



RESEARCH ARTICLE

Gas chromatography-mass spectrometry profiling and antioxidant evaluation of the wood rotting fungus *Ganoderma multipileum* Ding Hou collected from Mizoram, Northeast India

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Abstract

Macrofungi, particularly wood-rotting fungi, are increasingly recognised as valuable sources of bioactive compounds with potential therapeutic applications. The present study evaluated the antioxidant activity and volatile metabolite composition of *Ganoderma multipileum* collected from Mizoram University Campus, Aizawl, Mizoram, India. The antioxidant activity of the methanolic extracts of *G. multipileum* was evaluated using DPPH and ABTS radical scavenging assays, revealing IC₅₀ values of 564.7 µg/mL and 647.7 µg/mL respectively, indicating moderate radical scavenging activity. The gas chromatography-mass spectrometry (GC-MS) analysis revealed a broad range of chemical constituents, highlighting the chemical richness of the extract. The identified compounds include 3-ethoxy-6-(1-tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane, fluvalinate, methyl p-coumarate, nonanoic acid and 2-methyl heptanoic acid. These compounds belong to various structural group, including fatty acids, esters, phenolic derivatives and heterocyclic compounds. The presence of these chemically diverse metabolites indicates that the extract contains multiple bioactive constituents with a broad range of biological activities. Many of these chemical groups are well-known for their pharmacological properties, particularly their antioxidant, antimicrobial and anti-inflammatory activities. The combined antioxidant and chemical profiling reveal the pharmacological potential of *G. multipileum* as a source of natural bioactive metabolites. These findings provide a foundation for future studies aiming to isolate specific compounds and explore their therapeutic applications in nutraceutical, pharmaceutical and industrial contexts.

Keywords: antioxidant activity; gas chromatography-mass spectrometry; health; macrofungi; medicinal mushrooms; volatile compound; white rot fungi

Introduction

Increasing attention has been directed toward wild mushrooms, particularly wood rotting fungi, for their ability to produce novel bioactive compounds with potential applications in modern drug discovery and therapeutic development (1–3). These fungi are now recognised as prolific sources of structurally diverse metabolites exhibiting a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory and anticancer effects (4–7). The genus *Ganoderma* has been extensively studied, with over 20 species investigated, yielding more than 600 bioactive compounds, including triterpenes, meroterpenoids, steroids, alkaloids, nucleosides, nucleobases and polysaccharides, among which triterpenes constitute the predominant class and *Ganoderma lucidum* has emerged as the most prominent species (8–12).

Species of *Ganoderma* have been widely used for centuries in traditional Asian medicine because of their diverse therapeutic and medicinal properties (13). These mushrooms have long been valued in various traditional healing systems for the prevention and treatment of numerous health disorders, including microbial infections, chronic inflammation, liver related ailments, hypertension, diabetes and immune-associated diseases. Their extensive use in traditional medicine highlights their historical importance as natural remedies and has attracted increasing scientific attention in recent decades for their pharmacological and biomedical potential (14–15). Species of *Ganoderma* play a crucial ecological role as white-rot fungi involved in the degradation of lignocellulosic materials in forest ecosystems. They contribute significantly to nutrient recycling, carbon turnover, humus formation and maintenance of soil fertility through the decomposition of dead woody substrates. In addition, *Ganoderma* species support forest

biodiversity by creating microhabitats for various organisms and influencing natural ecosystem dynamics (16). *Ganoderma multipileum* is a medicinal mushroom rich in bioactive secondary metabolites, including triterpenoids and polyphenols (17). Studies have shown that caffeine, a cytochrome P450 inducer, can significantly enhance triterpenoid production by stimulating triterpenoid synthase activity, while limonene provides additional metabolic precursors to further boost metabolite levels (18). Beyond its pharmaceutical potential, purified laccase from *G. multipileum* has been optimised for Cr(VI) bioremediation, achieving over 94 % reduction to Cr(III) under environmentally friendly conditions (19). Additionally, this species has been successfully employed for the green synthesis of zinc oxide (ZnO) nanoparticles, demonstrating notable biological activity and versatility for applications in various biotechnological and environmental fields (20).

In Mizoram, more than 264 species of macrofungi have been documented, including around ten species of the genus *Ganoderma* (21). *Ganoderma* species have long been recognised for their remarkable medicinal value and have been widely utilised in traditional herbal medicine across Asia for thousands of years. However, in Mizoram, this particular species of *Ganoderma* has no known traditional use and is generally considered insignificant. Therefore, investigating its properties is valuable to determine whether it also possesses potential bioactive compounds. Past studies in Mizoram have investigated the taxonomy, phylogeny, nutritional composition, antioxidant properties, heavy metal content and associated health risk assessments of certain macrofungal taxa, studies specifically addressing the antioxidant activity and comprehensive volatile metabolite profiling of macrofungi, particularly wood rotting fungi, remain limited and insufficiently explored. Although *Ganoderma* species are globally recognised for their therapeutic and pharmacological significance, investigations addressing their bioactive properties within the regional context of Mizoram are scarce. Therefore, the present study aims to evaluate the antioxidant activity and characterize the volatile compounds of *G. multipileum*, to better understand its chemical composition and bioactive potential.

Materials and Methods

Collection and identification of specimens

The specimen was collected from Mizoram University Campus, Tanhril Aizawl Mizoram (23.737669° N, 92.661683° E) and identified based on the morphological characteristics and confirmed it with molecular analysis. For molecular analysis, DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method, amplified with polymerase chain reaction (PCR) using ITS1 and ITS4 primers and sequenced using Sanger sequencing.

Extraction of specimen

The fruiting bodies were initially dried at 60 °C for 24 hr until a constant weight was achieved. Following drying, the samples were finely ground into a uniform powder. For extraction, 10 g of each powdered mushroom sample was mixed with 100 mL of methanol and stirred at 150 rpm for 24 hr, after which the mixture was filtered through Whatman No. 4 filter paper. The residues were subjected to a second extraction using an additional portion of methanol (22). The combined methanolic extracts were concentrated under reduced pressure using a rotary evaporator. The extraction yield was found to be approximately 7–10 % (w/w), calculated as the

percentage of crude extract obtained relative to the initial dry sample weight. The concentrated extracts were stored at 4 °C in airtight amber containers and used within 1 month of preparation for subsequent analyses. Methanol was selected as the extraction solvent due to its high polarity and strong ability to dissolve a broad range of bioactive compounds present in mushrooms.

Antioxidant activity

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

The antioxidant potential of the mushroom species was evaluated using the DPPH free radical scavenging assay (23). A methanolic extract of the mushroom was prepared and diluted to final concentrations of 1000, 800, 600, 400 and 200 µg/mL. For the assay, 180 µL of each extract concentration was combined with 10 µL of 0.1 mM DPPH solution (dissolved in methanol) in 96-well microplates, resulting in a total volume of 190 µL per well. The reaction mixtures were thoroughly homogenised and incubated at 37 °C in the dark for 30 min to prevent photodegradation of the DPPH radicals. Absorbance was measured at 515 nm using a UV-visible spectrophotometer. The half-maximal inhibitory concentration (IC₅₀) was determined to quantify the free radical scavenging activity of the mushroom extract. Ascorbic acid was used as a standard reference and positive control to validate the assay and enable comparison of antioxidant activity.

The percentage of DPPH free radical scavenging activity was calculated as follows:

$$\text{RSA on DPPH (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}})] \times 100$$

Where:

A sample = the absorbance of the extract/reference;

A control = the absorbance of the DPPH solution without the addition of extract.

ABTS {2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)} assay:

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging activity of the mushroom species was evaluated following the method of Re et al. (24). The ABTS radical cation (ABTS^{•+}) was generated by reacting 5 mL of ABTS stock solution with 5 mL of 2.45 mM potassium persulfate solution and incubating the mixture in the dark at room temperature for 16 hr to allow complete radical formation. Prior to the assay, the ABTS^{•+} solution was diluted with distilled water to an absorbance of 0.700 ± 0.020 at 734 nm and equilibrated at 30 °C. The mushroom extract was prepared at different concentrations and further diluted with methanol to obtain the working solutions. For the assay, 20 µL of each extract solution was combined with 150 µL of ABTS^{•+} solution and incubated at room temperature for 6 min. The absorbance was measured at 734 nm using a UV-visible spectrophotometer. Blanks were included in all measurements and the percentage inhibition of ABTS^{•+} radicals were calculated to determine the antioxidant activity of the mushroom species.

The ABTS scavenging ability was expressed as IC₅₀ (µg/mL) and the inhibition percentage was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where,

A₀ is the absorbance of the control; A₁ is the absorbance of the sample.

Gas chromatography-mass spectrometry analysis

The analysis of volatile compounds present in the mushroom extract was performed using a Clarus 690 PerkinElmer gas chromatograph (Auto System XL) coupled with a TurboMass Gold PerkinElmer 5.1 mass spectrometer. Separation was achieved using an Elite-1 capillary column (100 % dimethylpolysiloxane; 123.5 m × 678 µm), which provides high-resolution analysis of complex volatile mixtures. The oven temperature program was carefully optimised to ensure efficient separation: starting at an initial temperature of 40 °C, the temperature was ramped at 5 °C/min to 115 °C and held for 5 min, then further increased at 5 °C/min to 140 °C with a hold of 5 min. Subsequently, the temperature was raised at a slower rate of 2 °C/min to 210 °C and maintained for 8 min to allow better separation of higher boiling point compounds. Finally, the oven temperature was increased to 250 °C at a rate of 5 °C/min and held for 9 min to ensure complete elution of all constituents. The injection port temperature was maintained at 250 °C and helium was used as the carrier gas at a constant flow rate of 1.5 mL/min to facilitate optimal chromatographic performance. Samples were injected in a 10 : 1 split mode with a 1 µL injection volume to avoid column overloading. Electron impact ionisation was performed at 70 eV and the mass spectral scanning range was set from 50 to 800 m/z to capture the relevant molecular ions and fragments. The ion source and interface temperatures were maintained at 230 °C and 240 °C respectively, to ensure efficient ionisation and transfer of analytes into the mass detector. Mass spectral acquisition was performed from 3 to 75 min, with a solvent delay of 3 min to prevent solvent interference. The resulting chromatograms and mass spectra of detected volatile compounds were systematically compared with the NIST 17 online library (Ver. 2.3) to identify and confirm the chemical constituents of the mushroom extract based on spectral matching and retention indices (25). This detailed analytical approach allowed precise identification and characterisation of the volatile metabolites present in the mushroom sample, providing insights into its biochemical composition.

Results and Discussion

The collected specimen was identified as *G. multipileum* based on morphological characteristics and confirmed with molecular analysis (GenBank Accession number: MG450346). Basidiocarp is annual, typically stipitate, occasionally sessile or with a reduced lateral base and corky in texture. Pileus is fan-shaped with a

yellowish to reddish-brown surface and an obtuse margin. The pore surface is cream in young stages, becoming pale brown with age and the pores are circular to subcircular. The stipe, when present, is lateral, ranging from flattened to subcylindrical and yellowish to reddish-brown. The context is corky, yellowish-brown to dark brown, sometimes with melanised deposits. Basidiospores are ovoid to ellipsoid, thick-walled, smooth and brown to dark brown.

Antioxidant activity

The antioxidative potential of the methanolic extract of *G. multipileum* against free radical-induced oxidative damage was evaluated using the DPPH scavenging assay. The extract exhibited concentration-dependent radical scavenging activity, with the highest inhibition of 70.96 % observed at 1000 µg/mL (Fig. 1). The IC₅₀ value for *G. multipileum* was determined to be 564.7 µg/mL, which is comparable to the positive control, ascorbic acid, showing 86.26 % inhibition and an IC₅₀ of 562.0 µg/mL. The results indicate that *G. multipileum* possesses significant *in vitro* antioxidant activity, likely due to its high content of phenolic compounds, which are known to contribute to radical scavenging.

The ABTS radical scavenging activity *G. multipileum* was also concentration-dependent, with the highest inhibition of 42.12 % at 1000 µg/mL (Fig. 2). The IC₅₀ value was measured as 647.7 µg/mL, while ascorbic acid, used as a positive control, exhibited 89.44 % inhibition with an IC₅₀ of 562.8 µg/mL. The relatively lower ABTS scavenging compared to DPPH may reflect differences in radical specificity and the extract's interaction with the ABTS radical.

The determined IC₅₀ values of 564.7 µg/mL (DPPH) and 647.7 µg/mL (ABTS) for the methanolic extract of *G. multipileum* indicate a moderate antioxidant capacity. This level of activity is consistent with findings for several other *Ganoderma* species, though significant interspecies variability is well-documented in the literature. For instance, some species like *Ganoderma lucidum* and *Ganoderma applanatum* have been reported to exhibit significantly stronger antioxidant in various studies (26–30). However, the observed potency of *G. multipileum* confirms its potential as a source of antioxidants. Variation can be attributed to several factors, including the fungal species, geographical origin, developmental stage, extraction solvent and the specific composition of phenolic compounds and terpenoids. The findings firmly establish that *G. multipileum* possesses measurable *in vitro* radical scavenging activity, warranting further investigation into the isolation and identification of its specific bioactive constituents.

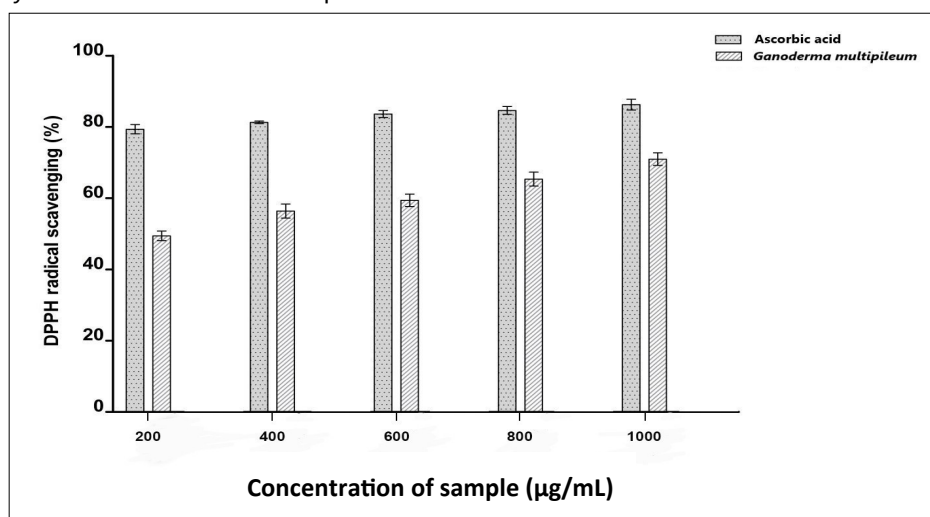


Fig. 1. DPPH radical scavenging activity of methanolic extract of *Ganoderma multipileum*.

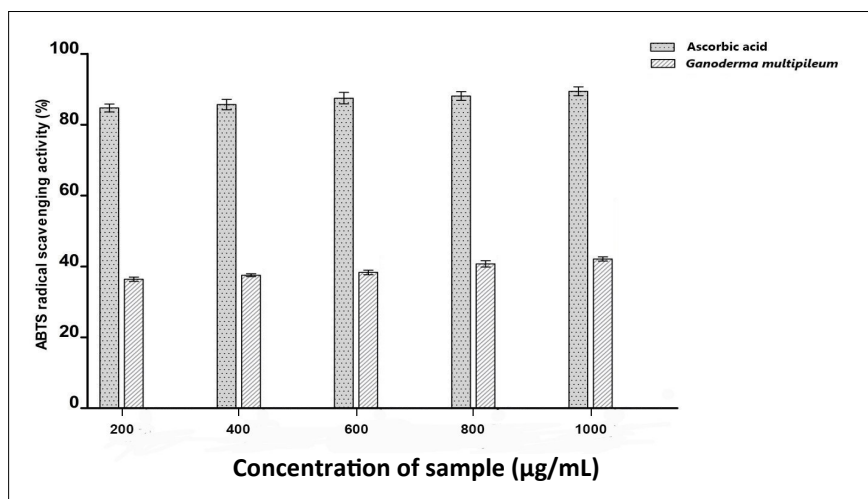


Fig. 2. ABTS radical scavenging activity of methanolic extract of *Ganoderma multipileum*.

Gas chromatography-mass spectrometry analysis of bioactive compounds

The GC-MS analysis revealed the presence of several bioactive compounds with diverse chemical structures and molecular weights. The detected compounds, their retention times, peak areas, molecular formulas and molecular weights are summarised in Table 1. The retention time (RT) of each compound reflects its volatility and interaction with the stationary phase of the GC column. In general, compounds with lower molecular weight and higher volatility elute earlier, resulting in shorter retention times, whereas compounds with higher molecular weight, increased polarity, or stronger interaction with the column matrix exhibit longer retention times. In the present study, early eluting peaks likely correspond to low-boiling or more volatile constituents, while compounds appearing at higher retention times represent less volatile, more complex, or higher molecular weight bioactive metabolites. The variation in retention times further indicates the chemical diversity of the methanolic extract, suggesting the presence of multiple classes of phytoconstituents such as phenolics, terpenoids and fatty acid derivatives. This wide distribution of retention times supports the complexity of the extract and highlights the potential contribution of these compounds to the observed biological activity.

The chromatogram displayed multiple peaks between retention times of 6.7–24.7 min, indicating a complex mixture of phytoconstituents (Fig. 3). Major compounds identified included 3-ethoxy-6-(1-tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane, fluvalinate, 1-methyl-2,5-dichloro-1,6-diazaphenylene, methyl p-coumarate (TMS derivative) and nonanoic acid, among others. These compounds are known for various biological and pharmacological properties such as antimicrobial, antioxidant and anti-inflammatory activities (31). Methyl p-coumarate are well known for their hydrogen-donating ability and free radical scavenging potential, which may directly contribute to the DPPH antioxidant activity observed in this study. Similarly, nonanoic acid and related fatty acid derivatives may also contribute to antioxidative and membrane-protective effects. Therefore, the antioxidant activity of *G. multipileum* is likely associated with the synergistic action of these bioactive compounds, particularly those detected with relatively higher abundance and recurring peak patterns in the GC-MS chromatogram.

Fluvalinate ($C_{26}H_{22}O_3N_2ClF_3$), a synthetic pyrethroid derivative, appeared at retention times 9.636 and 14.108 min, suggesting the presence of halogenated aromatic compounds used in agricultural application with potential pesticidal or protective roles (32). 3-Ethoxy-6-(1-tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane ($C_{20}H_{32}O_4$)

Table 1. Gas chromatography-mass spectrometry profiling of the identified compound from the methanol extract of *Ganoderma multipileum*

Sl. No.	RT	Area (%)	Name (compound)	MW	MF
1	6.795	1.537	3-Ethoxy-6-(1-Tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane	336	$C_{20}H_{32}O_4$
2	7.355	0.938	Pyrimidine, 5-Bromo-2-4-Bis(methylthio)-	250	$C_6H_7N_2BrS_2$
3	7.705	1.068	Pyrimidine, 5-Bromo-2-4-Bis(methylthio)-	250	$C_6H_7N_2BrS_2$
4	8.005	1.042	3-Ethoxy-6-(1-Tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane	336	$C_{20}H_{32}O_4$
5	8.376	0.941	3-Ethoxy-6-(1-Tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane	336	$C_{20}H_{32}O_4$
6	9.006	1.755	1-Methyl-2,5-Dichloro-1,6-Diazaphenylene	250	$C_{12}H_8N_2Cl_2$
7	9.636	1.508	Fluvalinate	502	$C_{26}H_{22}O_3N_2ClF_3$
8	10.647	1.327	Dibenzoic{C,H}Diazecine,6,13-Bis{2,5-Dimethylphenyl}-5,6,7,12,13,14-Hexane	502	$C_{36}H_{42}N_2$
9	11.567	1.207			
10	11.927	0.910	1-Methyl-2,5-Dichloro-1,6-Diazaphenylene	250	$C_{12}H_8N_2Cl_2$
11	12.317	1.173	3-Ethoxy-6-(1-Tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane	336	$C_{20}H_{32}O_4$
12	13.668	0.887	3-Ethoxy-6-(1-Tetrahydropyroneoxy-5-methyl-4-hexenyl)-2-cyclohexane	336	$C_{20}H_{32}O_4$
13	14.108	1.228	Fluvalinate	502	$C_{26}H_{22}O_3N_2ClF_3$
14	19.08	1.327	5,5-Dimethyl-2-(2,2,2-Trifluoromethylethyl)-4,5-DihydroF	249	$C_8H_9ONF_6$
15	23.82	0.911	Methyl P-Coumarate, TMS Derivative	250	$C_{13}H_{18}O_3Si$
16	23.89	1.168	2-Methylheptanoic acid	144	$C_8H_{16}O_2$
17	23.96	1.005	Benzenepropanoic acid, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-,MET	292	$C_{18}H_{26}O_3$
18	24.15	1.170	Nonanoic acid	158	$C_9H_{18}O_2$
19	24.482	0.917	[2,6-Bis(1-methylethyl)Phenoxy](Trimethyl)Silane	250	$C_{15}H_{26}OSi$
20	24.71	1.169	2-Propanoic acid, Cyclohexyl ester	154	$C_9H_{14}O_2$

RT: retention time; MW: molecular weight; MF: molecular formula.

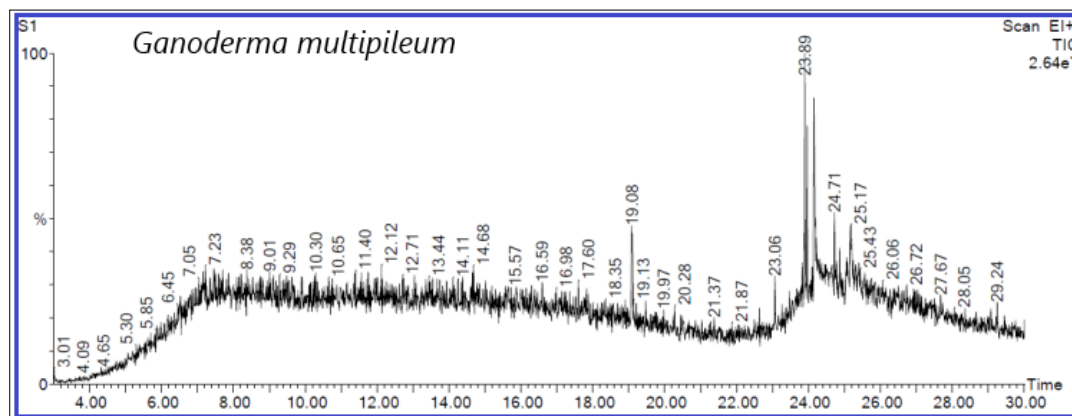


Fig. 3. Gas chromatography-mass spectrometry chromatogram of the methanolic extract of *Ganoderma multipileum*.

was observed as a recurring compound at multiple retention times (6.795, 8.005, 8.376, 12.317 and 13.668 min), indicating it as one of the dominant constituents in the extract.

Fatty acid derivatives, including 2-methylheptanoic acid ($C_8H_{16}O_2$), nonanoic acid ($C_9H_{18}O_2$) and 2-propanoic acid, cyclohexyl ester ($C_9H_{14}O_2$), were also detected, indicating the presence of lipid-related compounds with potential antioxidant and antimicrobial properties. Nonanoic acid is widely utilised in organic synthesis and industrial applications, including lacquers, plastics, hydrotropic salts, pharmaceuticals, synthetic flavours and fragrances, flotation agents, esters for turbojet lubricants, vinyl plasticisers and as a gasoline additive. Similarly, cyclohexyl acetate is a commonly used fragrance compound found in decorative cosmetics, fine fragrances, shampoos, soaps and other toiletries, as well as in non-cosmetic products such as household cleaners and detergents (33, 34).

Other compounds of interest included methyl p-coumarate ($C_{13}H_{18}O_3Si$), a phenolic ester derivative belonging to the hydroxycinnamic acid class. Hydroxycinnamic acids have attracted increasing attention due to their potent antioxidant properties (35). Additionally, benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- has been reported to exhibit strong antifungal and antioxidant activities, as observed in *Azadirachta indica* A.Juss. and *Thesium humile* Vahl (36, 37).

Overall, the GC-MS profile indicates that the sample contains a diverse range of chemical constituents, including aromatic hydrocarbons, fatty acids, esters and heterocyclic compounds. These findings provide insight into the potential pharmacological properties of the extract and support further investigation into its bioactive potential.

Conclusion

The present study demonstrates that *Ganoderma multipileum* possesses appreciable antioxidant activity and contains a chemically diverse range of bioactive metabolites. The methanolic extract showed concentration dependent radical scavenging activity in both DPPH and ABTS assays, confirming its ability to neutralise free radicals and indicating the presence of antioxidant constituents such as phenolics and other secondary metabolites. The gas chromatography-mass spectrometry (GC-MS) analysis further revealed a complex profile of compounds belonging to different chemical classes, including fatty acids, esters, phenolic derivatives and heterocyclic compounds, many of which are associated with

important biological activities. The findings of the present study are significant as they provide preliminary scientific validation for the medicinal potential of *G. multipileum*, a relatively underexplored macrofungus from Mizoram. The study highlights the potential of this species as a natural source of antioxidant compounds with possible applications in pharmaceutical, nutraceutical and industrial sectors. Furthermore, the work emphasises the importance of macrofungal biodiversity in Northeast India as a promising reservoir of valuable bioactive metabolites. Future research should focus on the isolation and purification of individual bioactive compounds, followed by detailed structural characterisation and evaluation of their pharmacological properties through *in vitro* and *in vivo* studies.

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Authors' contributions

BL, VLT and LB carried out the research work and experimental analysis. JZ assisted with DNA sequencing. PKR contributed to the preparation and revision of the manuscript. STL supervised and guided the overall research. The final version of the manuscript was written by BL and VLT. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The authors hereby declare that Artificial Intelligence (AI) tools were used for language editing. However, the final interpretation, conclusions and overall responsibility for the content of this manuscript remain the sole responsibility of the authors.

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