tehler *et al.*, 2004), is traditionally used in folk medicine and has been reported to possess a rich

secondary metabolite profile with potential in the management of various infections and diseases (Tatipamula *et al.*, 2019). *R. montagnei* is reported to contain several secondary metabolites, including orcinol, montagnitol, β -carotene, β -sitosterol, erythritol, roccellic acid, lecanoric acid, and methyl orsellinate (Neelakantan *et al.*, 1956; Mishra *et al.*, 2017). The diverse secondary metabolites reported from *R. montagnei* has also been associated with its biological significance. Previous studies have reported antimicrobial, antifungal, insecticidal, and anti-inflammatory activities of this species (Balaji *et al.*, 2006; Cetin *et al.*, 2008; Nanayakkara *et al.*, 2005).



Figure 1. Morphological characteristics of the lichen *R. montagnei*.

Gas Chromatography–Mass Spectrometry (GC–MS) is a widely used technique for identifying volatile and semi-volatile chemical constituents through comparison of mass spectral fragmentation patterns with NIST/WILEY libraries (Chandrasekar *et al.*, 2024). Despite the documented occurrence of diverse secondary metabolites and reported biological properties of *R. montagnei* studies focusing on its chemical composition, particularly GC–MS-based characterization of secondary metabolites in specimens collected from Thengapattanam, Kanyakumari District, Tamil Nadu, are limited. Therefore, the present study aimed to characterize the chemical constituents of *R. montagnei* collected from Thengapattanam using GC–MS, with emphasis on the identification and characterization of its secondary metabolites.

MATERIALS AND METHODS

Study Area

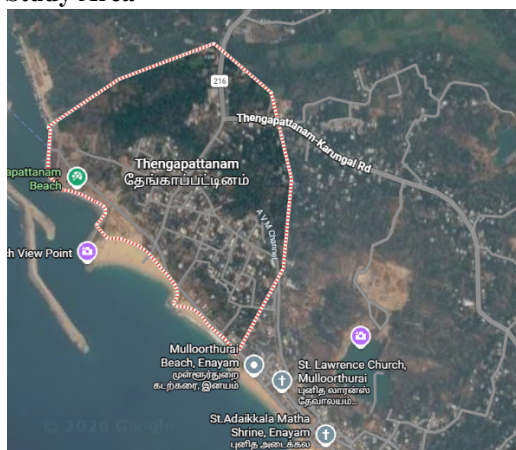


Figure 2. Location map of the study area

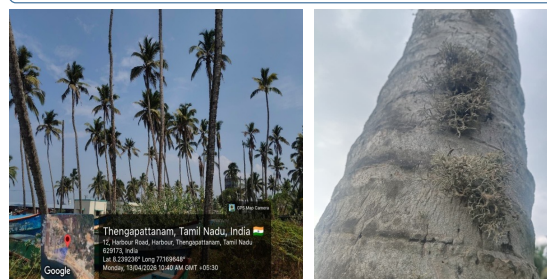
The study was conducted in Thengapattanam, Kanyakumari District, Tamil Nadu, India, located at 8.239152° N latitude and 77.169531° E longitude. The study site represents a coastal environment with vegetation and tree substrates suitable for the occurrence of corticolous lichens. During the field

survey, *R. montagnei* was observed growing on the bark of coconut trees (*Cocos nucifera* L.).

Sample Collection

Healthy thalli of *R. montagnei* were collected from the bark of coconut trees (*Cocos nucifera* L.) at the study site in Thengapattanam during the month of April 2026. The thalli were carefully detached from the bark and transferred to clean paper bags for transportation to the laboratory. The collected specimens were identified based on their morphological characteristics using standard taxonomic keys (Awasthi, 2007), and authenticated as *R. montagnei* (Roccellaceae) at the Botanical Survey of India, Southern Regional Centre, Coimbatore.

Figure 3. Study site and occurrence of *R. montagnei* on coconut tree bark.



a. Thengapattanam Study site

b. *R. montagnei* growing on tree bark

Sample Preparation

The freshly collected thalli of *R. montagnei* were gently rinsed with distilled water to eliminate surface dust and extraneous debris, avoiding prolonged exposure to water. The cleaned thalli were cut into small fragments and shade-dried at ambient room temperature until a constant weight was achieved. The dried material was then pulverized into a fine, uniform powder using a laboratory mixer grinder. The resulting lichen powder was wrapped in butter paper, packed into airtight zip-lock pouches, and stored under dry, moisture-free conditions until further use in phytochemical extraction and analysis.

Extraction of Lichen Sample

For extraction, 1 g of the powdered *R. montagnei* sample was weighed and transferred into a 25 mL test tube containing 5 mL of analytical-grade methanol. The mixture was vortexed for 2 min to ensure uniform mixing and subsequently agitated on a mini rotary flask shaker at 150 rpm for 14–16 h at room temperature to facilitate efficient extraction of the phytoconstituents. Following extraction, the mixture was filtered through Whatman No. 1 filter paper to remove particulate residues, and the clear filtrate was transferred into GC–MS vials and stored at 4 °C until subjected to GC–MS analysis.

GC–MS Analysis

GC–MS analysis of the methanolic extract of *R. montagnei* was carried out using an Agilent Gas Chromatography–Mass Spectrometry system fitted with an Rtx-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium gas served as

the carrier gas at a constant flow rate of 1 mL min⁻¹. A 2 µL aliquot of the extract was injected in split mode (10:1) with the injector port temperature set at 280 °C. The column oven temperature program was initiated at 110 °C (held for 3.5 min), ramped to 200 °C at 10 °C min⁻¹, followed by a further increase to 280 °C at 5 °C min⁻¹, and finally held at 280 °C for 12 min. Mass spectral detection was performed in electron ionization (EI) mode at 70 eV, scanning over a mass range of 50–550 m/z. The chemical constituents present in the extract were identified by comparing their mass spectral fragmentation patterns with reference spectra available in the National Institute of Standards and Technology (NIST) Mass Spectral Library, supported by standard spectral interpretation approaches (Silverstein *et al.*, 2005; Pavia *et al.*, 2001; Sasidharan *et al.*, 2011).

RESULT AND DISCUSSION

GC–MS Analysis of *R. montagnei*

GC–MS analysis of the methanolic extract of *R. montagnei* collected from Thengapattanam, Kanyakumari district, revealed the presence of six phytochemical constituents, identified based on their retention time (RT), molecular formula, molecular weight, and relative peak area percentage by comparison with the NIST Mass Spectral Library (Table 1 and Figure 4).

Among the identified compounds, Orcinol (RT 7.34 min, C₇H₈O₂) was found to be the predominant constituent, accounting for the highest relative abundance of 49.92%, followed by Methyl 2,6-dihydroxy-4-methylbenzoate (RT 11.22 min, C₉H₁₀O₄) with a peak area of 45.16%. Together, these two orcinol-derived phenolic compounds constituted approximately 95% of the total peak area, indicating that the extract is predominantly composed of orcinol-type secondary metabolites. The remaining constituents were detected in comparatively minor proportions, namely 1,1'-Biphenyl, 5-hydroxy-3,4'-dimethoxy- (RT 18.09 min, C₁₄H₁₄O₃, 1.69%), Norfenefrine (RT 14.25 min, C₈H₁₁NO₂, 1.35%), Erythritol (RT 6.37 min, C₄H₁₀O₄, 1.10%), and Palmitoleic acid (RT 16.69 min, C₁₆H₃₀O₂, 0.77%).

Table 1. GC–MS identified compounds

R.T	Compound Name	Molecular Weight	FORMULA	Peak Area %
6.37	Erythritol	122	C ₄ H ₁₀ O ₄	1.10
7.34	Orcinol	124	C ₇ H ₈ O ₂	49.92

11.22	Methyl 2,6-dihydroxy-4-methylbenzoate	182	C ₉ H ₁₀ O ₄	45.16
14.25	Norfenefrine	153	C ₈ H ₁₁ NO ₂	1.35
16.69	Palmitoleic acid	254	C ₁₆ H ₃₀ O ₂	0.77
18.09	1,1'-Biphenyl, 5-hydroxy-3,4'-dimethoxy-	230	C ₁₄ H ₁₄ O ₃	1.69

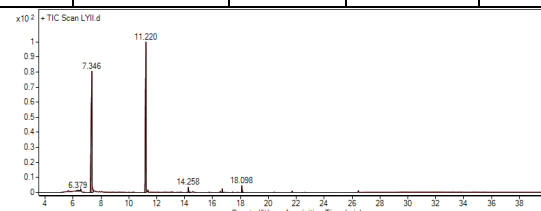


Figure 4. GC–MS chromatogram of *R. montagnei*.

Lichens are recognized as a rich source of biologically active natural products, producing more than 1000 specialized secondary metabolites, most of which are rarely found in other organisms. These compounds mainly arise from the acetate-malonate, shikimic acid, and mevalonic acid pathways (Newman *et al.*, 2020; Prashith *et al.*, 2019). Several lichen metabolites have shown antioxidant and antidiabetic potential, and many have been reported to inhibit enzymes linked to diabetes, obesity, and Alzheimer's disease, in addition to showing anticancer activity (Thadhan *et al.*, 2017; Prashith *et al.*, 2019).

Orcinol is a phenolic compound reported from various lichen species, and also from higher plants, fungi, and insects (Harborne *et al.*, 1969; Monde *et al.*, 1998; Iwata *et al.*, 2004; Jones *et al.*, 2004; Jorgensen *et al.*, 2014; Bunbamrung *et al.*, 2015; Hoenigsberger *et al.*, 2019). It has been reported to show antiproliferative activity against cancer cells, acetylcholinesterase inhibition, and antifungal activity against *Cladosporium* species (Oliveira *et al.*, 2009; Ivanova *et al.*, 2010). In the present study, GC–MS analysis of *R. montagnei* tentatively identified orcinol as the major constituent (49.92% of the total peak area), which is consistent with the phenolic-rich chemistry typical of lichens. Its occurrence in *R. montagnei* has been documented since the early chemical investigations of the species, with orcinol reported together with erythrin,

erythritol, lecanoric acid and roccellic acid (Rao and Seshadri, 1940).

However, Tatipamula *et al.*, (2019) isolated orcinol from the acetone extract of manglicolous *R. montagnei*, along with eight other known metabolites; notably, orcinol was the only metabolite among these nine that had been previously reported from the species. Within the genus *Roccella*, orcinol has also been isolated from *R. phycopsis*, together with methyl orsellinate, (+)-montagnetol and 4-chlororcinol (Zorrilla *et al.*, 2022). In *R. tinctoria*, orcinol was detected by SPME–GC–MS in an extract, further demonstrating its occurrence within the genus (Frezza *et al.*, 2024). Beyond *Roccella*, orcinol has been detected by GC–MS in the acetonitrile extract of *Parmotrema tinctorum* (Shiromi *et al.*, 2021), while a GC-based chemical profile of *Evernia prunastri* reported orcinol at approximately 25% (Stojanovic *et al.*, 2011). Collectively, these reports demonstrate the occurrence of orcinol across diverse lichen taxa. The relatively high proportion of orcinol *R. montagnei* in therefore represents a notable feature of the present chemical profile and is consistent with the established occurrence of orcinol among the secondary metabolites of *R. montagnei* and other *Roccella* species.

More recently, Roca *et al.*, (2026) investigated orchil dye synthesis using *R. fuciformis* and *R. phycopsis*, with experiments based on pure orcinol and subsequent characterization of the resulting orcein compounds using UV–Vis spectroscopy and HPLC–DAD–MS. Their findings further highlight the chemical relevance of orcinol as a precursor in *Roccella* derived pigment systems and demonstrate the influence of lichen species and synthesis conditions on the resulting molecular composition (Roca *et al.*, 2026).

Methyl 2,6-dihydroxy-4-methylbenzoate was detected as a second major constituent in the GC–MS profile of *R. montagnei* with peak area of 45.16%. Its detection of this compounds in *R. montagnei* is consistent with the isolation and spectroscopic characterization of methyl- γ -orsellinate ($C_9H_{10}O_4$) by Khader *et al.*, (2018). Related 2,6-dihydroxy-4-methylbenzoate chemistry has also been reported from *R. montagnei* through the isolation of Roccellatol, an erythrityl ester of 2,6-dihydroxy-4-methylbenzoate (Mallavadhani and Sudhakar, 2018). The same methyl ester was subsequently reported as a lichen metabolite in *Ramalina leiodea* (Tatipamula and Vedula, 2019). Tatipamula *et al.*, (2020) further isolated this compound from *R. leiodea* and reported DPPH, ABTS and superoxide radical-scavenging activities, with IC_{50} values of 40.0, 43.5 and 40.0 $\mu\text{g/mL}$, respectively. These reports provide biological relevance to the occurrence of this phenolic ester in lichens. The present detection at 45.16% represents relative GC–MS peak-area abundance and should

not be interpreted as an absolute concentration or as evidence that the compound alone is responsible for the biological activity of the extract.

Several minor constituents identified in the GC–MS profile of *R. montagnei* extract included erythritol, norfenefrine, palmitoleic acid and 1,1'-Biphenyl, 5-hydroxy-3,4'-dimethoxy-. Erythritol has previously been reported as a constituent of *R. montagnei* by Rao and Seshadri (1940), who identified it among the important chemical components of the species. Subsequent phytochemical investigations have further reported erythritol-derived metabolites from *R. montagnei*, indicating the occurrence of related erythritol-based chemistry in the species (Duong *et al.*, 2017). In the present analysis, erythritol was detected at a relative peak area of 1.10%, whereas norfenefrine and palmitoleic acid were detected at 1.35% and 0.77%, respectively. These compounds occurred at comparatively low relative abundances and therefore represented minor constituents of the analysed chemical profile.

Conclusion

GC–MS profiling of *R. montagnei*. revealed a phenolic-rich chemical profile, dominated by orcinol (49.92%) and methyl 2,6-dihydroxy-4-methylbenzoate (45.16%). The detection of these metabolites, together with minor constituents, provides further chemical evidence for the secondary-metabolite composition of *R. montagnei*. The findings establish a concise chemical profile of the species and provide a basis for subsequent targeted isolation and biological evaluation of its principal metabolites.

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