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ORIGINAL ARTICLE

Profiling secondary metabolites from lichen "*Parmotrema perlatum* (Huds.) M.Choisy" and antibacterial and antioxidant potentials

P. R. Dwarakanath[1] · K. Abinaya[1] · K. Nagasathya[1] · S. Meenakumari[1] · Subash C. B. Gopinath[2,3,4] · Pachaiappan Raman[1]

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Abstract
Parmotrema perlatum lichen is traditionally used as a spice in Indian households and also to treat diseases such as eczema, respiratory diseases, pulmonary diseases, and arthritis. This study emphasizes the extraction of secondary metabolites from foliose lichen *P. perlatum* with antioxidant and antibacterial activity. The secondary metabolites extracted from *P. perlatum* using hexane, chloroform, and methanol were analysed by gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC–MS/MS) shows that *P. perlatum* extracts possess 2,4-dihydroxy-3,6-dimethyl-methyl ester, 1,4-benzenediol, atraric acid, orcinol, benzoic acid, 2,5-dimethyl- resorcylic acid, and savinin, tetrahydroterotri-L- glutamate, primidolol, ustiloxin, mallotinic acid, and mecambrine. The efficacy of three solvent extracts of lichen against Gram-negative bacteria *Pseudomonas aeruginosa*, *Chromobacterium violaceum*, and Gram-positive *Lactobacillus plantarum* was tested. The methanol extract of *P. perlatum* has the highest antibacterial activity against the tested bacteria at a concentration of 10 µg/ml as compared with the other two solvent extracts. The antioxidant potential of *P. perlatum* was determined using ferric ion reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and nitric oxide scavenging activity assays, which exhibit that all extracts showed radical scavenging potential.

Keywords Kalpasi · Lichen · Antibacterial · Antioxidant · GC–MS · LC–MS · Secondary metabolites

1 Introduction

Lichens are a distinct group of algae and fungi that respond to environmental changes by producing biologically active compounds [1]. By most definitions, the organism reflects a symbiotic interaction between the mycobiont (fungi) and the photosynthetic partner as algae (photobiont), the lichen. The presence of photobionts alongside the mycobiont of lichens confers several advantages, none of which can be accomplished by either of the organisms alone [2]. The foliose lichens known as parameloid lichens are a complex and widespread genus of lichens. *Parmotrema* is a big genus that has been separated from *Parmelia* sp. lat. Based on morphological and chemical characteristics, other genera have been separated from this huge genus. This genus is a large lettuce-like leaf with broad lobes, a spongy apex, a multi-rooted margin on the underside, large thick-walled ellipsoid spores, accessory or filamentous spores with or without cilia margin, and large ellipsoid sporangia with thick-walled, accessory or filamentous spores and with or without cilia at the edges [3, 4].

Eight percent of the earth's land surface is covered with lichens, which is characteristic of symbiotic creatures found all over the globe. Even though they are among the slowest developing species, secondary metabolites provide them with a great deal of significance and interest. Chinese and Egyptian civilizations were among the first to describe their usage earlier. Lichens have been

✉ Pachaiappan Raman
pachaiar@srmist.edu.in; pachaitvm@gmail.com

1 Department of Biotechnology, School of Bioengineering, College of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur 603 203, Tamil Nadu, India

2 Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), 02600 Arau, Perlis, Malaysia

3 Institute of Nano Electronic Engineering, Universiti Malaysia Perlis (UniMAP), 01000 Kangar, Perlis, Malaysia

4 Micro System Technology, Centre of Excellence (CoE), Universiti Malaysia Perlis (UniMAP), Pauh Campus, 02600 Arau, Perlis, Malaysia

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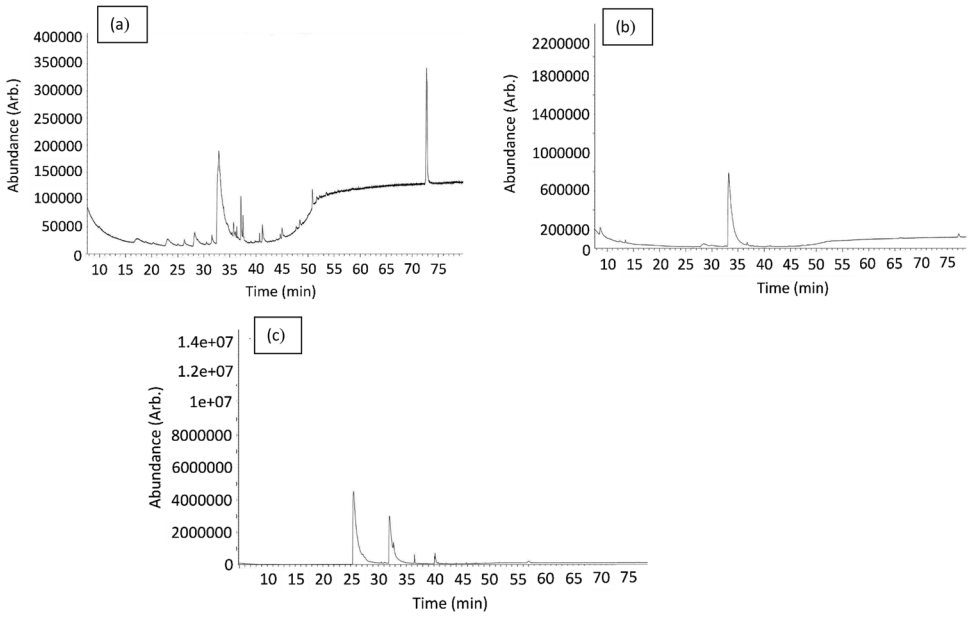


Fig. 1 GC–MS chromatogram of *P. perlatum* thallai secondary metabolites extracted with **a**) hexane, **b**) chloroform, and **c**) methanol

utilized in the perfume and cosmetic industries since the sixteenth century. Because of their distinctive colour, they are used as dyes for more than a century; lichen secondary metabolites have been studied for their pharmacological, biological, and environmental potential. There are several reports of small molecules for biological activities like antimicrobial [5] and analgesic [6, 7], anti-HIV, anti-oxidative, anti-proliferative, and cytotoxic actions [8–11]. The secondary metabolites derived from the genus *Parmotrema* including the *P. tinctorum* and *P. stuppeum* extracts were purified using column chromatography, and the compounds like orsellinic acid, atranorin, lecanoric acid, and, methyl orsellinate are identified first [12, 13].

The free radicals under the group of reactive oxygen species (ROS) like superoxide radicals, hydroxyl radicals, singlet oxygen, and H_2O_2 can trigger the development of cancer, inflammatory disorders, as well as cardiovascular diseases [14]. Unstable free radicals get stabilized by pairing with electrons of macromolecules like DNA, lipids, and, protein causing them damage. Antioxidant compounds can oxidize the free radicals and make them stable [15]. There are two ways for antioxidant activity to occur: direct radical scavenging and increasing the antioxidant enzyme with antioxidant compounds. Based on this concept, there is a surge of interest in the extraction of natural antioxidant molecules with low cytotoxicity to help the body reduce ROS-induced molecules and cell damage.

Metabolomics has become an important tool in contemporary life sciences over the past few years. Mass spectrometry-based metabolomics, both gas and liquid chromatography techniques are widely used. Natural products mainly secondary metabolites are used in nutrition and medicine as they function as important units in dealing with stress. The metabolic response of the system to a particular disturbance in addition to obvious links between primary and secondary metabolites is studied by metabolomics [16]. Recently, in our lab, an extensive study has been done in analysing the nutritional values of the oils by using these analytical techniques. Adulteration of price-based oils is a major concern as that can harm consumers' health [17]. Siddha's systematic studies of drugs in herbal formulations are being developed for the treatment of a wide variety of diseases. The chemical composition of black cumin (*Nigella sativa* L.), has been analysed [18]. Apart from the secondary metabolites, the medicinal value of the biologically active plant peptides has been extensively discussed in order to develop a drug against several human diseases [19]. Its potential antimicrobial activities promise to have anti-quorum sensing activity, and peptide research can be used to develop this *P. perlatum* study in the future [20, 21]. This study provides an insight into the chemical composition of *P. perlatum* using GC–MS and LC–MS/MS analysis, as well as its antibacterial activity against the Gram-negative bacteria *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, and Gram-positive bacteria *Lactobacillus plantarum*. The antioxidant activity of three *P. perlatum* extracts were tested *in vitro*.

2 Methodology

2.1 Sample collection

The *P. perlatum* was obtained from an herbal shop in Chennai, Tamil Nadu, India, and the voucher specimen was deposited in the SRM IST herbarium collection after

Table 1 List of *P. perlatum* compounds identified by GC–MS

S. No.	Retention time (min.)	Molecular weight	Compound name	Structure	Metabolites extracted with		
					Hexane	Chloroform	Methanol
1.	24.158	138	3-Methoxy-5-methylphenol		-	-	+
2.	25.517	124	Orcinol		-	-	+
3.	26.992	460	Succinic acid		+	-	-
4.	28.499	138	1,4-Benzenediol, 2,5-dimethyl		-	+	-
5.	31.081	210	Methyl haematommate		-	-	+
6.	31.564	324	Heptadecane, 9-hexyl-		+	-	-
7.	31.980	182	Atraric acid		-	-	+
8.	32.649	226	Dodecane, 5,8-diethyl-		-	+	-
9.	33.191	196	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester		+	+	-
10.	36.073	292	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester		+	-	-
11.	36.482	270	Palmitic acid, methyl ester		-	-	+
12.	36.779	276	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione		+	+	-
13.	37.128	282	Eicosane		+	-	+
14.	37.381	324	4-Methyldocosane		-	+	-
15.	37.537	272	Hibaene		+	-	-
16.	38.647	374	2-Methylcortisone		-	+	-
17.	40.092	294	Linoleic acid, methyl ester		-	-	+
18.	40.196	296	trans-13-Octadecenoic acid, methyl ester		-	-	+
19.	40.612	592	Oleic acid		-	+	-

Table 1 (continued)

20.	40.731	366	Heptadecanoic acid, 16-methyl-, methyl ester		-	-	+
21.	41.073	298	Methyl stearate		-	+	-
22.	42.061	324	Heptadecane, 9-hexyl		-	-	+
23.	44.683	366	Octadecane, 3-ethyl-5-(2-ethylbutyl)-		+	-	-
24.	45.790	338	Tetracosane		-	-	+
25.	47.759	487	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone		+	-	-
26.	50.849	344	Phosphinous chloride, bis[5-methyl-2-(1-methylethyl)cyclohexyl]		+	-	-
27.	51.726	412	Tetracosapentaene, 2,6,10,15,19,23-hexamethyl		-	-	+
28.	57.007	374	p-Xylenolphthalein		-	-	+

(+) indicates presence, (−) indicates the absence

identification by Dr. D. Narishimhan, Retired Professor, Madras Christian College, Chennai, Tamil Nadu, India.

2.2 Successive extraction

The lichens were cleaned and separated from the other contaminants. It was crushed into a coarse powder after being cut into small pieces, and secondary metabolites were extracted by sequential extraction method with selected solvents based on the polarity. Initially, 5 g of sample was mixed with 25 ml of hexane and left undisturbed at 20 ℃ for 24 h and collected the extract after filtration. This sample was mixed with 25 ml of chloroform and 25 ml of methanol as followed in hexane extract. The extracts were filtered using Whatman No.1 filter paper before changing each solvent. The filtrate was then concentrated using a rotary evaporator [22].

2.3 Gas chromatography-mass spectrometry (GC–MS) analysis

The conditions for GC–MS analysis on an Agilent, USA (Model 7890B- GC and 5977A MSD) are as follows: HP5 MS column 5% phenyl methyl siloxane (30 m × 0.25 µm × 250 µm) at 70 eV as electron impact mode. The constant flow of carrier gas of helium at a 1 ml/min flow rate was used. The 1 µl of lichen extracts was injected at a split ratio of 100:1, ion-source temperature 280 °C, and injector temperature 250 °C. The programmed oven temperature includes the rise from 110 °C (isothermal for 2 min) to 200 °C (10 °C/min), then to 280 °C (5 °C/min), finishing with a 9-min isothermal at 280 °C. The peaks determine the presence of compounds and were identified by the National Institute of Standard and Technology library (NIST) and quantification by using electron impact ionization at 70 eV, and data were analysed using total ion count (TIC) [23].

2.4 Liquid chromatography-mass spectrometry (LC–MS/MS) analysis

The study was carried out using an Impact HD MS/MS (Bruker, Germany) coupled to an LC system (Shimadzu, Japan). With an electrospray ionization (ESI) probe operating in active mode, detection is carried out in direct spray mode. The separation of lichen extract was carried out on the Shiseido C_{18} column (1.8 µm, 2.1 × 150 mm, Shimadzu, Japan). The 5 µl of injection volume was used with the flow rate of 0.3 ml/min for analysis. The auxiliary gas flow rate was set to 8 l/min, the spray voltage of 4.5 kV, and the capillary temperature was maintained at 280 °C with the MS acquisition range of 50–2200 m/z.

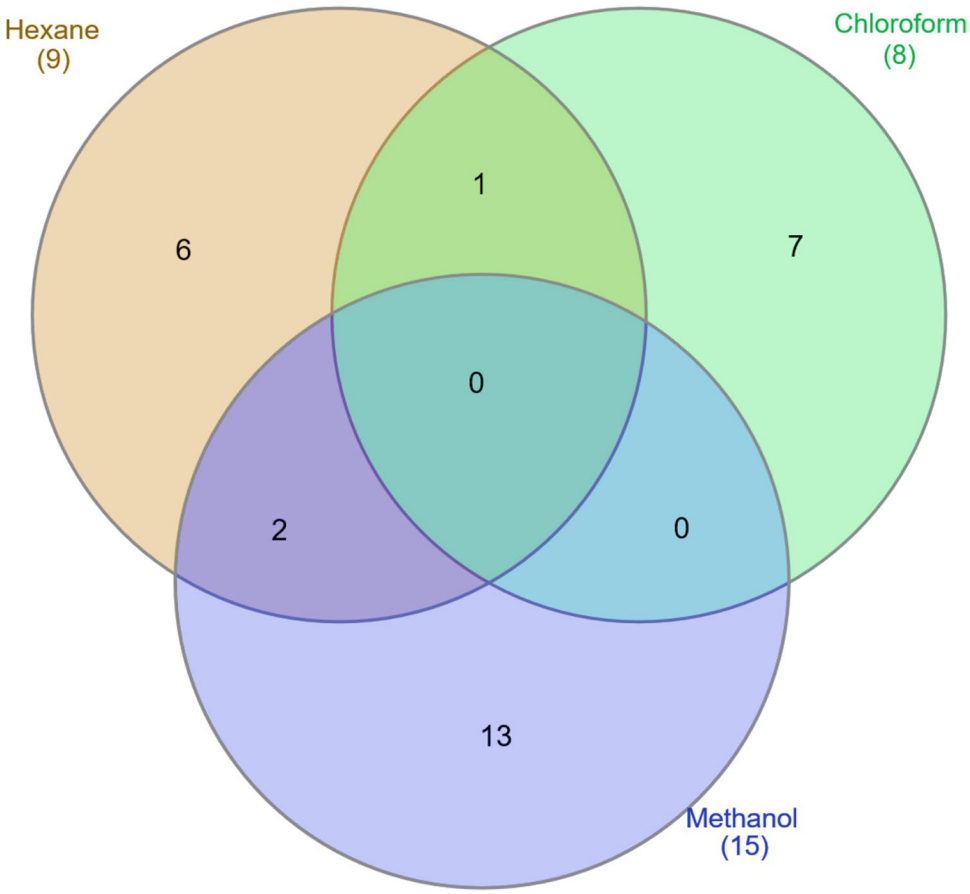


Fig. 2 Number of metabolites identified from different solvent extracts of *P. perlatum* analysed by GC–MS

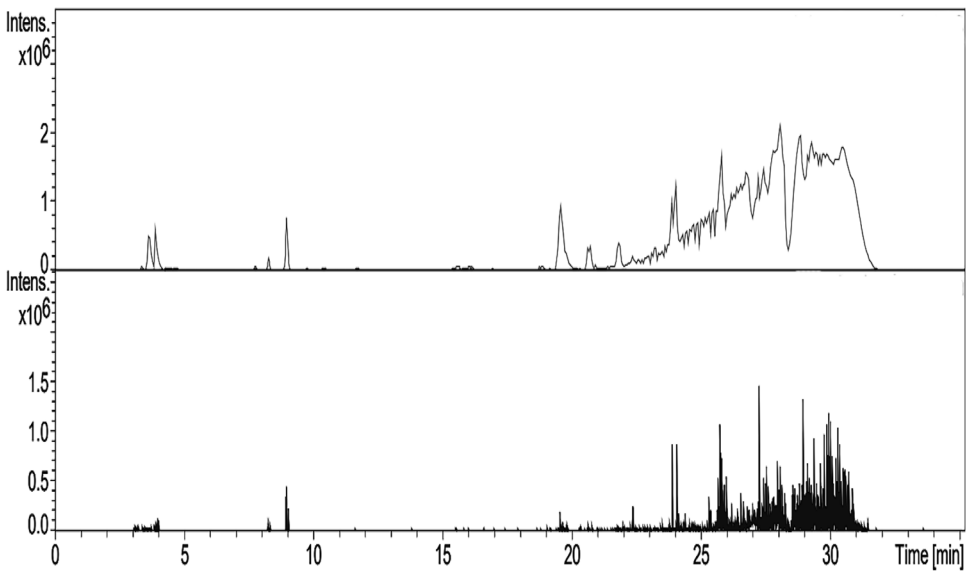
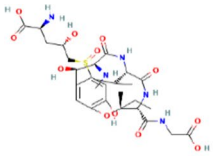
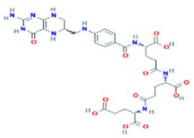
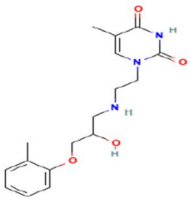
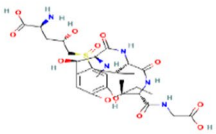
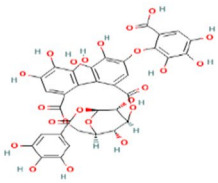
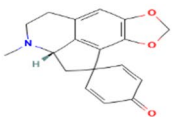


Fig. 3 LC–MS chromatogram of *P. perlatum* extracted with methanol

The collision dissociation energy (CID) in MS/MS is kept within a range of 10 and 45. As a mobile phase, acetonitrile and 0.1% formic acid were utilized. Initially, 5% acetonitrile was used, and it gradually reached 80% at 30 min. Then, acetonitrile was brought back to its initial condition. By manually transferring the analyte and

Table 2 List of compounds identified by LC–MS/MS from *P. perlatum* thallai extracted with methanol

S. No.	Retention time (min.)	Name of the Compound	Molecular weight	Structure	Biological function
1.	3	Savinin	352.09		Anticancer activity [34]
2.	4	Tetrahydroterotri - L- glutamate	703.25		Methionine Pathway [35]
3.	7.7	Primidolol	333.16		Beta adrenergic receptor antagonist [36]
4.	8.8	Ustiloxin	673.26		Fungal cyclic peptide [37]
5.	19.4	Mallotinic acid	802.08		Antioxidant activity [38]
6.	21.7	Mecambrine	295.12		Antimicrobial activity [39]

switching settings, the MS parameters of each compound have been optimized to ensure the best ionization conditions, optimal signal, and ion transfer from precursor ions and segments. All analytes have the same source parameters. [24].

2.5 Antibacterial activity

The well diffusion method on Mueller Hinton agar was used to determine the antibacterial activity of *P. perlatum* extracts as described by [25, 26]. The concentrated

Fig. 4 Antibacterial activity of *P. perlatum* extracts against **a**) *Pseudomonas aeruginosa*, **b**) *Chromobacterium violaceum*, **c**) *Lactobacillus plantarum* (The metabolites extracted with: H—hexane, M—methanol, Ch—Chloroform, and C—control)

Table 3 Determination of minimum inhibitory concentration (MIC) of *P. perlatum* extracts

P. perlatum metabolites extracted with	Concentration of *P. perlatum* extracts (μg/ml^{-1})	Diameter of zone of inhibition (mm)		
		L. plantarum	*C. violaceum*	*P. aeruginosa*
Methanol	50	19 ± 0.1	16 ± 0.1	04 ± 0.06
Chloroform	50	15 ± 0.05	11 ± 0.05	01 ± 0.05
Hexane	50	17 ± 0.05	13 ± 0.05	02 ± 0.1

*Each value represents mean ± standard error of three triplicates

1 mg/ml of dry methanol, chloroform, and hexane extracts was dissolved in the 5% dimethyl sulfoxide (DMSO). The extracts were tested for their antibacterial activity against Gram-negative *P. aeruginosa*, *C. violaceum*, and Gram-positive *L. plantarum*. The culture was overlaid on the Mueller Hinton agar, and the different extracts were loaded into the punctured wells with control in which the extracts were dissolved and incubated overnight at 37 °C.

2.6 Antioxidant activity

2.6.1 Ferric ion reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by mixing the 300 mM sodium acetate buffer pH 3.6, 10 mM TPTZ dissolved in 40 mM hydrochloric acid, and 20 mM $FeCl_3$ in a ratio of 10:1:1. The assay was performed in the 96-well plates at

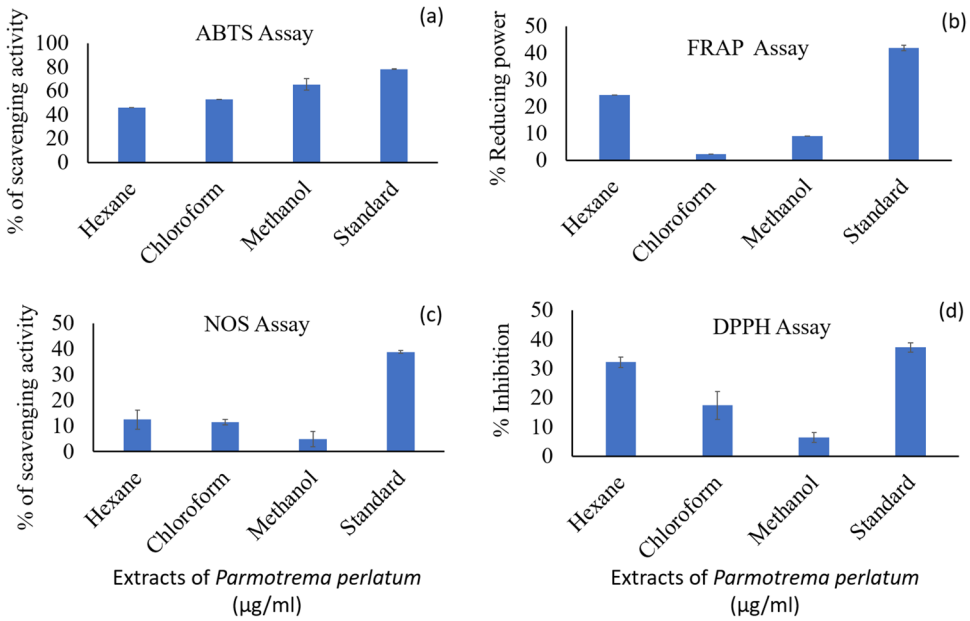


Fig. 5 Radical scavenging activity of *P. perlatum* thallai extracted with different solvents and quercetin served as a standard. **a**) 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), **b**) ferric reducing antioxidant power assay (FRAP), **c**) nitric oxide scavenging assay (NOS), **d**) 2,2-diphenyl-1-picrylhydrazyl (DPPH). (All experiments were performed in triplicate)

different concentrations of 10 to 50 µg/ml with quercetin as standard. The plates were protected away from the light for 30 min, and the absorbance was measured at 596 nm in the Multiskan GO (Thermo Fisher Scientific Corporation, Finland). The samples and standard quercetin absorbance were compared [27].

ABTS radicals cavenging (%) =[(Absorbance of control − Absorbance of sample) /Absorbance of control] × 100

Ferric reducing power = [(max . Abs. of standard − Abs. of test sample)/ max . Abs. of standard × 100]

2.6.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The assay was performed in a 96-well microplate method with minor modifications [28]. The 0.1 mM DPPH solution was made with HPLC-grade methanol and stored at − 20 °C for further use. A 20 µl sample solution and DPPH solution of 180 µl were mixed and stored in dark at room temperature for 30 min. A multimode microplate reader was then used to immediately read the optical density at 517 nm, and the percentage was calculated using absorbance value.

Inhibition (%) = [Control − Sample)/Control] × 100

2.6.3 Nitric oxide scavenging activity (NOS) assay

Different concentrations of the three extracts were mixed with 3 ml of sodium nitroprusside in phosphate-buffered saline (pH 7.4) incubated for 150 min. From this, 1.0 ml was taken, and freshly prepared Griess reagent (0.1% (w/v) naphthyl ethylenediamine dihydrochloride, 1% (w/v) sulphanilamide, and 2.0% (v/v) phosphoric acid) of 1.0 ml was added, and the OD value was observed at 546 nm [29]. The NO scavenging capacity was determined by comparing control and sample absorbance values. The % of NO scavenging capacity of extracts and ascorbic acid as standard was calculated by using the following formula:

Scavenged nitrite oxide % = $[(Abs._{control} - Abs._{sample})/Abs._{control}] \times 100$

2.6.4 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS stock was prepared by combining 7 mM of ABTS with 2.4 mM of potassium persulfate solution in equal volumes and incubated overnight at 4 °C in the dark. The dark green solution was then diluted with an ethanol absorbance value of 0.74 ± 0.02 at 734 nm. The dilution was carried out in the 96-well plates with ascorbic acid utilized as standard, and the diluted ABTS solution was added to the final volume of 200 µl. The scavenging activity was measured using a multimode reader at 734 nm after mixing well [30].

3 Results and discussion

3.1 GC–MS analysis

The three different solvents extracts of *P. perlatum* were analysed for secondary metabolites using GC–MS, and the chromatograms were represented in Fig. 1. The hexane extract of *P. perlatum* revealed the higher composition of benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester (62.2%) [31], and phosphinous chloride (1.737%). The chloroform extract of *P. perlatum* was composed of benzoic acid (92.47%) and 1,4-benzenediol, 2,5-dimethyl (4.518%) [32]. The methanolic extract of *P. perlatum* had orcinol (63.26%), and atraric acid (21.38%) was derived from a compound called atranorin [33].

The two pigments such as phosphinous chloride and p-xylenolphthalein were recorded in the extracts of *P. perlatum*. Some compounds with less composition such as succinic acid, oleic acid, methyl stearate, tetracosane were identified. Some other compounds had also been identified from the same extracts such as octadecane, 3-ethyl-5-(2-ethylbutyl)-. Eicosane and 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione were found in both hexane and methanolic extracts. Table 1 shows the list of compounds identified in hexane, chloroform, and methanol extracts of *P. perlatum*. The highest peak area percentage of compounds' names and their percentages are as follows: benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester (69.3%) in hexane, benzoic acid (92.5%) in chloroform, orcinol (63.3%), and atraric acid (21.4%) in methanol. Figure 2 depicts the number of compounds present in three extracts of *P. perlatum*. Thus, GC–MS analysis is used to identify new drug molecules with various properties such as antimicrobial agents and antioxidant drug candidates.

3.2 LC–MS/MS analysis

The methanol extract of *P. perlatum* was subjected to LC–MS/MS analysis, and the LC–MS chromatogram was shown in Fig. 3. The LC–MS analysis has identified 6 compounds such as savinin, tetrahydroterotri-L-glutamate, primidolol, ustiloxin, mallotinic acid, and mecambrine presented in Table 2. The identification of

these compounds is based on the *m/z* of molecular ion $[M+H]^+$ and interpretation of the MS data using Met Frag with Kegg Database. The presence of butyrolactone as a pharmacophore component has been suggested to possess an anti-inflammatory effect and also antioxidative [40]. The modified amino acid of glutamate is the neurotransmitter upon excitatory effects on nerve cells in the brain [41]. The malonic acid is the derivative of a unique class of antioxidant compounds that has the capability of eliminating the superoxide anion and hydrogen peroxide [42]. Mecambrine indicates that it contributes to the antibacterial effect which has been validated [43]. Among the two partners of lichens such as the autotrophic partner of algae and the saprophytic partner of fungi, metabolites were recorded based on the GC–MS and LC–MS data. Totally 6 compounds were detected in LC–MS/MS, 5 of them were from fungi. The mecambrine was recorded as the highest peak area percentage (36.5%) followed by ustiloxin (21.3%).

3.3 Antibacterial activity

The hexane, chloroform, and methanol extracts of *P. perlatum* were checked for its antibacterial activity against the *P. aeruginosa, C. violaceum*, and *L. plantarum*, represented in Fig. 4. It has been found that all extracts exhibited antibacterial activities against all the tested microbes. Methanol, hexane, and chloroform extracts of *P. perlatum* inhibited Gram-negative bacteria *P. aeruginosa* significantly at 50 µg/ml. Our results in consonance with [44] of hexane extract have a strong antibacterial activity. However, when compared to the hexane and chloroform extracts, the methanol extract demonstrated a higher zone of inhibition for all three tested microbes. Aside from antibacterial activity, the tested bacterial growth was used to calculate the minimum inhibitory concentration. The mean diameters of the zones of inhibition of *P. perlatum* were measured and presented in Table 3.

3.4 Antioxidant activity

Among the three extracts of *P. perlatum* analysed for antioxidant activity, each extract was found to have potential activity in scavenging the respective radicals in the antioxidant assay. The chloroform extract showed higher activity in the nitric oxide scavenging assay. Nitric oxide (NO) plays an important role in the central nervous system in synaptic plasticity, neurotransmitter release, neurodevelopment, neurotransmitter re-uptake, and regulation of gene expression in neurodegenerative diseases. However, excessive NO production can result in neurotoxicity [45] through conversion of peroxynitrite ($ONOO^-$). Thus, the scavenging of nitric oxide can be effective to reduce oxidative stress. Among all the assays, DPPH has limitations and low sensitivity. The methanolic extract was effective in ABTS radical-scavenging activity when compared with standard ascorbic acid, which shows significant antioxidant activity [46]. Ability to reduce the complex $[Fe^{3+}(TPTZ)_2]^{3+}$ in an acidic medium to ferrous complex $[Fe^{2+}(TPTZ)_2]^{2+}$ provides blue-coloured product at 593 nm determined in FRAP assay. The very low reducing abilities were absorbed in the methanol extract of *P. perlatum*. This *in vitro* analysis of antioxidants helps the researchers to go for further for their mode of action(s) *in vivo* and to find out the antioxidant compounds (Fig. 5).

4 Conclusion

This study aimed for extracting the secondary metabolites from *P. perlatum* using three different solvents based on polarity, ranging from hexane to chloroform to methanol, and its metabolites were analysed by GC–MS and LC–MS/MS. The bioactive compounds were profiled from *P. perlatum* and have various biological activity. The antibacterial activity of three different solvent extracts were tested against the test pathogens which includes one Gram positive and two Gram negative bacteria, which reveals that methanol extract has shown better antibacterial activity. The antioxidant activities of *P. perlatum* also evaluated for four different radical generating assays. As a result of the scavenging effects of *P. perlatum* lichens confirm that they have better radical scavenging potential and also comparable to that of ascorbic acid/quercetin used as a standard. Thus, this finding suggests the possibility of using them in the pharmaceutical industry.

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Author contribution DPR: investigation, conceptualization, writing original draft preparation, writing—reviewing and editing. AK: analysis, validation, writing original draft preparation. NK: validation, resources, writing—reviewing and editing. MS: validation, writing—reviewing and editing. SCBG: resources, writing—reviewing and editing. PR: conceptualization, validation, resources, supervision, funding, writing—reviewing and editing.

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Data availability All data and materials are available upon request.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

References

1. Huneck S (1999) The significance of lichens and their metabolites. Die Naturwiss Enschaften 86(12):559–570
2. Lucking R, Leavitt SD, Hawksworth DL (2021) Species in lichen-forming fungi: balancing between conceptual and practical considerations, and between phenotype and phylogenomics. Fungal Divers 109(1):99–154
3. Louwhoff SHJ, Crisp MD (2000) Phylogenetic analysis of *Parmotrema* (Parmeliaceae: lichenized Ascomycotina). Bryologist 103(3):541–554
4. Hale ME (1961) The typification of Parmelia perlata (Huds). Ach Brittonia 13(4):361–367
5. Malhotra S, Subban RAVI, Singh A (2008) Lichens—role in traditional medicine and drug discovery. Int J Alternat Med 5(2):1–5
6. Yousuf S, Choudhary MI (2014) Lichens: chemistry and biological activities. Stud Nat Prod Chem 43:223–259
7. Hussain M, Raza SM, Farooq U, Bakhsh H, Majeed A, Aziz A (2014) *In vitro* antimicrobial potential of lichen (*Parmelia perlata*) against different pathogenic microbes. Int J Pharmaceut Sci 4:666–670
8. Boustie J, Grube M (2005) Lichens—a promising source of bioactive secondary metabolites. Plant Genet Resour 3(2):273–287
9. Muller K (2001) Pharmaceutically relevant metabolites from lichens. Appl Microbiol Biotechnol 56(1):9–16
10. Ramya K, Thirunalasundari T (2017) Lichens: a myriad hue of bioresources with medicinal properties. Int J Life Sci 5(3):387–393
11. Dandapat M, Paul S (2019) Secondary metabolites from lichen *Usnea longissima* and its pharmacological relevance. Pharmacogn Res 11(2):103–109
12. Jayaprakasha GK, Jaganmohan Rao L, Singh RP, Sakariah KK (1998) Improved chromatographic method for the purification of phenolic constituents of the lichen *parmotrema tinctorum* (Nyl.) Hale. J Chromatogr Sci 36(1):8–10
13. Jayaprakasha GK, Rao LJ (2000) Phenolic constituents from the lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity. Zeitschrift für Naturforschung C 55(11–12):1018–1022
14. Sreeramulu D, Reddy CVK, Chauhan A, Balakrishna N, Raghunath M (2013) Natural antioxidant activity of commonly consumed plant foods in India: effect of domestic processing. Oxidat Med Cell Longev 2013:Article ID 369479
15. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J (2012) The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J 12(1):5
16. Doerfler H, Lyon D, Nägele T, Sun X, Fragner L, Hadacek F … Weckwerth W (2013) Granger causality in integrated GC–MS and LC–MS metabolomics data reveals the interface of primary and secondary metabolism. Metabolomics 9(3):564–574
17. Parthasarathy S, Soundararajan P, Krishnan N, Karuppiah KM, Devadasan V, Prabhu D… Raman P (2022) Detection of adulterants from common edible oils by GC–MS. Biomass Convers Biorefin 1–21. https://doi.org/10.1007/s13399-022-02913-3
18. Pachaiappan R, Nagasathiya K, Singh PK, Gopalakrishnan AV, Velusamy P, Ramasamy K, Gopinath SC (2022) Phytochemical profile of black cumin (*Nigella sativa* L.) seed oil: identification of bioactive anti-pathogenic compounds for traditional Siddha formulation. Biomass Convers Biorefin 1–13. https://doi.org/10.1007/s13399-022-02951-x
19. Saiprahalad M, Bhatt SB, …. & Pachaiappan, R. (2022) The updated review on plant peptides and their applications in human health. Int J Pept Res Ther 28:135. https://doi.org/10.1007/s10989-022-10437-7
20. Pachaiappan R, Rajamuthu TP, Sarkar A, Natrajan P, Krishnan N, Sakthivelu M… Gopinath SC (2022) N-acyl-homoserine lactone mediated virulence factor (s) of *Pseudomonas aeruginosa* inhibited by flavonoids and isoflavonoids. Process Biochem 116:84–93
21. Champalal L, Kumar US, Krishnan N, Vaseeharan B, Mariappanadar V, Raman P (2018) Modulation of quorum sensing-controlled virulence factors in *Chromobacterium violaceum* by selective amino acids. FEMS Microbiol Lett 365(23):fny252
22. Calla-Quispe E, Robles J, Areche C, Sepulveda B (2020) Are ionic liquids better extracting agents than toxic volatile organic solvents? A combination of ionic liquids, microwave and LC/MS/MS, applied to the lichen *Stereocaulon glareosum*. Front Chem 8:450
23. Pratibha P, Mahesh CS (2016) GC-MS analysis and biological activities of medicinally important lichen: *Parmelia perlata*. Int J Pharmacogn Phytochem Res 8(12):1975–1985
24. Parrot D, Peresse T, Hitti E, Carrie D, Grube M, Tomasi S (2015) Qualitative and spatial metabolite profiling of lichens by a LC–MS approach combined with optimised extraction. Phytochem Anal 26(1):23–33
25. Chauhan R, Abraham J (2013) In vitro antimicrobial potential of the lichen *Parmotrema sp*. extracts against various pathogens. Iran J Basic Med Sci 16(7):882
26. Mogana R, Adhikari A, Tzar MN, Ramliza R, Wiart C (2020) Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. BMC Complement Med Ther 20(1):1–11
27. Benzie IF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol (Academic press) 299:15–27
28. Reis FS, Martins A, Barros L, Ferreira IC (2012) Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between *in vivo* and *in vitro* samples. Food Chem Toxicol 50(5):1201–1207
29. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L (1994) The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem Biophys Res Commun 201(2):748–755
30. Hoon LY, Choo C, Watawana MI, Jayawardena N, Waisundara VY (2015) Evaluation of the total antioxidant capacity and antioxidant compounds of different solvent extracts of Chilgoza pine nuts (*Pinus gerardiana*). J Funct Foods 18:1014–1021
31. Mallavadhani UV, Tirupathamma RS, Sagarika G, Ramakrishna S (2019) Isolation, chemical modification, and anticancer activity of major metabolites of the lichen *Parmotrema mesotropum*. Chem Nat Compd 55(5):825–831
32. Yu XL, Yang XY, Gao XL, Bai RF, Yin X, Su GZ, Qu CH, Chai XY, Tu PF (2016) Zhongguo Zhong yao za zhi, Zhongguo zhongyao zazhi. China J Chin Mater Med 41(10):1864–1869
33. Bourgeois G, Suire C, Vivas N, Vitry C (1999) Atraric acid, a marker for epiphytic lichens in the wood used in cooperage: identification and quantification by GC/MS/(MS). Analusis 27(3):281–283
34. Cho JY, Park J, Kim PS, Yoo ES, Baik KU, Park MH (2001) Savinin, a lignan from *Pterocarpus santalinus* inhibits tumor necrosis factor-alpha production and T cell proliferation. Biol Pharm Bull 24(2):167–171
35. Radparvar S, Houghton PJ, Houghton JA (1989) Effect of polyglutamylation of 5,10-methylenetetrahydrofolate on the binding of 5-fluoro-2′-deoxyuridylate to thymidylate synthase purified from

a human colon adenocarcinoma xenograft. Biochem Pharmacol 38(2):335–342
36. Augstein J, Cox DA, Ham AL, Leeming PR, Snarey M (1973) Beta-adrenoceptor blocking agents. 1. Cardioselective 1-aryloxy-3- aryloxyalkylamino] propan-2-ols. J Med Chem 16(11):1245–1251
37. Tsukui T, Nagano N, Umemura M, Kumagai T, Terai G, Machida M, Asai K (2015) Ustiloxins, fungal cyclic peptides, are ribosomally synthesized in *Ustilaginoidea virens*. Bioinformatics 31(7):981–985
38. Tabata H, Katsube T, Tsuma T, Ohta Y, Imawaka N, Utsumi T (2008) Isolation and evaluation of the radical-scavenging activity of the antioxidants in the leaves of an edible plant, *Mallotus japonicus*. Food Chem 109(1):64–71
39. Manyelo TG, Sebola NA, Hassan ZM, Mabelebele M (2020) Characterization of the phenolic compounds in different plant parts of *Amaranthus cruentus* grown under cultivated conditions. Molecules 25(18):4273
40. Salat K, Moniczewski A, Librowski T (2013) Nitrogen, oxygen or sulfur containing heterocyclic compounds as analgesic drugs used as modulators of the nitroxidative stress. Mini Rev Med Chem 13(3):335–352
41. Zhou Y, Danbolt NC (2014) Glutamate as a neurotransmitter in the healthy brain. J Neural Transm (Vienna, Austria: 1996) 121(8):799–817
42. Dugan LL, Lovett EG, Quick KL, Lotharius J, Lin TT, O'malley, K. L. (2001) Fullerene-based antioxidants and neurodegenerative disorders. Parkinsonism Relat Disord 7(3):243–246
43. Jafaar HJ, Isbilen O, Volkan E, Sariyar G (2021) Alkaloid profiling and antimicrobial activities of *Papaver glaucum* and *P. decaisnei*. BMC Res Notes 14(1):1–7
44. Hoda S, Vijayaraghavan P (2015) Evaluation of antimicrobial prospective of *Parmotrema perlatum* hexane extract. Int J Pharm Res Allied Sci 4(2):47–53
45. Etim OE, Ekanem SE, Sam SM (2013) *In vitro* antioxidant activity and nitric oxide scavenging activity of *Citrullus Lanatus* Seeds. In Vitro 3(12):126–132
46. Solarova Z, Liskova A, Samec M, Kubatka P, Busselberg D, Solar P (2020) Anticancer potential of lichens' secondary metabolites. Biomolecules 10(1):87