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GC–MS Profiling of Lipid and Sterol Metabolites in the Biomass of Inonotus hispidus (Bull.) P. Karst.

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Abstract This study aimed to characterize the gas chromatography–mass spectrometry (GC–MS) profile of a lipophilic extract obtained from naturally grown fruiting bodies of Inonotus hispidus and to identify putative lipid- and sterol-related metabolites. Dried and milled biomass (54 g) was extracted with 1 L of 96% ethanol for 5–7 days. The concentrated extract was fractionated with hexane and chloroform and subsequently subjected to GC–MS analysis. A total of 28 chromatographic peaks were detected. Comparison with the NIST mass spectral library showed that the predominant peak, observed at 10.752 min, matched methyl linoleate with 99% similarity and accounted for 27.86% of the total peak area. Methyl palmitate (12.62%), ethyl linoleate (8.46%), methyl stearate (7.75%), palmitic acid (5.16%), and linoleic acid (5.35%) were also major constituents. Squalene represented 1.61% of the total peak area, whereas peaks at 20.282 and 20.995 min showed putative matches to an ergosta-type sterol and ergosterol, respectively. A phthalate derivative was interpreted as a possible laboratory contaminant. These findings indicate that the lipophilic fraction of I. hispidus fruiting bodies may be rich in fatty-acid derivatives and compounds associated with sterol biosynthesis. Because compound assignments were based on library matching, they should be regarded as tentative and confirmed using authentic standards, retention indices, and complementary LC–MS and/or NMR analyses.

Keywords Inonotus hispidus, GC–MS, Lipid metabolites, Fatty acids, Methyl linoleate, Palmitic acid, Squalene, Ergosterol, Sterols, Bioactive compounds

1. Introduction

Medicinal and biologically active fungi are important sources of natural metabolites, including polysaccharides, phenolic compounds, terpenoids, sterols, fatty acids, organic acids, and numerous other secondary metabolites. These compounds may exhibit antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and, in some cases, cytotoxic activities. Therefore, detailed characterization of the chemical composition of macrofungi is of considerable scientific and practical relevance to pharmacology, food biotechnology, nutraceutical development, and the cosmetics industry.

Inonotus hispidus (Bull.) P. Karst. is a wood-decaying basidiomycete belonging to the family Hymenochaetaceae and occurs predominantly on the trunks of deciduous trees. Its fruiting bodies are typically yellowish brown to dark brown, with a distinctly hispid surface, and may develop as either parasites or saprotrophs. In recent years, the bioactive constituents, pharmacological properties, and potential food and health-related applications of *I. hispidus* have attracted increasing scientific attention [11,12].

Previous studies have reported phenolics, flavonoids, polysaccharides, terpenoids, fatty acids, and sterol-type compounds in *I. hispidus*. Ergosterol and its derivatives are particularly important because they are major structural components of fungal cell membranes. Ergosterol can therefore serve as a chemical marker of fungal biomass and sterol metabolism. Variations in ergosterol peroxide and other ergostane derivatives have also been associated with the physiological state and developmental stage of *I. hispidus*.

The metabolite profile of fungi may vary markedly with growth substrate, host-tree species, geographic origin, sampling season, developmental stage of the fruiting body, extraction procedure, and solvent system. Consequently, profiling naturally occurring *I. hispidus* collected from local habitats is scientifically relevant. Such analyses can help assess its value as a biological resource, identify promising metabolites, and provide a basis for subsequent bioactivity studies.

Gas chromatography–mass spectrometry is a powerful analytical technique for detecting volatile and semivolatile organic compounds and for the preliminary identification of fatty-acid derivatives, aliphatic hydrocarbons, terpenoids, and sterols. Library-based spectral matching enables tentative assignment of extract constituents; however, particularly in

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complex natural matrices, GC–MS identifications should be verified using authentic standards or complementary analytical approaches.

Accordingly, this study aimed to characterize lipid and sterol metabolites in an extract prepared from naturally occurring *I. hispidus* fruiting bodies by GC–MS and to assess their potential biotechnological relevance.

To the best of our knowledge, this work provides the first systematic GC–MS description of a lipophilic extract obtained from naturally grown *I. hispidus* fruiting bodies collected in Uzbekistan [13-16]. Major fatty-acid derivatives, squalene, and putative ergosta-type sterols were evaluated together on the basis of their relative peak areas.

The specific objectives of this study were to: (i) prepare dried biomass from I. hispidus fruiting bodies and obtain a 96% ethanolic extract; (ii) partially fractionate the extract using hexane, chloroform, and ethanol while reducing pigmented impurities; (iii) determine the GC–MS chemical profile of the resulting extract; (iv) evaluate the major fatty acids, aliphatic hydrocarbons, squalene, and ergosterol-like compounds detected; and (v) assess the potential biological and biotechnological significance of the identified metabolites.

2. Materials and Methods

2.1. Collection and Preparation of Fungal Material

Figure 1. Dried and milled biomass prepared from an I. hispidus fruiting body collected from a tree trunk

Fruiting bodies of naturally occurring *I. hispidus* were collected from a tree trunk and transported to the laboratory. Visible debris and remnants of tree bark were removed. The material was then dried, milled, and homogenized to obtain a uniform biomass fraction. The prepared dry biomass was weighed on an electronic balance and had a total mass of 54 g.

2.2. Preparation of the Ethanolic Extract

Figure 2. Filtration of the 96% ethanolic extract prepared from 54 g of dried I. hispidus biomass

Milled dry I. hispidus biomass (54 g) was placed in a glass vessel and mixed with 1 L of 96% ethanol. Extraction was carried out in the dark under laboratory conditions for 5–7

days. The mixture was agitated periodically to improve the transfer of extractable constituents into the solvent.

At the end of extraction, the dark-brown suspension was filtered through filter paper. The filtrate was separated from the solid residue and concentrated for subsequent processing.

2.3. Partial Purification of the Extract with Organic Solvents

To reduce co-extracted compounds and obtain a cleaner fraction for GC–MS analysis, the concentrated extract was sequentially treated with organic solvents. First, the viscous extract was washed with hexane to separate part of the nonpolar and strongly lipophilic constituents.

The sample was subsequently treated with chloroform to recover a lipophilic metabolite fraction. To reduce pigments and other strongly colored compounds, the resulting fraction was washed again with 96% ethanol. This procedure yielded a relatively purified, dark-colored extract fraction suitable for GC–MS analysis.

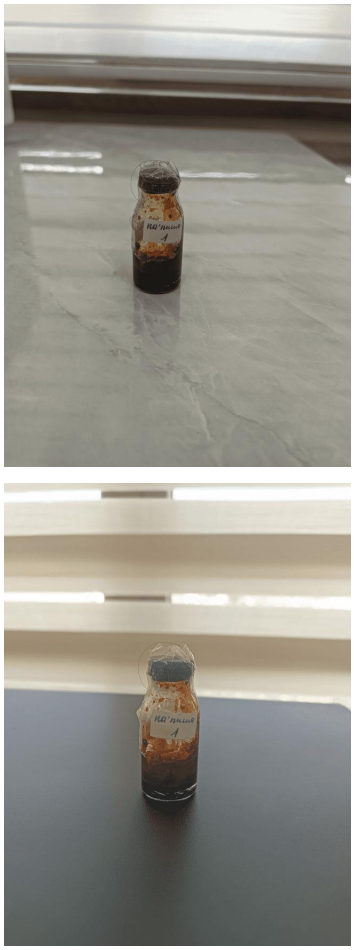

Figure 3. Concentrated fraction of the I. hispidus extract after sequential solvent treatment

2.4. GC–MS Analysis

The prepared extract fraction was analyzed by gas chromatography–mass spectrometry to obtain a preliminary profile of lipids, sterols, and other semivolatile organic constituents. Chromatographic peaks were evaluated according to retention time and mass-spectral characteristics. Preliminary compound identification was performed by comparison with a mass-spectral library; consequently, all reported assignments were regarded as tentative, and definitive identification of selected compounds requires authentic standards and/or complementary spectroscopic analyses.

GC–MS analysis was performed in full-scan mode using an automated sample-introduction system. Data were acquired with the instrumental method "Drugs_SCAN_MED_22_757 _auto.M". The mass spectrometer was operated under electron ionization at 70 eV. Peaks were evaluated on the basis of retention time, integrated peak area, and diagnostic mass fragments. Experimental spectra were compared with the NIST17.L mass-spectral library using a minimum match threshold of 80%. In total, 28 chromatographic peaks were recorded between 4.277 and 20.995 min.

2.5. Criteria for Compound Identification

Preliminary identification was based on comparison of experimental mass spectra with the NIST library. Table 1 reports the library match, retention time, and relative peak area for each peak. Identification confidence should be strengthened in future work by calculating linear retention indices using an n-alkane series, confirming major compounds with authentic standards, and examining the sterol fraction by LC–MS or NMR. Names based solely on spectral-library similarity are therefore designated as tentative or putative.

2.6. Experimental Design and Statistical Analysis

The GC–MS data presented here represent a descriptive chemical profile of a single extract; therefore, inferential statistics were not applied. Relative abundance was calculated as the percentage contribution of each integrated peak to the total area of all detected peaks in the total-ion chromatogram. At least three independent biological replicates, together with technical replicates for each sample, should be analyzed in follow-up work, with results reported as mean ± standard deviation.

2.7. Control of Contamination and Analytical Artifacts

To distinguish phthalate derivatives and solvent-related artifacts from genuine sample constituents, solvent blanks, extraction blanks, and predominantly glass laboratory ware with minimal plastic contact are recommended. Interpretation of methyl and ethyl esters should explicitly document the solvents used, any derivatization step, and the complete sample-preparation workflow. Because no separate derivatization was performed in this experiment, methyl- and ethyl-ester assignments should be treated as tentative library-based identifications.

3. Results and Discussion

3.1. GC–MS Profile of the Inonotus hispidus Extract

GC–MS analysis of the *I. hispidus* extract revealed 28 chromatographic peaks. Spectral-library comparison indicated

the presence of aliphatic hydrocarbons, aldehydes, fatty acids and their methyl and ethyl esters, terpenoids, sterol-like compounds, and several possible exogenous contaminants.

The largest peak was detected at 10.752 min and showed 99% similarity to methyl 9,12-octadecadienoate (methyl linoleate), accounting for 27.86% of the total peak area. The 99% spectral match is a very high similarity score, indicating a high level of confidence in the tentative library identification of this peak; nevertheless, because no authentic standard or retention-index confirmation was performed, the assignment is still reported as tentative. Other abundant constituents included methyl hexadecanoate, ethyl 9,12-octadecadienoate, methyl stearate, palmitic acid, and linoleic acid.

Table 1. Major compounds tentatively identified in the I. hispidus extract by GC–MS.

Peak No.	RT (min)	Area (%)	Best library match	Brief interpretation
1	4.277	0.68	Glycerol	Trihydric alcohol; may reflect polar constituents or residual moisture.
2	6.337	0.95	Dodecane	C12 aliphatic hydrocarbon belonging to the lipophilic fraction.
3	7.130	0.72	2,4-Decadienal	Unsaturated aldehyde potentially associated with lipid oxidation or the volatile aroma fraction.
4	7.530	1.18	Tetradecane	C14 saturated hydrocarbon; a constituent of wax-like and lipophilic fractions.
5	8.483	0.71	1-Hexadecene	Unsaturated long-chain hydrocarbon; may be associated with lipid metabolites or a hydrophobic surface layer.
6	8.507	0.94	Hexadecane	C16 alkane occurring in the lipophilic fungal fraction.
7	9.393	0.74	1-Octadecene	C18 unsaturated hydrocarbon associated with membrane lipids or wax-like fractions.
8	9.983	12.62	Methyl hexadecanoate	Methyl palmitate; saturated fatty-acid ester and a major lipid-fraction constituent.
9	10.143	5.16	Hexadecanoic acid	Palmitic acid; a common saturated fatty acid in fungal lipids.
10	10.266	4.19	Ethyl hexadecanoate	Ethyl palmitate; the ethyl ester of palmitic acid in the oily extract fraction.
11	10.752	27.86	Methyl 9,12-octadecadienoate	Methyl linoleate; the largest-area peak and an unsaturated fatty-acid derivative.
12	10.844	7.75	Methyl octadecanoate	Methyl stearate; methyl ester of stearic acid and a saturated lipid constituent.
13	10.930	5.35	9,12-Octadecadienoic acid	Linoleic acid; a biologically relevant unsaturated fatty acid.
14	11.028	8.46	Ethyl 9,12-octadecadienoate	Ethyl linoleate; a major ester of linoleic acid in the lipid fraction.
15	11.127	2.20	Ethyl octadecanoate	Ethyl stearate; the ethyl ester of stearic acid.
16	11.736	0.98	Methyl eicosanoate	Methyl arachidate; a long-chain saturated fatty-acid ester.
17	12.055	1.47	Tetracosane	C24 alkane; a wax-like hydrophobic-fraction constituent.
18	12.566	2.83	Octadecane	C18 saturated hydrocarbon; its spectrum also resembled eicosane and nonadecane.
19	12.762	0.91	Methyl docosanoate	Methyl behenate; a long-chain fatty-acid ester.
20	13.008	2.56	Mono(2-ethylhexyl) benzene-1,2-dicarboxylate	Phthalate-group compound; a possible contaminant from plastics, filters, syringes, or laboratory vessels.
21	13.150	1.52	Eicosane	C20 saturated hydrocarbon; a lipophilic and wax-like constituent.
22	13.808	1.35	Heptacosane	C27 long-chain alkane interpreted as a hydrophobic coating or wax-like component.
23	13.968	2.76	Hydroxylated glycerol ester of 9,12-octadecadienoic acid	Complex linoleic-acid-related ester; tentatively interpreted as a glyceride-like lipid.
24	14.576	1.24	Eicosane	Long-chain aliphatic hydrocarbon; may represent the same or a closely related component as peak 21.
25	15.013	1.61	Squalene	Triterpenoid and a key intermediate in sterol biosynthesis.
26	15.492	0.84	Eicosane	Lipophilic hydrocarbon detected at low abundance.
27	20.282	1.12	Putative ergosta-type sterol	Spectrum resembled ergosta-5,7,9(11),22-tetraen-3-ol, but alternative library hits were obtained; standard confirmation is required.
28	20.995	1.29	Putative ergosterol	Matched ergosterol in the library, although alternative hits were also present; therefore reported as putative ergosterol.

3.2. Chemical Classes of the Detected Compounds

Fatty acids and their esters constituted the largest chemical class in the extract. This group included methyl palmitate, palmitic acid, ethyl palmitate, methyl linoleate, methyl stearate, linoleic acid, ethyl linoleate, ethyl stearate, methyl arachidate, and methyl behenate, demonstrating the predominance of lipid-related metabolites. This predominance likely reflects both a biological and a methodological factor: fatty-acid esters are typical storage and membrane-associated

lipids of basidiomycete fruiting bodies, and the sequential hexane–chloroform–ethanol treatment used here was specifically designed to enrich nonpolar, lipophilic constituents while removing polar pigments and other co-extracted compounds, so the resulting fraction was expected to be dominated by this chemical class.

Methyl linoleate was the dominant component, representing 27.86% of the total peak area. Methyl palmitate, ethyl linoleate, and methyl stearate contributed 12.62%, 8.46%, and 7.75%, respectively. These compounds may be major constituents of the oily extractive fraction of the fruiting body.

Long-chain hydrocarbons, including dodecane, tetradecane, hexadecane, octadecane, eicosane, tetracosane, and heptacosane, were also detected, suggesting the presence of hydrophobic and wax-like components. Such compounds may be associated with the protective surface layer, intracellular lipid droplets, or the natural wax fraction of the fruiting body. This interpretation is supported by their chain lengths (C12–C27) and saturation pattern, which are typical of cuticular wax hydrocarbons that form a hydrophobic barrier on fungal fruiting-body surfaces, protecting against desiccation and microbial colonization, as well as of hydrocarbons stored within intracellular lipid droplets.

Squalene, detected at 15.013 min, is an important intermediate in triterpenoid and sterol biosynthesis. Its occurrence supports the possible presence of lipophilic metabolites associated with fungal sterol-forming pathways.

Peaks at 20.282 and 20.995 min produced spectra resembling an ergosta-type compound and ergosterol, respectively. Because alternative library hits were also obtained, these compounds are more appropriately described as a putative ergosta-type sterol and putative ergosterol; confirmation requires comparative analysis with an authentic ergosterol standard and/or LC–MS analysis.

The phthalate-related compound detected at 13.008 min was interpreted as a likely laboratory contaminant rather than a natural fungal metabolite, because phthalates may leach from plastic containers, caps, syringes, filters, or other solvent-contact materials.

3.3. Relative Abundance and Biological Relevance of the Major Components

The 28 detected peaks were classified, on the basis of library matches, as aliphatic hydrocarbons, fatty acids and their esters, terpenoids, sterol-like compounds, and possible exogenous contaminants. Fatty acids and their derivatives clearly predominated, confirming that the analyzed fraction was enriched in lipophilic metabolites.

The most intense chromatographic signal occurred at 10.752 min and showed a high library match to methyl 9,12-octadecadienoate, commonly known as methyl linoleate. This unsaturated fatty-acid derivative is the methyl ester of linoleic acid. Its predominance indicates that unsaturated lipid constituents may represent an important component of I. hispidus biomass.

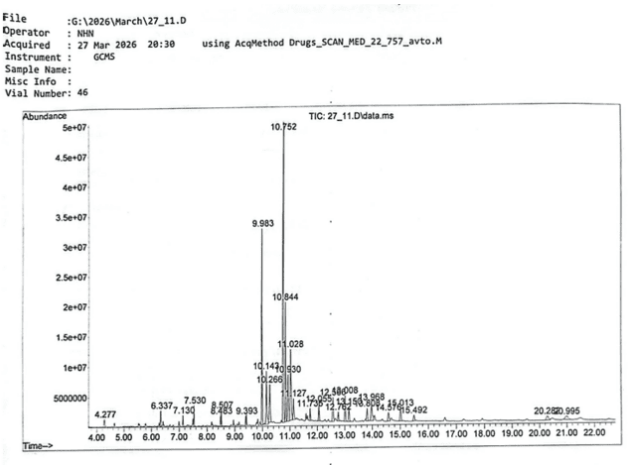

Figure 4. Total-ion chromatogram of the I. hispidus extract obtained by GC–MS

Methyl linoleate is a biologically interesting constituent of natural lipids and has potential relevance as a lipophilic ingredient in cosmetic formulations, as a marker in chemical profiling of natural extracts, and as a candidate component in bio-based product development. This relevance stems from its unsaturated C18 structure, which confers emollient and skin-barrier-supporting properties, together with reported antioxidant and anti-inflammatory activity of linoleate-type esters, making such compounds attractive as lipophilic active ingredients in topical and cosmetic formulations. Nevertheless, its exact concentration and biological activity must be confirmed using an authentic standard and dedicated bioassays.

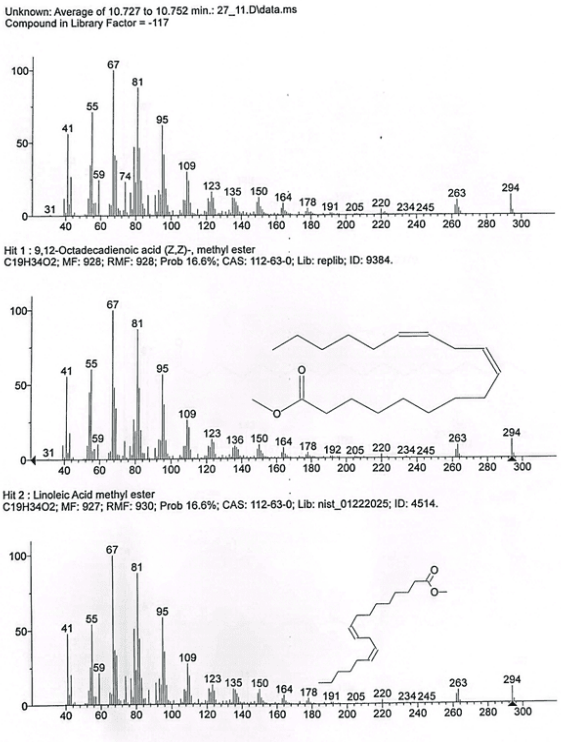

Figure 5. Mass spectrum of the peak detected at 10.752 min and its library match to methyl 9,12-octadecadienoate

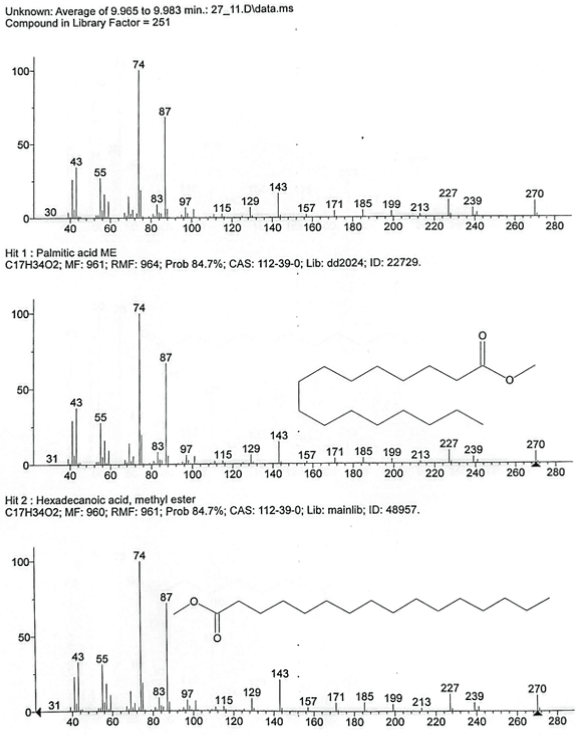

Figure 6. Experimental mass spectrum of palmitic acid detected in the I. hispidus extract and comparison with the library spectrum

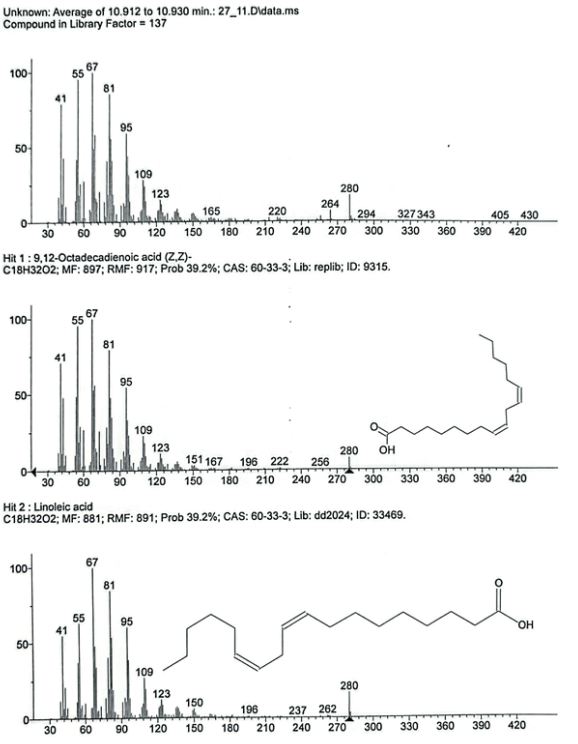

Figure 7. Experimental mass spectrum of linoleic acid detected in the I. hispidus extract and comparison with the library spectrum

Components matching palmitic acid and its methyl and ethyl esters were detected between 9.983 and 10.266 min. Methyl palmitate, palmitic acid, and ethyl palmitate were therefore considered important representatives of the saturated lipid fraction. Palmitic acid is a common saturated fatty acid in fungal membranes and contributes to membrane stability and biophysical properties.

Compounds resembling methyl stearate, linoleic acid, ethyl linoleate, and ethyl stearate were detected between 10.844 and 11.127 min. Their co-occurrence indicates the presence of both saturated and unsaturated fatty-acid derivatives. Saturated fatty acids lack carbon–carbon double bonds and are therefore less susceptible to autoxidation than their unsaturated counterparts, a property that may contribute to the relatively stable lipophilic fraction and to its resistance to degradation during extraction and storage, whereas unsaturated fatty acids and their esters, being more chemically reactive, may be more directly associated with active fungal lipid metabolism and bioactive-metabolite systems.

The extract also contained aliphatic hydrocarbons such as dodecane, tetradecane, hexadecane, 1-octadecene, octadecane, eicosane, tetracosane, and heptacosane. These compounds may originate from hydrophobic, wax-like, or otherwise lipophilic fractions of the fungal biomass. Detection of long-chain hydrocarbons also confirms that nonpolar constituents were retained after hexane and chloroform treatment.

Squalene, detected at 15.013 min, is of particular interest as a terpenoid and a central intermediate in sterol biosynthesis. Its presence in the I. hispidus extract supports the occurrence of metabolic processes associated with terpenoid and sterol formation. As a bioactive lipophilic compound, squalene is relevant to cosmetic, pharmaceutical, and biotechnological research.

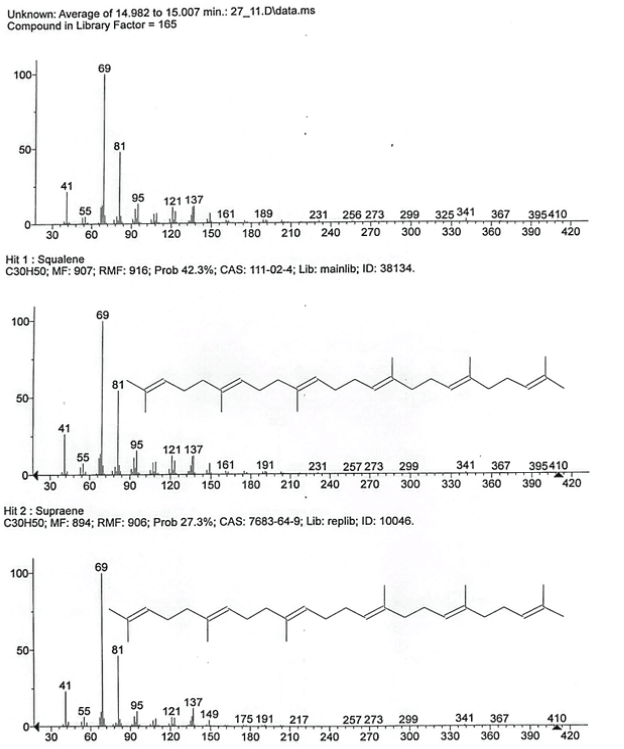

Figure 8. Mass spectrum of squalene detected in the I. hispidus extract and comparison with library data

At longer retention times, high-molecular-weight sterol-type peaks were observed at 20.282 and 20.995 min. Their spectra showed library similarity to an ergosta-type compound and ergosterol, respectively. Ergosterol is a major sterol component of fungal cell membranes and is widely used as a chemical marker of fungal biomass.

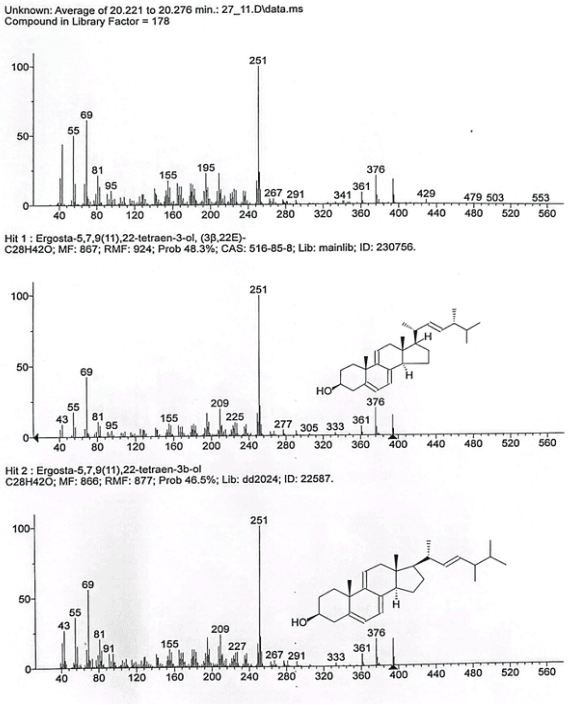

Figure 9. Mass spectrum of the putative ergosta-type sterol detected at 20.282 min

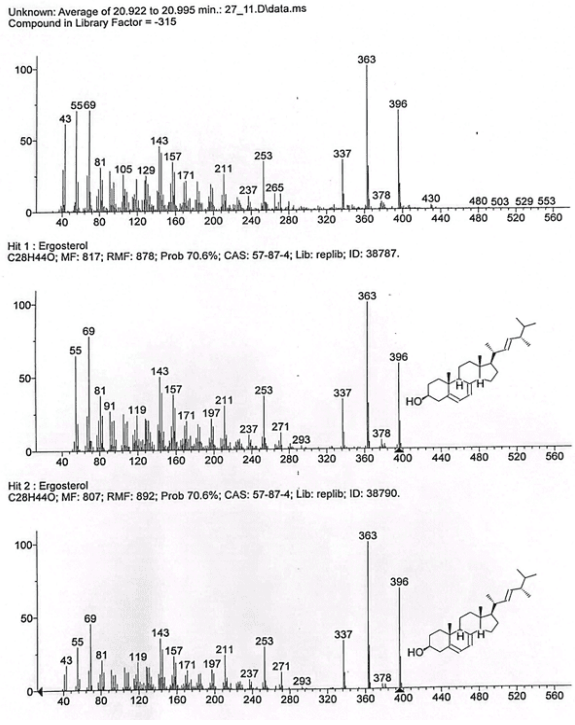

Figure 10. Mass spectrum of the putative ergosterol detected at 20.995 min and comparison with the library spectrum

Because these assignments were based solely on mass-spectral library matching, the compounds should be reported conservatively as a putative ergosta-type sterol and putative ergosterol. Definitive confirmation requires comparison with an authentic ergosterol standard and complementary LC–MS and/or NMR analysis.

A phthalate-group compound was detected at 13.008 min. Such compounds are commonly interpreted as contaminants originating from plastic vessels, caps, syringes, filtration materials, or other laboratory equipment rather than as natural fungal metabolites. It was therefore excluded from the group of biologically relevant constituents.

Overall, the GC–MS profile of the I. hispidus extract consisted primarily of fatty acids and their esters, long-chain hydrocarbons, squalene, and sterol-type constituents. The principal findings were the dominant methyl-linoleate-like peak, derivatives of palmitic and linoleic acids, squalene, ergosta-type compounds, and putative ergosterol. These observations justify further investigation of I. hispidus as a potential natural source of bioactive lipophilic metabolites.

3.4. Comparison with Previous Studies

The GC–MS profile obtained in this study was dominated by fatty acids and their methyl and ethyl esters, together with long-chain hydrocarbons, squalene, and sterol-like compounds. The predominance of the methyl-linoleate-like peak and the detection of palmitic-, stearic-, and linoleic-acid derivatives indicate that lipid metabolism makes a substantial contribution to the chemical composition of I. hispidus biomass.

These observations are broadly consistent with earlier studies of wood-decaying basidiomycetes, in which palmitic, stearic, and linoleic acids have frequently been reported among the major fatty acids; in some fungal species, linoleic acid represents a substantial proportion of the total fatty-acid pool [1,2,9].

The chemical diversity of I. hispidus has also been documented in previous experimental and review articles. Polyphenols, triterpenoids, and other bioactive secondary metabolites have been reported, and their abundance may vary substantially with geographic origin, developmental stage, host-tree species, and extraction procedure [3–5].

A squalene-like peak was detected at 15.013 min in the present study. Because squalene is a key intermediate in sterol biosynthesis, its occurrence indicates the presence of lipophilic metabolites associated with terpenoid and sterol metabolism. Previous reports describing the concurrent detection of palmitic and stearic acids, long-chain hydrocarbons, and squalene in selected wood-decaying basidiomycetes support this interpretation [6].

The peaks observed at 20.282 and 20.995 min showed library similarity to an ergosta-type compound and ergosterol, respectively. Ergosterol is one of the principal sterols in fungal membranes and is widely applied as a biomarker of fungal biomass; it has been reported as the predominant sterol in several edible and medicinal mushrooms [2,7,8,10].

However, the ergosterol and ergosta-type assignments in this study were based exclusively on spectral-library similarity. They should therefore be interpreted as putative ergosterol and a putative ergosta-type sterol. Definitive identification requires comparative GC–MS analysis with authentic standards and confirmation by LC–MS or NMR.

The results suggest that unsaturated fatty-acid derivatives may predominate in the lipophilic fraction of I. hispidus, particularly methyl linoleate, ethyl linoleate, and linoleic-acid-like compounds. Nevertheless, GC–MS profiling alone cannot establish pharmacological or cosmetic efficacy; antioxidant, antimicrobial, cytotoxicity, and safety assays are required before any functional claims can be made.

An important contribution of this study is the preliminary lipid and sterol profile generated from naturally grown I. hispidus collected under local conditions. Future comparative studies should include samples from different host trees, developmental periods, and solvent systems to clarify the variability of the chemical composition of this fungus.

3.5. Study Limitations

The principal limitations were the use of a single biological sample, the absence of technical replication and inferential statistics, reliance primarily on NIST library matching, lack of retention-index data and authentic-standard confirmation, and the absence of experimental antioxidant, antimicrobial, or cytotoxicity assays. The results should therefore be interpreted as a preliminary chemical profile.

4. Conclusions

This study provides a preliminary GC–MS assessment of lipid and sterol metabolites in an extract prepared from naturally occurring I. hispidus fruiting bodies. Dried biomass (54 g) was extracted with 1 L of 96% ethanol for 5–7 days, and the concentrated extract was sequentially treated with hexane, chloroform, and ethanol before GC–MS analysis.

A total of 28 chromatographic peaks were recorded. Library-based assignments indicated that the extract contained mainly aliphatic hydrocarbons, fatty acids and their esters, triterpenoids, and sterol-type compounds. The major conclusions are as follows: (i) fatty acids and their esters were the dominant chemical group, with palmitic, linoleic, and stearic acids and their methyl and ethyl esters indicating that products of lipid metabolism are prominent in I. hispidus biomass; (ii) the most intense peak, at 10.752 min, showed a high library match to methyl linoleate, suggesting that unsaturated fatty-acid derivatives are important constituents of the extract; (iii) detection of long-chain hydrocarbons confirmed the hydrophobic and lipophilic character of the extract; (iv) the detection of squalene suggests the presence of metabolites associated with terpenoid and sterol biosynthesis; and (v) peaks at 20.282 and 20.995 min showed library similarity to an ergosta-type sterol and ergosterol, respectively, supporting the possible occurrence of characteristic fungal sterol metabolism, although definitive identification requires authentic-standard comparison and complementary LC–MS or NMR analyses. The phthalate-related compound was considered a likely laboratory contaminant and was therefore excluded from the natural metabolites of I. hispidus.

Taken together, the results indicate that the lipophilic fraction of the analyzed I. hispidus sample may contain fatty-acid derivatives, squalene, and sterol-like compounds. Because this conclusion is based on a descriptive, library-assisted profile of a single sample, species-level generalization requires biological replication, quantitative confirmation with authentic standards, and biological-activity testing.

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Conflict of Interest

The author declares no conflict of interest.

Data Availability

The data presented in this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Wang, Z.; et al. Diverse metabolites and pharmacological effects from the medicinal mushroom Inonotus hispidus: A review. Antibiotics 2022, 11, 1097. https://doi.org/10.3390/antibiotics11081097.

[2] Xue, R.; et al. Inonotus hispidus has development potential in health and future food fields: A review. J. Future Foods 2025.

[3] Huo, Y.; et al. Transcriptional responses for biosynthesis of triterpenoids in Inonotus hispidus. J. Fungi 2022, 8, 1276.

[4] Dong, S.; et al. Improving triterpenoid extraction efficiency from Inonotus hispidus. Foods 2025, 14, 1069.

[5] Zhang, G.; et al. Cultivation of Inonotus hispidus on jujube wood waste. J. Fungi 2026, 12, 143.

[6] Parsi, Z.; et al. Determination of ergosterol as an indicator of fungal contamination by gas chromatography–mass spectrometry. J. Chromatogr. A 2006, 1130, 43–54.

[7] Lau, A.P.S.; Lee, A.K.Y.; Chan, C.K.; Fang, M. Ergosterol as a biomarker for the quantification of fungal biomass in atmospheric aerosols. Atmos. Environ. 2006, 40, 249–259.

[8] Parkinson, D.R.; et al. Analysis of ergosterol for the detection

of mold in soils by solid-phase microextraction–gas chromatography–mass spectrometry. Anal. Chem. 2010, 82, 10094–10100.

[9] Nieto, I.J.; et al. The effect of different substrates on triterpenoid metabolites and fatty acids in cultivated Pleurotus species. J. Chil. Chem. Soc. 2013, 58, 1604–1608.

[10] Albero, B.; et al. Development of a gas chromatography–mass spectrometry method for the determination of ergosterol in soil. Eur. J. Soil Sci. 2025.

[11] Bergmann, P.; Takenberg, M.; Frank, C.; et al. Cultivation of Inonotus hispidus in stirred tank and wave bag bioreactors to produce the natural colorant hispidin. Fermentation 2022, 8, 541.

[12] Gao, Y.; Li, X.; Xu, Y.; et al. Optimization of Inonotus hispidus liquid fermentation medium and evaluation of exopolysaccharide activities. Front. Microbiol. 2024.

[13] Sherqulova, J.; Qo'ziboev, X. Isolation of pure culture of medicinal herb macrofungus Inonotus hispidus (Bull.) P. Karst. in Uzbekistan. Universum Khimiya.

[14] Sherqulova, J.; Quziboev, X. Molecular analysis of medicinal Inonotus hispidus. Khorezm Ma'mun Acad. Bull. 2023, 12-1.

[15] Quziboev, X. Technology for isolating a pure culture of Inonotus hispidus (Bull.) P. Karst. Khorezm Ma'mun Acad. Bull. 2025, 12/1.

[16] Quziboev, X. Preparation of seed mycelium of Inonotus hispidus (Bull.) P. Karst. and its growth characteristics on different substrates. Qo'qon DPI Sci. Bull. 2025, 11.