Comparative Analysis of Bioactive Compounds of four edible Fungal Biomass by using GC-MS

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Abstract

This study investigates the comparative analysis of bioactive compounds in the mycelia of *Hericium erinaceus, Pleurotus ostreatus, Ganoderma lucidum,* and *Agaricus bisporus,* aiming to characterize their biochemical activities and highlight their economical cultivation, significant nutritional and medicinal properties. To achieve this, the selected mycelial samples were cultivated for 15–20 days on Potato Dextrose Agar under controlled conditions of temperature (25 ± 2 °C), ensuring optimal mycelial growth for subsequent morphological characterization and bioactive metabolite extraction. After cultivation, bioactive compounds were extracted and analysed using GC–MS, revealing 45 compounds in *Hericium erinaceus*, 43 in *Pleurotus ostreatus*, 52 in *Ganoderma lucidum*, and 62 in *Agaricus bisporus* respectively. Detailed morphological analysis using the lactophenol blue test revealed septate, branched, and clamp-connected hyphae, confirming typical Basidiomycete characteristics. Moreover, comparative analysis showed that, every mycelium is rich in polyunsaturated and unsaturated fatty acids mainly octadecanoic, linoleic, and palmitic acids which support heart health, lower cholesterol, and offer antimicrobial and emulsifying benefits, making them useful as food additives. This in-depth study not only confirms the bioactive compound in edible mycelia but also explore their nutritional value, underscoring their potential for future drug discovery, disease management, and functional food development. To our knowledge, this is the first comparative research integrating metabolomic profile with functional knowledge across these species and providing a valuable reference for future research and biotechnological applications.

Keywords: Mycelia, Bioactive Compounds, *Hericium erinaceus, Pleurotus ostreatus*, *Ganoderma lucidum. Agaricus bisporus.*

1.Introduction:

Mushrooms have recently attracted considerable scientific attention due to their numerous health benefits. Mycelia, the root-like structures of fungi, have a wide range of applications in the food,

pharmaceutical, and industrial sectors[1]. In the food industry, mycelia are utilized in the cultivation of edible mushrooms, which are valued for their nutritional benefits, including low carbohydrate content, unsaturated fatty acids, high levels of essential fatty acids, sterols, vitamins, and minerals [2]. Additionally, mycelia serve as a sustainable protein source and are being increasingly incorporated into plant-based food products [1, 2]. In addition to being a common food source, mushrooms contribute significantly to the prevention and management of various diseases, highlighting their importance in the human diet [3]. In the medical field, mycelia contain several bioactive compounds with significant therapeutic properties such as antioxidant, anti-inflammatory, and antimicrobial effects, making them promising candidates for the development of natural medicines and various supplements [4]. Most edible mushrooms, including *Hericium erinaceus*, *Pleurotus ostreatus*, *Ganoderma lucidum* and *Agaricus bisporus* belong to the phyla Basidiomycota. These mushrooms are widely recognized not only for their culinary value but also for their health-promoting properties [5, 6]. As we all know, bioactive compounds are widely recognized for their ability to inhibit various disease mechanisms through their antimicrobial, anticancer, antioxidant, and anti-inflammatory properties [7, 8]. Numerous studies have shown that consuming bioactive compounds derived from mycelia can potentially lower cancer risk, strengthen the immune system, and support cardiovascular health [9]. These compounds offer potential alternatives to synthetic drugs by providing a range of therapeutic effects beneficial to human health. Beyond their health benefits, edible mushrooms have attracted attention for their potential in bioremediation [10], the process of using living organisms to break down or eliminate environmental contaminants [11]. Species like *Pleurotus ostreatus* (oyster mushroom) and *Ganoderma lucidum* have shown the capacity to degrade a wide range of organic pollutants, such as petroleum hydrocarbons, pesticides, and heavy metals, through their inherent metabolic activities [12]

As mentioned earlier, the pharmaceutical industry is increasingly focused on extracting phytochemical compounds from mycelia due to their anticancer, anti-inflammatory, anti-allergic, and antioxidant properties [13]. Similarly, the cosmetic industry has also started incorporating mycelial phytochemicals into skincare products, recognizing their multifunctional therapeutic potential [14]. The antiinflammatory, antimicrobial, and anti-allergic properties of these bioactive compounds make them a subject of growing interest across multiple disciplines [15]. Although many studies have highlighted mushrooms as valuable sources of food and bioactive macromolecules such as proteins, lipids, and polysaccharides, there remains a gap in comparative analyses of bioactive metabolite profiles specifically at the mycelial stage across multiple edible species remain relatively limited [16]. In particular, integrated approaches that combine metabolite identification with functional interpretation of bioactive compounds across species are still emerging[17,18]. To address this gap, the present study

performs a comparative analysis of bioactive compounds in the mycelia of four edible mushroom species: Hericium erinaceus, Pleurotus ostreatus, Ganoderma lucidum, and Agaricus bisporus together. Previous studies have also demonstrated that metabolite composition in mushrooms varies significantly depending on species, developmental stage and environmental conditions, leading to fragmented and species -specific datasets [19]. Therefore, present study aims to provide a systematic and integrative evaluation of species-specific metabolite profiles and their associated biological activities. The current findings will provide valuable insights into the therapeutic potential of these mycelial compounds, highlighting their relevance for nutraceutical development and explore their potential medicinal benefits [20]. In contrast to earlier studies that largely emphasize fruiting bodies or individual species, the present work focuses on the mycelial stage and further associates identified metabolites with reported biological functions using the Dr. Duke phytochemical database, thereby providing a functional perspective on metabolite diversity. This approach provides insight into the potential of mushroom mycelia as a sustainable source of bioactive metabolites for nutraceutical and pharmaceutical applications. Further investigations can be done to evaluate the reported bioactive compounds for targeted medical applications through comprehensive in silico analyses, including molecular docking, pharmacokinetic (ADMET) profiling, and target–pathway interaction studies [19,20,21]

2.Materials and Methods:

2.1 Chemicals and reagents:

The fungal mycelia were grown on Potato Dextrose Agar (PDA) plates using PDA powder (Hi-Media Laboratories, India) followed by pure spawn cultures of *H. erinaceus*, *A. bisporus*, *G. lucidum*, and *P. ostreatus*. The cultivation process utilized sterile 90 mm Petri dishes and autoclavable Erlenmeyer flasks with capacities of 250 mL and 500 mL. For inoculation and sub-culturing, sterile scalpels and inoculation loops were used. All fungal cultures were aseptically prepared and maintained under laminar airflow conditions to minimize contamination, with 80% ethanol employed for sterilization.

2.2 Mycelia Growth conditions in Laboratory:

The present study was conducted at the Department of Biotechnology, Jaypee Institute of Information Technology (JIIT), Noida, India, over a period of six months in 2024. The mushroom cultures were derived from pure spawn cultures that were prepared in-house and maintained internally at the JIIT laboratory. Moreover, the mycelia were cultivated on a medium prepared by dissolving 35 g of Potato

Dextrose Agar (PDA) in 1 L of distilled water, followed by sterilization at 121 °C for 15–20 minutes. Furthermore, cultivation was carried out at 25 ± 2 °C in sterile conditions in lab incubator (BR BIOCHEM LIFE SCIENCES PVT. LTD) to prevent microbial contamination [20,21]. After sufficient growth, the mycelial biomass was harvested through filtration, washed with distilled water, and dried at 40°C to a constant weight [21]. In addition, after three weeks, growth pattern of mycelium was measured on the basis of growth rate, texture, and color of the mycelia. Furthermore, the dried mycelia were ground into a fine powder and subjected to ethanol extraction using a Soxhlet apparatus for 6–8 h [22, 23]. The ethanolic extracts were concentrated using a rotary evaporator under reduced pressure and analysed by Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent India) [19]. Bioactive compounds were identified by comparing their retention times and mass spectra with Wiley library databases [24].

2.3 Mycelia Growth observation by Lactophenol Blue Staining method:

The morphological analysis of grown mycelia was conducted using prepared petri plates containing Potato Dextrose Agar (PDA) as the growth medium [25]. After 15 days of cultivation, mycelial growth, colony traits, and structural features were observed for *G. lucidum*, *P. ostreatus*, and *H. erinaceus*, *A. bisporus*. Moreover, Lactophenol Cotton Blue staining was used for microscopic (OLYMPUS BX51) examination at 50× magnification. The analysis focused on key morphological traits such as the shape, colour, and texture of the mycelial colonies, as well as the development of hyphal structures [26]. The rate of growth, branching patterns, and any distinctive features like pigmentation or spore formation were also carefully observed, which is providing valuable insights into the development and physical characteristics of the mycelia, contributing to a deeper understanding of their growth dynamics under controlled conditions [26].

2.4 Solvent Extractions:

After sufficient growth, the mycelia were carefully removed from the plates, and the extracted mycelia were subsequently used for the metabolite extraction process. Four mycelial samples of *G. lucidum, P. ostreatus, H. erinaceus,* and *A. bisporus* were weighed and 0.38 g, 1.760 g, and 0.427 g and 0.026 g of dry weight was recovered respectively. After that, the scraped mycelia were soaked in 80 mL of 80% ethanol and shaken at room temperature for 24 hours. The mixture was then centrifuged at 6000 rpm for 5–10 minutes, and the supernatant was collected for further analysis [27]. Following extraction in Soxhlet apparatus (J. K. International) by 3 consecutive cycles. All the four samples were concentrated using a rotary evaporator and subsequently air-dried at room temperature for GC-MS analysis [27, 28].

2.5 GC-MS analysis for detection of Bioactive Compounds:

These ethanolic extracts of mycelia were subsequently analysed using Gas Chromatography–Mass Spectrometry (GC-MS) to identify and evaluate the various metabolites present in each mycelium. For the analysis, 1 mL of each mycelial extract was used. These ethanolic extracts of mycelia were subsequently analysed using Gas Chromatography–Mass Spectrometry (GC-MS) to identify and evaluate the various bioactive compounds present in each mycelium. The ethanolic extract was examined with a Shimadzu QP-2010 GC-MS system at the Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), using manufacturer-recommended tuning protocols to ensure mass accuracy and sensitivity. No internal standard was employed in the present study , the gas chromatograph was fitted with an HP-5 MS capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness, 5% phenylmethyl siloxane). Helium was used as the carrier gas at a flow rate of 1.61 mL/min with a split ratio of 1:10 [29]. The oven temperature was programmed from 60°C (held for 2 minutes) to 250°C [30], increasing at a rate of 20°C per minute and held for 10 minutes. The ion source temperature was set at 250°C with an electron ionization energy of 70 eV. Moreover, 1 μL of ethanolic sample was injected into the column. In our study, compound identification was carried out by comparing the acquired mass spectra with the in-built NIST and GC-MS mass spectral library (version available on the Shimadzu QP-2010 Plus GC–MS system at AIRF, JNU). The identified compounds were subsequently compiled and presented in tabulated form [31]. Quantification of the detected compounds was performed using peak area normalization, and the relative abundance of each compound was expressed as a percentage of the total ion chromatogram (TIC).

2.6 Determination of bioactive compounds:

Dr. Duke's database was used for the characterization of all the bioactive compounds for phytochemical and ethnobotanical along with literature mining [34].

3. Results and Observations:

3.1 Morphological study and chemical compounds of four mycelia under microscope:

After a 15-day incubation period, a morphological analysis of four mycelial samples such as *H. erinaceus, P. ostreatus, G. lucidum,* and *A. bisporus* was performed (Fig. 1). Along with that, GC–MS analysis was also conducted to identify bioactive compounds present in all selected mycelial samples. These analyses include variations in colony colour, texture, radial growth rate, and overall morphology, with each species displaying distinct and identifiable features (Table 1). Moreover, microscopic examination revealed the presence of both septate and aseptate hyphal structures across all fungal samples. As per the analysis*, P. ostreatus* displayed the most rapid and widespread mycelial growth, while G. lucidum formed dense, compact colonies.

Furthermore, the first fungal isolate, *Hericium erinaceus*, also exhibits dense and comparatively quick radial development on culture media. Microscopic examination revealed septate, hyaline, thin-walled hyphae with clamp connections, which are characteristic features of Basidiomycota. The lack of septa supports fast nutrient transport and growth in moist, nutrient-rich substrates. Additionally, GC–MS analysis revealed that Ethyl oleate was the most abundant compound detected in the *H. erinaceus* sample. Whereas, the second isolate *Agaricus bisporus* exhibited sparse hyphal branching and active vegetative mycelial growth. The isolate exhibits sparse branching with formation of aerial hyphae indicating active vegetative growth; however, no asexual spore-producing structures were observed, consistent with the nature of basidiomycetes. GC–MS analysis of the biomass revealed Hexadecanoic acid ethyl ester as the most predominant compound. Moreover, the third isolate, *G. lucidum*, exhibits extensive hyphal development characterized by long, highly branched hyphae with distinct transverse septa, confirming its classification as a septate fungus, likely belonging to the phylum Basidiomycota. The hyphae maintained a consistent diameter and exhibited regular dichotomous branching patterns, indicative of organized and directional growth under nutrient-rich conditions. In addition, GC–MS analysis revealed that Isopropyl methyl-pentadecanoate was the most abundant compound detected in the *G. lucidum* sample.

Whereas the fourth sample *P. ostreatus* showed compact, dense mycelial organization with irregular hyphal branching and occasional chlamydospore formation, and Ethyl linoleate was identified as the predominant compound. Overall, the morphological characterization provided valuable insights into the developmental strategies of the isolated fungi. These observations not only aid in preliminary species identification but also offer clues about their ecological roles ranging from decomposers and mutualists to potential pathogens. Further molecular identification will complement these morphological findings, ensuring accurate classification and deeper understanding of their functional attributes in various environments.

3.2 GC-MS Analysis of all four different mycelia:

The Gas Chromatography–Mass Spectrometry (GC–MS) spectra of *H. erinaceus, P. ostreatus, G. lucidum,* and *A. bisporus* extracts revealed a diverse range of bioactive compounds with varying molecular weights and retention times. The identified compounds are presented in Tables 2, 3, 4 and 5, along with their peak area percentages, retention times, molecular weights, molecular formulas, and reported therapeutic applications. According to the GC–MS analysis, a total of 45 bioactive compounds were identified in *H. erinaceus,* 43 *in P. ostreatus,* 52 *in G. lucidum,* and 62 in *A. bisporus*. Based on the observations, octadecenoic acid and hexadecanoic acid were identified as common compounds present in all four samples. In addition, linoleic acid (octadecadienoic acid) was another compound commonly detected across all samples, showing its highest relative abundance in *P. ostreatus* (20.17 percent), followed by *A. bisporus* (7.91 percent), *H. erinaceus* (7.42 percent), and *Ganoderma lucidum* (1.13 percent). Whereas, Oleate was detected in relatively low concentrations, with *A. bisporus* containing 3.34% and *P. ostreatus* only 0.02%. Nevertheless, *A. bisporus* showed the highest level of oleic acid among the samples, at 13.85%. These major findings highlight linoleic acid and octadecenoic acid as common polyunsaturated fatty acids present almost in all the samples of investigated mycelia with essential health benefits. The identified bioactive compounds are reported to exhibit anticancer, antitumor, antihistaminic, antiarthritic, antieczemic, and anti-inflammatory activities, making them promising candidates for cosmeceutical and pharmaceutical applications. Some compounds with no reported biological activity were also observed in the GC–MS analysis.

3.2.1 Quantitative analysis of phytochemical compounds present in *Hericium erinaceus:*

As stated earlier, in the *H. erinaceus* sample, around 45 bioactive compounds were identified. Among these, ten compounds exhibited the highest peak intensities (Figure 2). These major bioactive compounds were further classified based on their retention times (RT), chemical classes, and biological significance. Among them, the majority belong to fatty acids and their derivatives, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Table 2) [34]. Among the most abundant compounds, Ethyl oleate ((E)-9-octadecenoic acid ethyl ester; $C_{20}H_{38}O_2$, 310.50 g/mol) was detected at RT 10.770, representing a derivative of oleic acid (MUFA)

with reported anti-inflammatory activity. Similarly, ethyl stearate (octadecanoic acid ethyl ester; $C_{20}H_{40}O_2$, 312.50 g/mol) was identified at RT 10.974 and is associated with anticancer and antitumor properties. In addition, ethyl linoleate ($C_{20}H_{36}O_2$, 308.50 g/mol), a PUFA, was detected at RT 10.726 and is known for antiarthritic, antieczemic, antihistaminic, anti-inflammatory, and antileukotriene-D4 activities. Moreover, hexadecanoic acid ethyl ester ($C_{18}H_{36}O_2$, 284.50 g/mol) was detected at RT 9.397 and is a saturated fatty acid (palmitic acid derivative) reported to possess anti-inflammatory, hypocholesterolemic, hepatoprotective, nematicidal, anticancer, antitumor, and antihistaminic properties. Additionally, octadec-9-enoic acid ($C_{18}H_{34}O_2$, 282.50 g/mol), detected at RT 10.598, is a MUFA with known anti-inflammatory activity. Palmitic acid (TMS derivative) ($C_{19}H_{40}O_2Si$, 328.60 g/mol) was identified at RT 9.754 and is also reported to exhibit anti-inflammatory effects. Other prominent compounds include (9Z,12Z)-octadeca-9,12-dienoic acid ($C_{18}H_{32}O_2$, 280.45 g/mol) at RT 10.553, ethyl nonadecanoate ($C_{21}H_{42}O_2$, 326.60 g/mol) at RT 8.178, and linoleic acid (TMS derivative) ($C_{21}H_{40}O_2Si$, 352.60 g/mol) at RT 11.095. These compounds further support the dominance of fatty acids and their derivatives in the extract. TMS derivatives of linoleic acid are categorized under PUFAs, while compounds such as ethyl nonadecanoate belong to saturated fatty acid derivatives. Additionally, 2-undecanone ($C_{11}H_{22}O$, 170.29 g/mol) detected at RT 5.942 exhibited antioxidant activity. However, several compounds, including benzene, 1,4-dimethoxy-2-methyl-5-isopropyl- (RT 6.160), dodecane, 6-cyclohexyl- (RT 6.391), acetamide derivative (RT 8.877), isopropyl tetradecyl ether (RT 9.039), benzocyclodecene derivative (RT 11.801), ethyl 14-methyl-hexadecanoate (RT 12.722), benzphetamine (RT 13.069), tritetracontane (RT 13.338), showed no clearly reported biological activity.

3.2.2 Quantitative analysis of phytochemical compounds present in *Pleurotus ostreatus:*

According to the GC–MS analysis of the *P. ostreatus* sample, a total of 43 bioactive compounds were identified (Table 3) [34]. Among these, the top ten compounds were selected based on their higher peak area percentages, reflecting their relative abundance in the extract (Figure 3). These main compounds were predominantly fatty acids and their esters, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and a limited proportion of polyunsaturated fatty acids (PUFA), indicating a lipidrich phytochemical composition. Among the identified compounds, ethyl linoleate ($C_{20}H_{36}O_2$, 308.50 g/mol), a PUFA, exhibited the highest peak area (20.17%) at RT 11.678, suggesting its dominance in the extract. This compound is widely reported for anti-inflammatory, antihistaminic,

antiarthritic, antieczemic, and cardioprotective activities. Similarly, ethyl hexadecanoate ($C_{18}H_{36}O_2$, 284.50 g/mol), a saturated fatty acid ester, was detected at RT 10.210 with a peak area of 12.95%, and is associated with anti-inflammatory, hypocholesterolemic, hepatoprotective, nematicidal, anticancer, and antihistaminic properties. Hexadecanoic acid ($C_{16}H_{32}O_2$, 256.42 g/mol), identified at RT 10.060 with a peak area of 6.52%, represents a main saturated fatty acid contributing to the biological activity of the extract. In addition, ethyl stearate ($C_{20}H_{40}O_2$, 312.50 g/mol) was detected at RT 11.952 with a peak area of 9.91%, and is known for its antioxidant and anticancer properties.

Oleoyl chloride ($C_{18}H_{33}ClO$, 300.90 g/mol), identified at RT 15.589 with a peak area of 6.59%, also represents a significant lipid-derived compound with reported antimicrobial activity. Furthermore, ethyl erucate ($C_{24}H_{46}O_2$, 366.60 g/mol), detected at RT 14.548 with a peak area of 4.20%, is a longchain MUFA derivative. Additional major compounds include butanoic acid derivative ($C_{20}H_{32}O_3$, 320.50 g/mol) at RT 10.287 (3.84%), acetamide derivative ($C_{14}H_{22}N_2O$, 234.34 g/mol) at RT 9.636 (3.81%), hexadecanoic acid glycerol ester ($C_{19}H_{38}O_4$, 330.50 g/mol) at RT 14.200 (2.86%), and 17pentatriacontene ($C_{35}H_{70}$, 490.90 g/mol) at RT 14.078 (2.67%). In addition, several compounds such as ethyl α-D-glucopyranoside (RT 8.070), ethyl pentadecanoate (RT 9.434), 3-dodecanol, 3,7,11trimethyl- (RT 9.896), 2-heptadecanol (RT 10.344 and 13.520), pentadecyl trifluoroacetate (RT 10.401), octacosanol (RT 11.107), and octadecyl trifluoroacetate (RT 11.212 and 12.911) were also detected; however, these compounds lack well-documented biological activities. Overall, the predominance of fatty acids and their esters, particularly ethyl linoleate and ethyl hexadecanoate, suggests that the P. ostreatus extract may exert its biological effects primarily through lipid-mediated mechanisms, including anti-inflammatory, antimicrobial, and cardioprotective activities.

3.3.3 Quantitative analysis of phytochemical compounds present in *Ganoderma lucidum:*

As compared to other samples, the *G. lucidum* extract revealed a total of 52 bioactive compounds through GC–MS analysis (Table 4; Figure 4). Among these, several compounds, including 1-dodecene (RT 5.147), dodecane (RT 5.220), tridecyl fluoro-benzoate derivative (RT 7.055), and multiple TMSderivatized compounds, showed no clearly reported biological activity. The top 10 compounds were selected based on their higher peak area percentages, indicating their relative abundance and potential biological significance. Among these, isopropyl methyl-pentadecanoate ($C_{19}H_{38}O_2$, 298.50 g/mol) was detected at RT 9.398 with the highest peak area (10.30%) and is reported to exhibit antifungal activity. Similarly, methyl stearate derivative ($C_{20}H_{40}O_2$, 312.50 g/mol), identified at RT

10.968 with a peak area of 9.51%, represents a saturated fatty acid derivative associated with anticancer properties. Ethyl nonadecanoate ($C_{21}H_{42}O_2$, 326.60 g/mol) was detected at RT 8.178 with a peak area of 7.84%, and is also reported for its anticancer potential. In addition, the phthalate ester (butyl octyl) ($C_{20}H_{30}O_4$, 334.40 g/mol) identified at RT 9.213 exhibited a peak area of 5.05% and is known for antimicrobial activity. Palmitic acid (TMS derivative) ($C_{19}H_{40}O_2Si$, 328.60 g/mol), detected at RT 9.752 with a peak area of 3.83%, represents a saturated fatty acid with anti-inflammatory properties.

Furthermore, Oleic acid ($C_{18}H_{34}O_2$, 282.50 g/mol), identified at RT 11.140 with a peak area of 2.02%, is a monounsaturated fatty acid (MUFA) known for its anticancer and antihistaminic activities. Its corresponding TMS derivative ($C_{21}H_{42}O_2Si$, 326.63 g/mol) was also detected at RT 11.192 (1.03%), further confirming its presence in the extract. Stearic acid (TMS derivative) ($C_{21}H_{46}O_2Si$, 358.68 g/mol), identified at RT 11.355 with a peak area of 2.61%, is another main saturated fatty acid derivative with reported antibacterial and cholesterol-lowering effects. Additional significant compounds include 1-tetradecene ($C_{14}H_{28}$, 196.37 g/mol) at RT 6.500 (1.89%), which exhibits anticancer and emollient properties, and ammonium benzoate derivative ($C_{28}H_{34}N_2O_3$, 446.60 g/mol) at RT 13.501 (2.36%), known for antimicrobial activity. Moreover, Oleamide ($C_{18}H_{35}NO$, 281.50 g/mol), detected at RT 15.225 with a peak area of 0.24%, is a bioactive fatty acid amide with reported antioxidant properties. Overall, the results indicate that saturated fatty acid derivatives constitute the predominant class of compounds in the G. lucidum extract, followed by monounsaturated fatty acids, while polyunsaturated fatty acids (PUFAs) were present in comparatively lower abundance. This lipid-dominated profile suggests that the biological activities of G. lucidum may primarily be mediated through fatty acid–related mechanisms, including antimicrobial, antifungal, anti-inflammatory, and anticancer effects.

3.3.4 Quantitative analysis of phytochemical compounds present in *Agaricus bisporus:*

GC–MS analysis of the *A. bisporus* sample identified a total of 62 bioactive compounds (Table 5; Figure 5). Among these, several compounds such as sulfurous acid, octadecyl 2-propyl ester (RT 12.044), glycidyl palmitate (RT 12.766), benzeneethanamine derivative (RT 13.768), and α-tocopheryl acetate (RT 21.206) showed no clearly reported biological activity. The major compounds were selected based on their higher peak area percentages, indicating their relative abundance and potential biological significance. Among these, hexadecanoic acid ethyl ester ($C_{18}H_{36}O_2$, 284.50 g/mol) was the most abundant compound, detected at RT 10.196 with the highest peak area (15.29%). This compound represents a saturated fatty acid (C16:0) and is reported to possess anti-inflammatory,

hypocholesterolemic, hepatoprotective, nematicidal, and anticancer properties. Similarly, Oleic acid ($C_{18}H_{34}O_2$, 282.50 g/mol), a monounsaturated fatty acid (MUFA), was identified at RT 11.514 with a peak area of 13.85%, highlighting its significant contribution to the lipid profile and its known anticancer and antihistaminic activities. Linoleic acid ethyl ester ($C_{20}H_{36}O_2$, 308.50 g/mol), a polyunsaturated fatty acid (PUFA), was detected at RT 11.621 with a peak area of 7.91%, and is associated with anti-inflammatory, antihistaminic, and cardioprotective effects. Ethyl oleate ($C_{20}H_{38}O_2$, 310.50 g/mol), detected at RT 11.665 with a peak area of 3.34%, represents another MUFA derivative with reported HMG-CoA inhibitory activity. Octadecanoic acid ethyl ester ($C_{20}H_{40}O_2$, 312.50 g/mol), identified at RT 11.901 with a peak area of 6.45%, is a saturated fatty acid associated with antioxidant and anticancer properties.

In addition, butyl undecyl ester ($C_{23}H_{36}O_4$, 376.50 g/mol), detected at RT 9.991 with a peak area of 11.50%, exhibited antimicrobial and cosmetic properties, making it one of the most prominent nonfatty acid contributors. Other notable compounds include ethyl pentadecanoate ($C_{17}H_{34}O_2$, 270.50 g/mol) at RT 8.783 (2.87%), and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester ($C_{19}H_{38}O_4$, 330.50 g/mol) at RT 14.171 (2.01%), both associated with anti-inflammatory and anticancer activities. Furthermore, 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester ($C_{21}H_{40}O_4$, 356.50 g/mol), detected at RT 11.901 with a peak area of 7.47%, represents a monounsaturated fatty acid derivative with reported anticancer and antihistaminic properties. Additionally, Oleamide ($C_{18}H_{35}NO$, 281.50 g/mol), identified at RT 13.213 with a peak area of 0.44%, contributes to the antioxidant profile of the extract. Overall, the results indicate that saturated fatty acids constitute the dominant class of compounds in the A. bisporus extract, followed by monounsaturated and polyunsaturated fatty acids. The predominance of lipid-derived bioactive compounds suggests that the biological activities of A. bisporus may be largely mediated through fatty acid–associated mechanisms, including antiinflammatory, antimicrobial, hepatoprotective, and anticancer effects.

4. Current analysis and future directions of the study:

The current study revealed that all four mycelial samples were highly enriched with various bioactive compounds, including saturated fatty acids, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), among others. As previously mentioned, saturated fatty acid esters were the most dominant bioactive compounds in A. bisporus, followed by monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). The most abundant compound detected in A. bisporus was hexadecanoic acid ethyl ester (palmitic acid ethyl ester) (RT 10.196), a saturated fatty acid recognized

for its anti-inflammatory properties and widely used in nutraceutical and cosmetic products for its skin-conditioning and emollient effects. The second most abundant compound was oleic acid (RT 11.514), followed by butyl undecyl ester (RT 9.991) and linoleic acid ethyl ester (RT 11.621), an esterified form of linoleic acid and particularly notable for its antihistaminic, antiinflammatory, and antileukotriene-D4 activity, making it beneficial for allergic and inflammatory conditions. Moreover, its cardioprotective properties make it a valuable dietary component. In *P. ostreatus,* saturated and monounsaturated fatty acid esters were the predominant bioactive compounds, with linoleic acid ethyl ester (RT 11.678) being the most abundant compound, rather than the only PUFA identified. Linoleic acid, an omega-6 PUFA, was the most abundant compound, known for its vital role in maintaining cell membrane integrity, regulating immune function, and exerting antiinflammatory effects. This was followed by hexadecanoic acid ethyl ester (RT 10.210) and ethyl stearate (RT 11.952), while ethyl oleate (RT 11.746) was present in lower abundance. In *Ganoderma lucidum,* saturated fatty acid esters were the most prevalent, along with oleic acid and its derivatives such as TMS esters and amides, classified as monounsaturated fatty acids (MUFAs). The most prominent compound identified was isopropyl methyl-pentadecanoate (RT 9.398), a saturated fatty acid ester known for its antifungal properties. Another major compound, methyl stearate derivative (RT 10.968), is recognized for its anticancer potential. Ethyl nonadecanoate (RT 8.178) was also present in significant quantity and is associated with anticancer activity. Additionally, oleic acid (RT 11.140) and its TMS derivative (RT 11.192) were identified, contributing to the MUFA profile of the sample.

In *H. erinaceus,* saturated fatty acids were the most dominant, followed by monounsaturated and polyunsaturated fatty acids. The most abundant compound was ethyl oleate (RT 10.770), an ethyl ester of oleic acid, noted for its anti-inflammatory activity. The second major compound was octadec-9-enoic acid (RT 10.598), followed by hexadecanoic acid ethyl ester (RT 9.397) and butyl undecyl ester (RT 9.208). Linoleic acid ethyl ester (RT 10.726) was also present and contributes to the PUFA profile of the sample. This study demonstrates that all four mycelial types possess a wide range of biological activities, including antibacterial, antimicrobial, anti-inflammatory, anticancer, antitumor, and antioxidant effects. The identified bioactive compounds exhibit diverse pharmacological properties, underscoring their potential applications in the food, pharmaceutical, and cosmetic industries. Utilizing mushroom mycelia-derived products may offer significant health benefits by helping to combat diseases such as cancer, cardiovascular conditions, and microbial infections. Notably, saturated fatty acids contribute to improved nutrient absorption, brain and cellular health, cooking stability, and increased levels of high-density lipoprotein (HDL). This research highlights the therapeutic potential of these fungal species, providing a foundation for further exploration into their roles in disease

prevention and health promotion. The rich phytochemical diversity found in the mycelia, particularly bioactive fatty acids and their esters, suggests these mushrooms could serve as valuable natural sources of therapeutic agents. Their integration into functional foods, nutraceuticals, pharmaceuticals, and cosmetics could offer a range of health benefits, including inflammation control, allergy relief, cancer prevention, and skin care. Overall, the findings support the expanded use of mushroom-derived bioactives in enhancing human health and well-being.

Future prospects for the comparative analysis of bioactive compounds in four edible fungal biomasses using GC-MS are promising, with significant potential to advance both scientific understanding and practical applications. Further research could focus on exploring the specific mechanisms through which these bioactive compounds exert their pharmacological effects, enabling the development of targeted nutraceuticals and functional foods. Additionally, expanding the study to include a wider variety of fungal species and cultivation conditions may uncover novel compounds with unique health benefits. Integration of GC-MS data with other analytical techniques, such as metabolomics and proteomics, could provide a more comprehensive profile of fungal bioactivity. Moreover, scaling up the extraction and purification processes for these compounds could facilitate their incorporation into pharmaceuticals, cosmetics, and dietary supplements. Ultimately, this line of research holds great potential to promote sustainable utilization of edible fungi as natural sources of therapeutic agents, supporting innovations in health, nutrition, and disease prevention. Future research should also prioritize the isolation and characterization of individual metabolites and assess their efficacy through in vitro and in vivo studies. Additionally, advances in biotechnology and metabolic engineering can further optimize compound yields, enabling large-scale production and expanding commercial applications of these promising mycelial resources.

4. Discussion:

The dry biomass yield of mycelia compounds varied considerably among the studied species, which may have regulated the extraction efficiency and subsequent detection of metabolites. Species yielding higher biomass could potentially provide a greater quantity of extractable compounds, thereby enhancing the detectability of minor constituents. In contrast, lower biomass may limit the identification of low-abundance metabolites. However, in the present study, GC–MS analysis was performed using equal volumes of extract, and compound abundance was expressed as relative percentages based on peak area normalization. Therefore, the results primarily reflect relative metabolite composition rather than absolute concentrations. Nonetheless, the potential influence of biomass variation on metabolite detection should be considered when interpreting interspecies

differences. Future studies employing biomass normalization and absolute quantification approaches may provide more accurate comparative insights. Furthermore, several compounds were categorized as having "no reported activity" based on the Dr. Duke phytochemical database. It is important to clarify that the absence of documented biological activity in this database does not necessarily indicate that these compounds are biologically inactive. Rather, it may reflect limited available studies or insufficient experimental validation. Therefore, such compounds should not be interpreted as inactive but rather as underexplored, warranting further investigation to elucidate their potential biological roles.

5. Conclusion:

In conclusion, this study underscores the potential of fungal mycelia as sustainable bio factories for producing a diverse array of bioactive compounds with significant therapeutic and industrial value. A total of 202 bioactive metabolites were identified across four species *A. bisporus* (62), *G. lucidum* (52), *H. erinaceus* (45), and *P. ostreatus* (43), with A. bisporus yielding the highest number. The comparative GC–MS profiling revealed common pharmacologically active fatty acids such as linoleic, stearic, oleic, and palmitic acids, known for their antioxidant, anti-inflammatory, anticancer, antimicrobial, hepatoprotective, and cosmetic properties. These findings highlight the practical utility of mushroom mycelia in pharmaceuticals, nutraceuticals, cosmeceuticals, agriculture, and sustainable material sciences. However, challenges remain in compound purification, structural elucidation, and biological validation.

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Author Contributions:

Apeksha Rathi contributed to the conceptualization, overall conducting the experiments, design of the study, data analysis, and interpretation of results. Prof. Neeraj Wadhwa provided valuable inputs regarding literature review and conduct of experiment. Dr. Ekta Bhatt provided valuable input in the literature review, data interpretation, and drafting of the manuscript. Dr. Chakresh Kumar Jain assisted

with data collection and the preparation of figures and tables. All authors reviewed and revised the manuscript, and approved the final version for publication.

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Clinical trial number: Not applicable.

Graphical Abstract: A Comparative study of the bioactive compounds in the biomass of four edible fungi using GC-MS analysis.

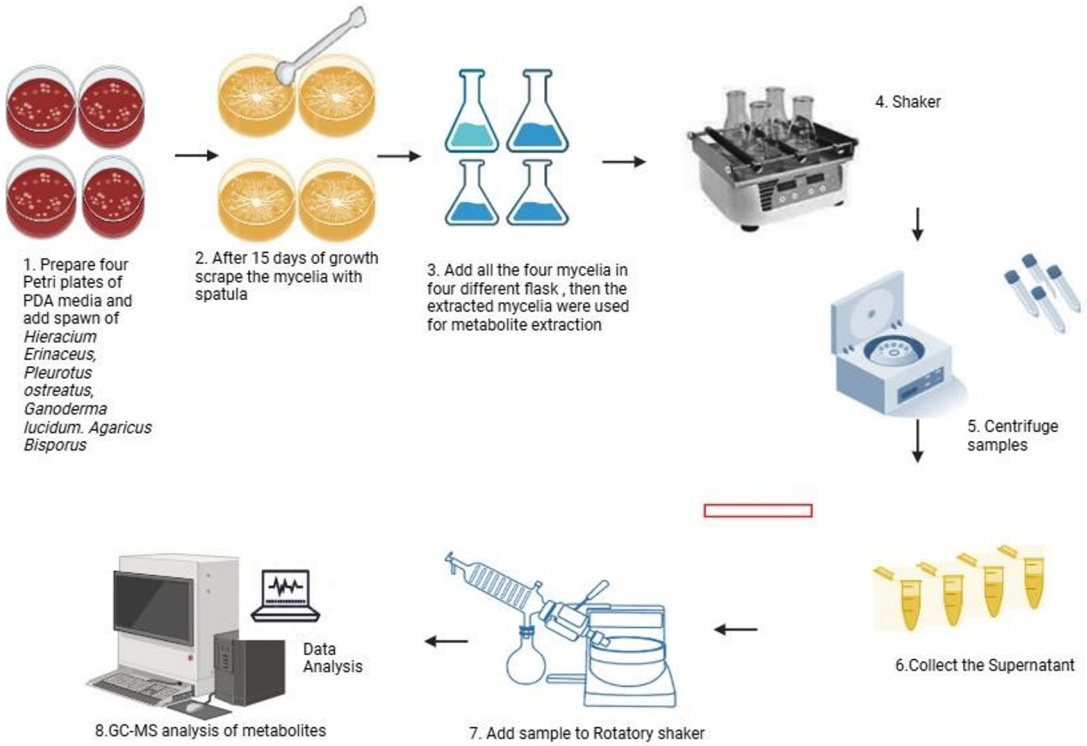
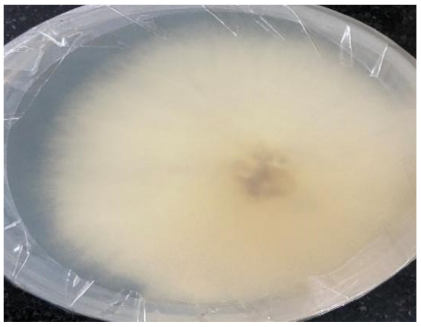
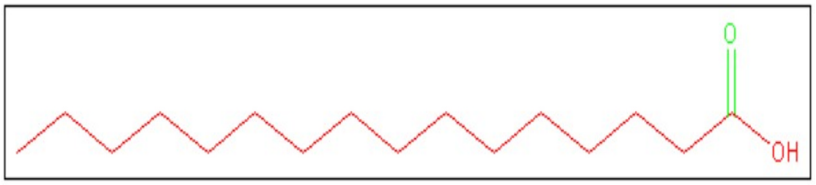
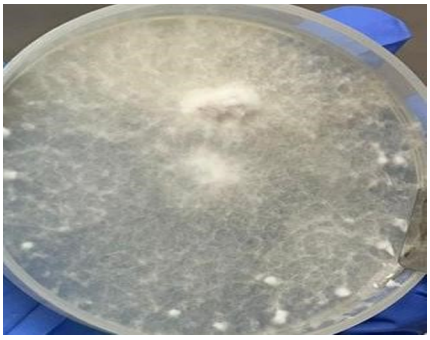
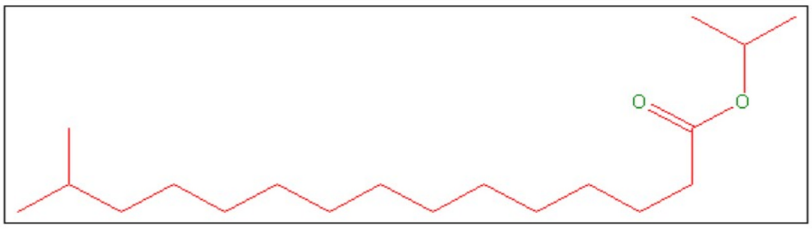


Table 1. Highest chemical compounds investigated in different mycelial growth

Growth of four mycelia on PDA	Chemical structure of highest bioactive compund present in the sample
 *Hericium Erinaceus*	 Octadecenoic acid Molecular Formula -$C_{18}H_{34}O_2$, Molecular Weight- 282.5 g/mol
 *Agaricus Bisporus*	 Hexadecanoic acid/ Palmitic acid Molecular Formula-$C_{16}H_{32}O_2$ Molecular Weight- 256.42 g/mol



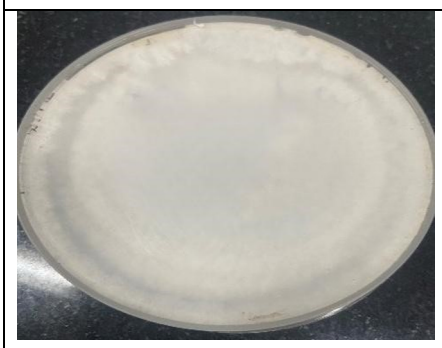
Ganoderma Lucidum



i-Propyl 14-methyl-pentadecanoate

Molecular Formula-$C_{19}H_{38}O_2$

Molecular Weight-298.5 g/mol



Pleurotus Ostreatus

CCCCC/C=C\C/C=C\CCCCCCCC(=O)O

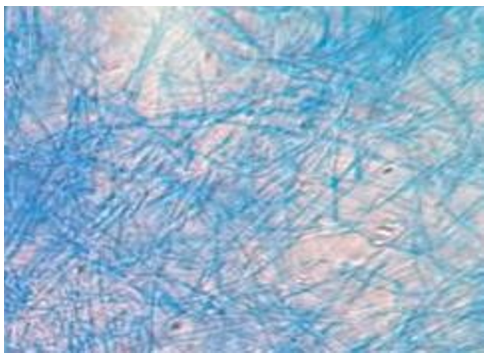
Linoleic acid ethyl ester

Molecular Formula-$C_{20}H_{36}O_2$

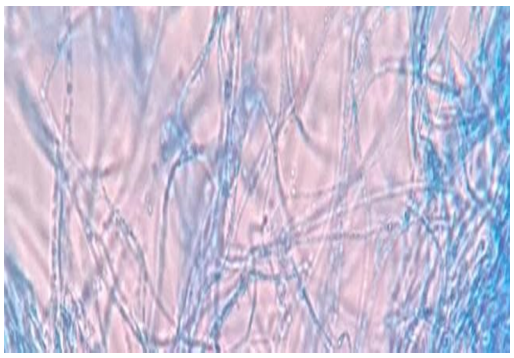
Molecular Weight-308.5 g/mol

Figure 1: Filamentous structure of all four different mycelia of *Ganoderma lucidum, Pleurotus ostreatus*, *Hieracium Erinaceus, Agaricus Bisporus*

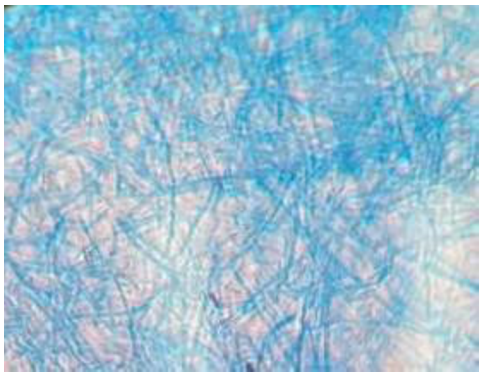
Morphological study of four mycelia under microscope



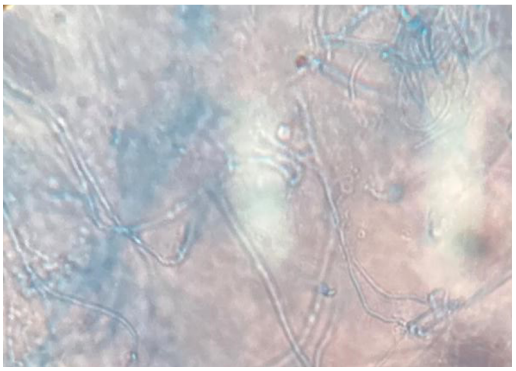
[A] ***Ganoderma Lucidum***



[B] ***Pleurotus Ostreatus***



[C] ***Hericium Erinaceus***



[D] ***Agaricus Bisporus***

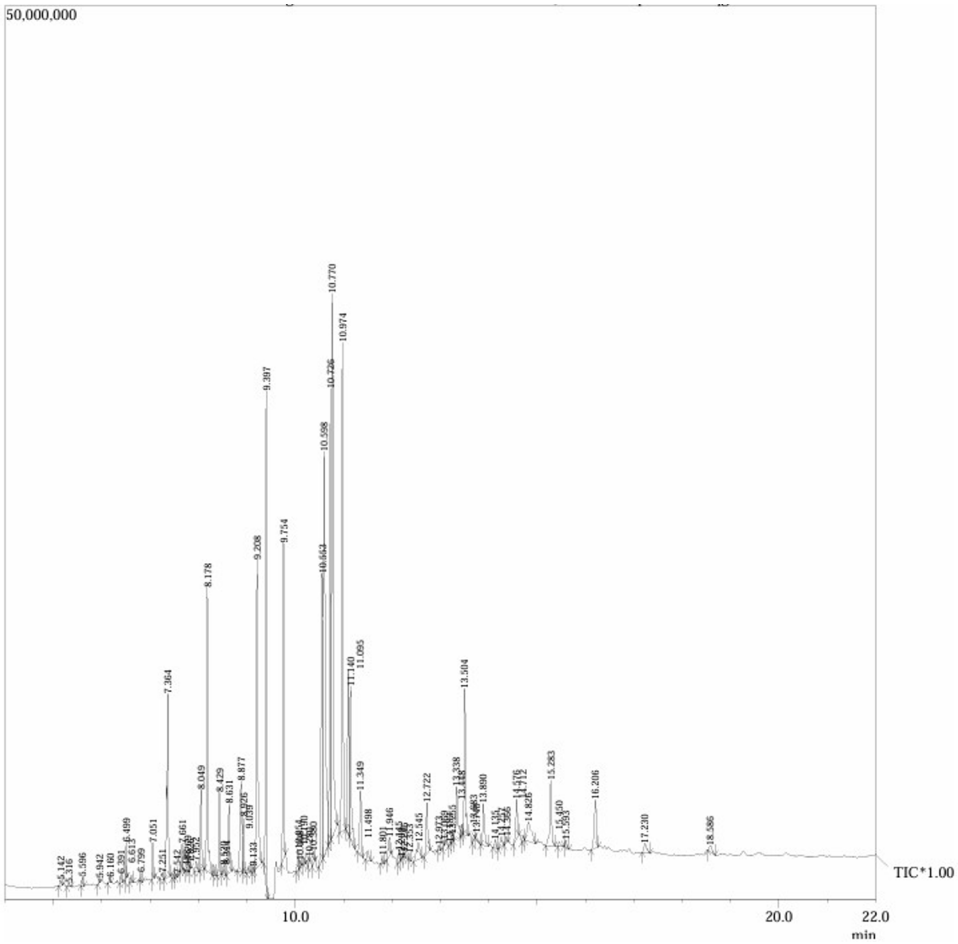


Figure 2: GC–MS chromatogram of *Hericium Erinaceus* mycelial extract showing the distribution of detected metabolites. Prominent peaks corresponding to major fatty acids and their derivatives, including linoleic acid, oleic acid, and their ethyl esters, indicate a high relative abundance of unsaturated fatty acid compounds.

Table 2. Peak report of *H. erinaceus* and its investigated Bioactive compounds

Peak	Retention Time	Area	Area%	Compound Name	Molecular formula	Molecular weight (g/mol)	Compound activity	References
1.	5.142	792781	0.12	Cyclopropane, nonyl-	$C_{12}H_{24}$	168.32	Antimicrobial, anti-viral	[34,35]
2.	5.316	435291	0.07	Cyclohexane, 1,1'-(1,2-dimethyl-1,2ethanediyl)bis-	$C_{16}H_{30}$	222.41	Antimicrobial	[34,36]
3.	5.596	809365	0.12	Cyclohexane, hexyl-	$C_{12}H_{24}$	168.32	Antibacterial, Anti-microbial	[34,36]
4.	5.942	374719	0.06	2-Undecanone	$C_{11}H_{22}O$	170.29	Antioxidant activity	[34,37]
5.	6.160	563709	0.09	Benzene, 1,4dimethoxy-2methyl-5-isopropyl-	$C_{12}H_{18}O_2$	194.27	Not reported	[34]
6.	6.391	926362	0.14	Dodecane, 6cyclohexyl-	$C_{18}H_{36}$	252.50	Not reported	[34]
7.	6.499	2518236	0.39	1-Tetradecene	$C_{14}H_{28}$	196.37	Anti-cancer, emollient	[34,63]
8.	6.613	570128	0.09	Cyclohexane, 1,2,4,5-tetraethyl-	$C_{14}H_{28}$	196.37	Antimicrobial	[34,36]
9.	6.799	499382	0.08	Cyclohexane, octyl-	$C_{14}H_{28}$	196.37	Antimicrobial	[34,36]
10.	7.051	2588391	0.40	Phenol, 2,4-bis(1,1dimethylethyl)-	$C_{14}H_{22}O$	206.32	Antitumor, anticancer, Hallucinogenic, Suppress osteoblast activity, Xanthine-oxidase inhibitor	[34,38]
11.	7.661	2497684	0.39	8-Pentadecanone	$C_{15}H_{30}O$	226.40	Anti-microbial	[34,42,65]

12.	7.952	1516197	0.23	Hexacosane	$C_{26}H_{54}$	366.70	Antibacterial, Antimicrobial	[34,40]
13.	8.049	7587307	1.17	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antioxidant	[34,43]
14.	8.178	29684166	4.58	Ethyl nonadecanoate	$C_{21}H_{42}O_2$	326.60	Anticancer	[34,44]
15.	8.429	7796243	1.20	Myristic acid, TMS derivative	$C_{17}H_{36}O_2Si$	300.54	Anticancer, Antifungal	[34,45]
16.	8.631	8266170	1.28	8-Octadecanone	$C_{18}H_{36}O$	268.50	antimicrobial and anti-inflammatory	[34,46]
17.	8.877	13851004	2.14	Acetamide derivative	$C_{14}H_{22}N_2O$	234.34	Not reported	[34]
18.	8.926	4908702	0.76	Oxaspiro compound	$C_{17}H_{24}O_3$	276.40	Anticancer, Antitumor	[34,53]
19.	9.039	841779	0.13	Isopropyl tetradecyl ether	$C_{17}H_{36}O$	256.50	Not reported	[34]
20.	9.208	40262263	6.21	Butyl undecyl ester	$C_{15}H_{30}O_2$	242.40	Anti-microbial, cosmetic	[34,47]
21.	9.397	43631910	6.73	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.50	Antiinflammatory, Hypocholesterolaemia, Hepatoprotective, Nematicide, Anticancer, Antitumor, Antihistaminic	[34,37]
22.	9.754	32177957	4.97	Palmitic acid, TMS derivative	$C_{19}H_{40}O_2Si$	328.60	Antiinflammatory	[34,48]
23.	10.108	475330	0.07	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364.70	Antimicrobial	[34,37]
24.	10.190	478047	0.07	Methyl octadecadienoate	$C_{19}H_{34}O_2$	294.50	Anticancer	[34,49]

25.	10.553	46338928	7.15	(9Z,12Z)-Octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$	280.45	Antiinflammatory, anti-cancer Hypocholesterolae mia,	[34,37]
26.	10.598	59914051	9.25	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.50	Antiinflammatory	[34,50]
27.	10.726	48060112	7.42	Ethyl linoleate	$C_{20}H_{36}O_2$	308.50	Antiarthritic Antieczemic, Antihistaminic Antiinflammatory, Antileukotriene-D4. Supports heart health	[34,41]
28.	10.770	67644720	10.44	Ethyl oleate (E)	$C_{20}H_{38}O_2$	310.50	Antiinflammatory	[34,51]
29.	10.974	53780507	8.30	Ethyl stearate	$C_{20}H_{40}O_2$	312.50	Anticancer, Antitumor	[34,52]
30.	11.095	18523348	2.86	Linoleic acid, TMS derivative	$C_{21}H_{40}O_2Si$	352.60	Antiinflammatory, anti-cancer Hypocholesterolae mia	[34,37]
31.	11.349	8566919	1.32	Stearic acid, TMS derivative	$C_{21}H_{46}O_2Si$	358.68	Anti-cancer, anticholesterol, Anti-bacterial	[34,53]
32.	11.498	1597578	0.25	1-Decanol, 2-octyl-	$C_{18}H_{38}O$	270.50	Antibacterial	[34,59]
33.	11.801	750492	0.12	Benzocyclodecene derivative	$C_{14}H_{26}$	194.36	Not reported	[34]
34.	11.946	3035277	0.47	Iron complex	$C_{21}H_{14}FeN_2O_3$	398.20	Fungicidal	[34,56]

35.	12.145	1285930	0.20	1-Tridecene	$C_{13}H_{26}$	182.35	Antibacterial activity	[34,57]
36.	12.246	478167	0.07	Pentatriacontane	$C_{35}H_{72}$	492.90	Antimicrobial, Antioxidant	[34,58]
37.	12.353	552077	0.09	1-Decanol, 2-octyl-	$C_{18}H_{38}O$	270.50	Antibacterial	[34,59]
38.	12.722	6552678	1.01	Ethyl 14-methylhexadecanoate	$C_{19}H_{38}O_2$	298.50	Not reported	[34]
39.	12.973	496521	0.08	Arachidic acid, TMS derivative	$C_{23}H_{48}O_2Si$	384.70	Antiinflammatory	[34,60]
40.	13.069	547444	0.08	Benzphetamine	$C_{17}H_{21}N$	239.35	Not reported	[34]
41.	13.338	8170185	1.26	Tritetracontane	$C_{43}H_{88}$	604.20	Not reported	[34]
42.	13.504	15839496	2.44	Ammonium benzoate derivative	$C_{28}H_{34}N_2O_3$	446.60	Antimicrobial	[34,61]
43.	13.683	682187	0.11	Hexadecanoic acid derivative	$C_{23}H_{48}O_3Si$	400.70	Antioxidant	[34,36]
44.	13.890	5430603	0.84	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368.60	Hallucinogenic, antitumor	[34,62]
45.	14.135	1048109	0.16	Behenic acid, TMS derivative	$C_{25}H_{52}O_2Si$	412.80	Antiinflammatory	[34]

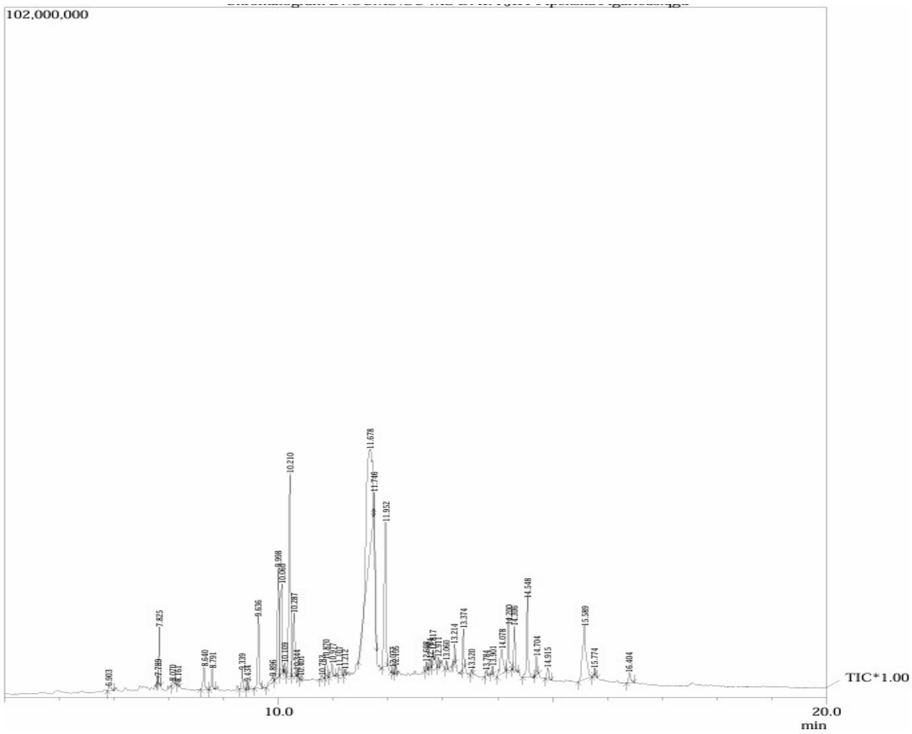


Figure 3: GC–MS chromatogram of *Pleurotus Ostreatus* mycelial extract illustrating a diverse metabolite profile. The chromatogram is characterized by dominant peaks of fatty acid esters and long-chain hydrocarbons, suggesting a rich composition of bioactive lipid-derived compounds.

Table 3: Showing to Gas Chromatography-Mass Spectrometry results of *Pleurotus Ostreatus*

Peak	**Retention Time**	**Area**	**Area%**	**Compound Name**	**Molecular formula**	**Molecular weight (g/mol)**	**Compound activity**	**References**
1.	6.903	2237853	0.40	1-Tetradecene	$C_{14}H_{28}$	196.37	Anti-cancer, emollient	[34,63]
2.	7.789	420650	0.08	1-Hexadecene	$C_{16}H_{32}$	224.42	Anti-microbial, anti-oxidant	[34,64]
3.	8.070	1147438	0.21	Ethyl α-Dglucopyranoside	$C_8H_{16}O_6$	208.21	Not reported	[34]
4.	8.161	622449	0.11	8-Pentadecanone	$C_{15}H_{30}O$	226.40	Anti-microbial	[34,42,65]
5.	8.640	6542756	1.17	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antioxidant	[34,43]
6.	8.791	5780888	1.04	1-Hexadecene	$C_{16}H_{32}$	224.42	Anti-microbial, anti-oxidant	[34,64]
7.	9.339	4548461	0.81	8-Octadecanone	$C_{18}H_{36}O$	268.50	antimicrobial and anti-inflammatory	[34,46]
8.	9.434	1408332	0.25	Ethyl pentadecanoate	$C_{17}H_{34}O_2$	270.45	Not reported	[34,38]
9.	9.636	21249178	3.81	Acetamide derivative	$C_{14}H_{22}N_2O$	234.34	Not reported	[34]
10	9.896	3968823	0.71	3-Dodecanol, 3,7,11trimethyl-	$C_{15}H_{32}O$	228.41	Not reported	[34]
11	10.060	36374004	6.52	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Anti-inflammatory, Hypocholesterolaemia, Hepatoprotective, Nematicide, Anticancer, Antitumor, Antihistaminic	[34,36]

12	10.109	1324009	0.24	Ethyl hexadecenoate	$C_{18}H_{34}O_2$	282.50	T-cell stimulant, anticancer, antitumor, cosmetic, collagensparing	[34]
13	10.210	72254205	12.95	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284.50	Anti-inflammatory, Hypocholesterolaemia, Hepatoprotective, Nematicide, Anticancer, Antitumor, Antihistaminic	[34,36]
14	10.287	21434418	3.84	Butanoic acid derivative	$C_{20}H_{32}O_3$	320.50	Bactericide, Chronotropic AChE-Inhibitor	[34]
15	10.344	901633	0.16	2-Heptadecanol	$C_{17}H_{36}O$	256.50	Not reported	[34]
16	10.401	660126	0.12	Pentadecyl trifluoroacetate	$C_{17}H_{31}F_3O_2$	324.40	Not reported	[34]
17	10.783	1075963	0.19	Ethyl (9Z,12Z)octadecadienoate	$C_{20}H_{36}O_2$	308.50	Not reported	[34]
18	10.870	5590939	1.00	10-Nonadecanone	$C_{19}H_{38}O$	282.50	Not reported	[34]
19	10.977	4475291	0.80	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.50	Not reported	[34]
20	11.107	4191901	0.75	Octacosanol	$C_{28}H_{58}O$	410.80	Not reported	[34]
21	11.212	1374774	0.25	Octadecyl trifluoroacetate	$C_{20}H_{37}F_3O_2$	366.50	Not reported	[34]

22	11.678	112581999	20.17	Ethyl linoleate	$C_{20}H_{36}O_2$	308.50	Antiarthritic Antieczemic, Antihistaminic, Anti-inflammatory, Antileukotriene-D4, Supports heart health	[34,41]
23	11.746	125954	0.02	Ethyl oleate	$C_{20}H_{38}O_2$	310.50	Not reported	[34]
24	11.952	55286075	9.91	Ethyl stearate	$C_{20}H_{40}O_2$	312.50	Antioxidant, Anticancer	[34,53]
25	12.077	1232115	0.22	Hexacosyl propyl ether	$C_{29}H_{60}O$	424.80	Not reported	[34]
26	12.135	1596299	0.29	Cyclooctane, (methoxymeth oxy)-	$C_{10}H_{20}O_2$	172.26	Not reported	[34]
27	12.698	1531103	0.27	Indene derivative	$C_{25}H_{48}$	348.60	Not reported	[34]
28	12.764	1702112	0.30	Heptadecanoli de	$C_{17}H_{32}O_2$	268.40	Not reported	[34]
29	12.817	2488146	0.45	1-Nonadecene	$C_{19}H_{38}$	266.50	Not reported	[34]
30	12.911	1808719	0.32	Octadecyl trifluoroacetate	$C_{20}H_{37}F_3O_2$	366.50	Not reported	[34]
31	13.060	1915639	0.34	2-Methyltetracos ane	$C_{25}H_{52}$	352.70	Not reported	[34]
32	13.214	3086124	0.55	Ethyl oleate (E)	$C_{20}H_{38}O_2$	310.50	Anti-inflammatory	[34,51]
33	13.374	9330342	1.67	Ethyl docosanoate	$C_{24}H_{48}O_2$	368.60	Hallucinogenic, antitumor	[34,62]
34	13.520	831001	0.15	2-Heptadecanol	$C_{17}H_{36}O$	256.50	Not reported	[34]
35	13.784	1505956	0.27	Benzeneethan amine derivative	$C_{13}H_{18}ClN$	223.74	Not reported	[34]

36	14.078	14914269	2.67	17-Pentatriaconte ne	$C_{35}H_{70}$	490.90	Anti-cancer, antiinflammatory	[34,66]
37	14.200	15946549	2.86	Hexadecanoic acid glycerol ester	$C_{19}H_{38}O_4$	330.50	Anti-inflammatory	[34,36]
38	14.548	23444243	4.20	Ethyl erucate	$C_{24}H_{46}O_2$	366.60	Not reported	[34]
39	14.704	4486389	0.80	Ethyl docosanoate	$C_{24}H_{48}O_2$	368.60	Hallucinogenic, antitumor	[34,62]
40	14.915	3498760	0.63	1,16-Dichlorohexad ecane	$C_{16}H_{32}Cl_2$	295.30	Pesticide	[34,67]
41	15.589	36786410	6.59	Oleoyl chloride	$C_{18}H_{33}ClO$	300.90	Antimicrobial	[34,68]
42	15.774	2613435	0.47	Glyceryl stearate	$C_{21}H_{42}O_4$	358.60	Antimicrobial, Anti-cancer	[34,52]
43	16.404	3992980	0.72	Ethyl tetracosanoate	$C_{26}H_{52}O_2$	396.7	Antimicrobial	[34]

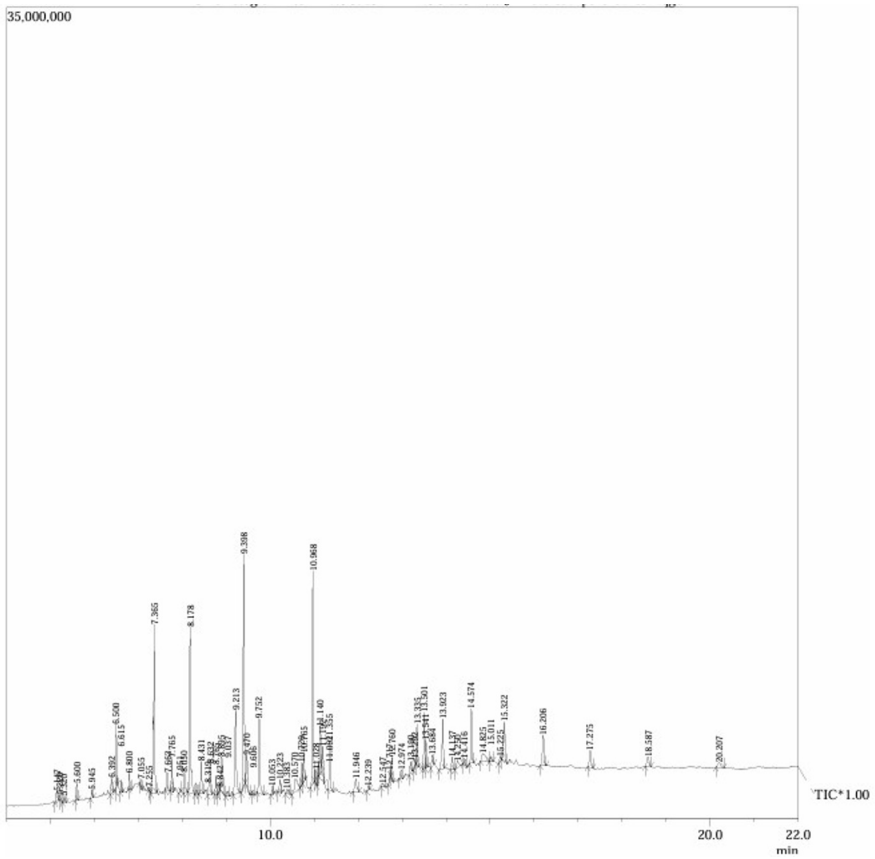


Figure 4: Gas Chromatography-Mass Spectrometry Chromatogram of ethanolic extract of *Ganoderma lucidum*

Table.4: GC–MS chromatogram of *Ganoderma lucidum* mycelial extract showing the presence of key bioactive compounds. Notable peaks represent fatty acids, esters, and phenolic derivatives, reflecting moderate metabolite diversity with emphasis on compounds associated with antioxidant and antimicrobial activity.

Peak	Retention Time	Area	Area%	Compound Name	Molecular formula	Molecular weight (g/mol)	Compound activity	References
1.	5.147	1108220	0.65	1-Dodecene	$C_{12}H_{24}$	168.32	Not reported	[34]
2.	5.220	326549	0.19	Dodecane	$C_{12}H_{26}$	170.33	Not reported	[34]
3.	5.320	580456	0.34	Cyclohexane derivative	$C_{16}H_{30}$	222.41	Antimicrobial	[34,36]
4.	5.600	1125265	0.66	Cyclohexane	C_6H_{12}	84.16	Antimicrobial	[34,36]

5.	5.945	558349	0.33	2-Undecanone	$C_{11}H_{22}O$	170.29	Antioxidant activity	[34,37]
6.	6.392	1169874	0.69	Dodecane, 6cyclohexyl-	$C_{18}H_{36}$	252.50	Not reported	[34]
7.	6.500	3223434	1.89	1-Tetradecene	$C_{14}H_{28}$	196.37	Anti-cancer, emollient	[34,63]
8.	6.615	656731	0.39	Cyclohexane, tetraethyl-	$C_{14}H_{28}$	196.37	Antimicrobial	[34,36]
9.	6.800	746813	0.44	Cyclohexane, octyl-	$C_{14}H_{28}$	196.37	Antimicrobial	[34,36]
10.	7.055	381568	0.22	Tridecyl fluorobenzoate derivative	$C_{21}H_{30}F_4O_2$	390.50	Not reported	[34]
11.	7.663	1489031	0.87	8-Pentadecanone	$C_{15}H_{30}O$	226.40	Anti-microbial	[34,42,65]
12.	7.765	2095690	1.23	Heptadecane	$C_{17}H_{36}$	240.50	Not reported	[34]
13.	8.050	1717755	1.01	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antioxidant	[34,43]
14.	8.178	13353451	7.84	Ethyl nonadecanoate	$C_{21}H_{42}O_2$	326.60	Anticancer	[34,44]
15.	8.431	2377706	1.40	Myristic acid, TMS derivative	$C_{17}H_{36}O_2Si$	300.54	Anticancer, Anti-fungal	[34,45]
16.	8.632	2978040	1.75	2-(Octadecyloxy)ethanol	$C_{20}H_{42}O_2$	314.50	Anti-microbial	[34,69]
17.	8.765	1150193	0.68	Indole, TMS derivative	$C_{13}H_{19}NSi$	217.38	Not reported	[34]
18.	8.895	4135965	2.43	Acetamide derivative	$C_{14}H_{22}N_2O$	234.34	Anesthetic	[34,70]
19.	9.213	8606989	5.05	Phthalate ester (butyl octyl)	$C_{20}H_{30}O_4$	334.40	Anti-microbial	[34,71]
20.	9.398	17542953	10.30	Isopropyl methyl-pentadecanoate	$C_{19}H_{38}O_2$	298.50	Anti-fungal	[34,72]
21.	9.470	2728557	1.60	Phenolic acrylate derivative	$C_{21}H_{32}O_3$	332.50	Antioxidant	[34,73]

22.	9.752	6528727	3.83	Palmitic acid, TMS derivative	$C_{19}H_{40}O_2Si$	328.60	Antiinflammatory	[34,48]

23.	10.053	700937	0.41	10-Nonadecanone	$C_{19}H_{38}O$	282.50	Not reported	[34]
24.	10.223	1068949	0.63	Heneicosane	$C_{21}H_{44}$	296.60	Not reported	[34]
25.	10.383	764296	0.45	Octadecyl carbonic ester	$C_{21}H_{40}O_3$	340.50	Antioxidant	[34,37]
26.	10.570	2934970	1.72	(Z)-Octadec-9en-1-ol	$C_{18}H_{36}O$	268.50	Emollient, Surfactant	[34,38]
27.	10.720	1929901	1.13	Propyl linoleate	$C_{21}H_{38}O_2$	322.50	Antiinflammatory	[34,37]
28.	10.765	2119562	1.24	Ethyl oleate (E)	$C_{20}H_{38}O_2$	310.50	Antiinflammatory	[34,51]
29.	10.968	16188040	9.51	Methyl stearate derivative	$C_{20}H_{40}O_2$	312.50	Anticancer	[34,52]
30.	11.028	768834	0.45	Docosane	$C_{22}H_{46}$	310.60	Not reported	[34]
31.	11.140	3436906	2.02	Oleic acid	$C_{18}H_{34}O_2$	282.50	Anticancer, Antihistaminic	[34,39]
32.	11.192	1750459	1.03	Oleic acid, TMS derivative	$C_{21}H_{42}O_2Si$	326.63	Anticancer, Antihistaminic	[34,39]
33.	11.355	4439852	2.61	Stearic acid, TMS derivative	$C_{21}H_{46}O_2Si$	358.68	Anti-cancer, anti-cholesterol, Anti-bacterial	[34,53]
34.	11.946	1782845	1.05	Heneicosane	$C_{21}H_{44}$	296.60	Not reported	[34]
35.	12.239	338673	0.20	Methoxyphenyl acrylate ester	$C_{18}H_{26}O_3$	290.40	Antiandrogenic activity	[34,54]
36.	12.547	472856	0.28	Dimethyl decanol	$C_{12}H_{26}O$	186.33	Antibacterial	[34,55]
37.	12.717	695474	0.41	Ethyl tetradecanoate	$C_{16}H_{32}O_2$	256.42	Antioxidant	[34,43]
38.	12.760	1177354	0.69	Nonadecane	$C_{19}H_{40}$	268.50	Not reported	[34]

39.	12.974	671681	0.39	Arachidic acid, TMS derivative	$C_{23}H_{48}O_2Si$	384.70	Antiinflammatory	[34,60]
40.	13.190	970673	0.57	Monopalmitin, di-TMS derivative	$C_{25}H_{54}O_4Si_2$	474.90	Anti-cancer	[34]
41.	13.292	543331	0.32	Decane bis-TMS derivative	$C_{16}H_{38}O_2Si_2$	318.64	Not reported	[34]
42.	13.335	2688639	1.58	Pentacosane	$C_{25}H_{52}$	352.70	Not reported	[34]
43.	13.501	4022394	2.36	Ammonium benzoate derivative	$C_{28}H_{34}N_2O_3$	446.60	Antimicrobial	[34,61]
44.	13.541	1676766	0.98	Phthalic acid	$C_8H_6O_4$	166.13	Anti-microbial	[34,37]
45.	13.684	1128245	0.66	Palmitate TMS derivative	$C_{22}H_{46}O_3Si$	386.70	Not reported	[34]
46.	13.923	4420576	2.60	Pentacosane	$C_{25}H_{52}$	352.70	Not reported	[34]
47.	14.137	1132602	0.67	Behenic acid, TMS derivative	$C_{25}H_{52}O_2Si$	412.80	Antiinflammatory	[34]
48.	14.416	396012	0.23	Dioxolane derivative	$C_{12}H_{21}NO_4$	243.30	Not reported	[34]
49.	14.574	4302176	2.53	Pentacosane	$C_{25}H_{52}$	352.70	Not reported	[34]
50.	14.825	1815666	1.07	Oleamide derivative	$C_{18}H_{35}NO$	281.50	Antioxidant	[34,37]
51.	15.011	549109	0.32	Glycerol ester, di-TMS derivative	$C_{29}H_{62}O_4Si_2$	531.00	Not reported	[34]
52.	15.225	403261	0.24	Oleamide	$C_{18}H_{35}NO$	281.50	Antioxidant	[34]

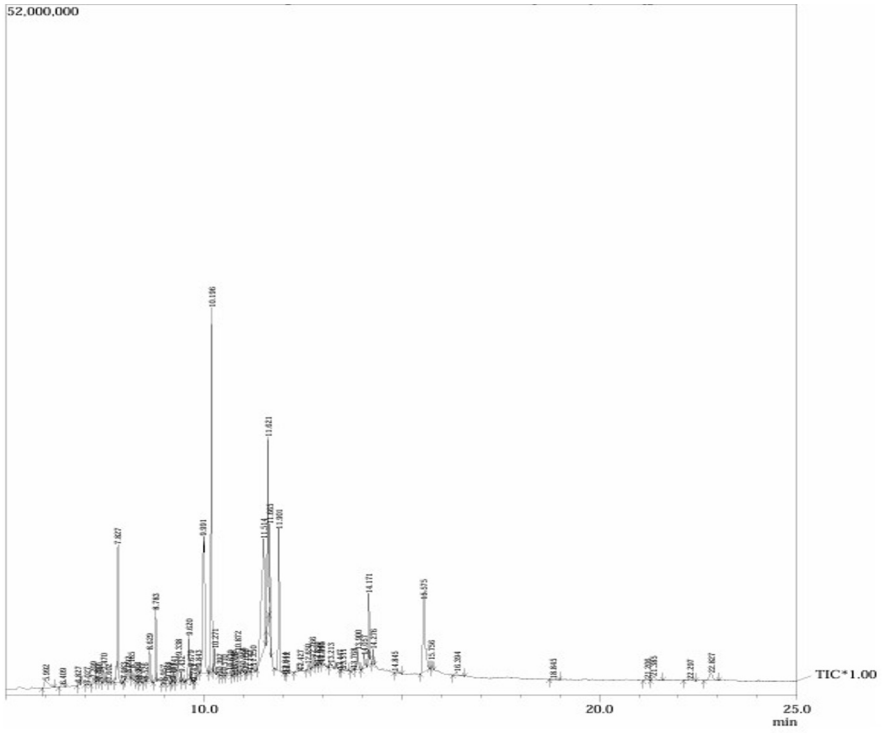


Figure 5: GC–MS chromatogram of *Agaricus bisporus* mycelial extract highlighting comparatively fewer and lower-intensity peaks. The chromatographic profile indicates relatively lower metabolite diversity and abundance, with detectable fatty acids and their derivatives as the primary constituents.

Table 5: Gas Chromatography-Mass Spectrometry results of *Agaricus Bisporus*

Peak	Retention Time	Area	Area%	Compound Name	Molecular formula	Molecular weight (g/mol)	Compound activity	References
1.	5.992	4381897	1.39	*5-Hydroxymethylfurfural*	$C_6H_6O_3$	*126.11 g/mol*	HMG-CoAinhibitor, Improve cerebral Hypoxia	[34]
2.	6.827	933761	0.13	*Nonane, 1,1-Diethoxy-*	$C_{13}H_{28}O$	*216.36 g/mol*	Antitumor, Anticancer, calcium antagonist	[34]

3.	7.027	413323	0.06	*1,7-Dimethyl-4(1-Methylethyl) Cyclodecane*	$C_{15}H_{30}$	*210.40 g/mol*	5-HT-Inhibitor , Antidote (Cadmium), Hallucinogenic, Increase T-helper cells	[34]
4.	7.209	175228	0.40	*2-Pentyl-Cyclopentanone*	$C_{10}H_{18}O$	*154.25 g/mol*	HMG-CoA-inhibitor, Anti-HIV-Integrase, Histamine inhibitor	[34]
5.	7.297	1261898	0.03	*4-Decenal, (E)-*	$C_{10}H_{18}O$	*154.25 g/mol*	Acetyl-cholineantagonist, HMG-CoAinhibitor	[34]
6.	7.350	253848	0.08	*Decane*	$C_{10}H_{22}$	*142.28 g/mol*	Narcotic, Cosmetic	[34]
7.	7.470	1674591	0.53	*Phenol, 2,4-Bis(1,1Dimethylethyl)-*	$C_{14}H_{22}O$	*206.32 g/mol*	Antitumor, anticancer, Hallucinogenic, Suppress osteoblast activity, Xanthine-oxidase inhibitor	[34,38]
8.	7.602	225985	0.07	*Cyclandelate*	$C_{17}H_{24}O_3$	*276.4 g/mol*	Anticancer (Cervix), Antidote (Heavy Metals), Antitumor (Colon)	[34]
9.	8.092	1720164	0.54	*Beta. -D-Glucopyranoside, Methyl*	$C_{29}H_{32}O_{13}$	*588.6 g/mol*	Increase T helper cells, low oxalate, Ovulation stimulant, anticancer	[34]
10.	8.165	1064036	0.34	*8-Pentadecanone*	$C_{15}H_{30}O$	*226.40 g/mol*	Anti-microbial	[34,42,65]

11.	8.301	430771	0.14	*Eicosane*	$C_{20}H_{42}$	*282.5 g/mol*	Acetyl-cholineantagonist, Antit	[34]
							umor, CNS-Suppressant	
12.	8.364	432038	0.14	*Behenic Alcohol*	$C_{22}H_{46}O$	*326.6 g/mol*	Allogenic. Antit umor Anti-cAMP-Phosphodiesteras e, Anticancer	[34]
13.	8.526	416364	0.13	*Hexadecane*	$C_{16}H_{34}$	*226.44 g/mol*	Antioxidant, pesticide, Anticancer, Cholesterolytic	[34]
14.	8.629	4435570	1.40	*Tetra Decanoic Acid*	$C_{14}H_{28}O_2$	*228.37 g/mol*	Antitumor, Anticancer, cosmetic, CNS stimulant	[34]
15.	8.783	9067478	2.87	*EthylPentadecanoate*	$C_{17}H_{34}O_2$	*270.5 g/mol*	Antitumor,Anticancer, Colorant, Anti-cAMP-Phosphodiesteras e	[34]
16.	8.957	307962	0.10	*Sulfurous Acid,Octadecyl 2-PropylEster*	$C_{21}H_{44}O_3S$	*376.6 g/mol*	Hallucinogenic, Anticancer	[34]
17.	9.088	1246275	0.39	*2-Methylhexacosane*	$C_{27}H_{56}$	*380.7 g/mol*	Anticancer, Antitumor	[34]
18.	9.184	352600	0.11	*Octadecanoic Acid,Ethyl Ester*	$C_{20}H_{40}O_2$	*312.5 g/mol*	Anticancer, Antitumor	[34,53]
19.	9.241	989741	0.31	*Ethyl Nonadecanoate*	$C_{21}H_{42}O_2$	*326.6 g/mol*	Anticancer	[34,44]
20.	9.338	3929981	1.24	*8-Octadecanone*	$C_{18}H_{36}O$	*268.5 g/mol*	antimicrobial and anti-inflammatory	[34,46]

21.	9.432	1193638	0.38	*Pentadecanoic Acid,Ethyl Ester*	$C_{17}H_{34}O_2$	*270.5 g/mol*	Anti-cAMP-Phosphodiesteras e, Anticancer, Antitumor	[34,37,38]
22.	9.620	6631020	2.10	*Acetamide, 2-(Diethylamino)N-(2,6-Dimethyl*	$C_{14}H_{22}N_2O$	*234.34 g/mol*	Cationic, antidemocraticcancer	[34]
23.	9.679	1282693	0.41	*7,9-Di-Tert-Butyl-1-Oxaspiro (4,5) Deca-6,9-Diene-2,8-Dione*	$C_{17}H_{24}O_3$	*276.4 g/mol*	Anticancer, Antitumor	[34]
24.	9.843	1950012	0.62	*Palmitoleic Acid*	$C_{16}H_{30}O_2$	*254.41 g/mol*	Anti-5-HT, Anticancer, Antitumor,	[34]
25.	9.991	36361007	11.50	*Butyl Undecyl Ester*	$C_{23}H_{36}O_4$	*376.5 g/mol*	Anti-microbial, cosmetic	[34,47]
26.	10.196	48371681	15.29	*Hexadecanoic Acid,Ethyl Ester*	$C_{18}H_{36}O_2$	*284.5 g/mol*	Antiinflammatory, Hypocholesterolaemia, Hepatoprotective, Nematicide, Anticancer, Antitumor, Antihistaminic	[34,36]
27.	10.271	2942460	0.93	*2-(2,4-DitertPentylphenoxy) Butanoic Acid #*	$C_{20}H_{32}O_3$	*320.5 g/mol*	Anticancer, Antitumor, Antihistaminic	[34]
28.	10.397	205131	0.06	*9-Octadecenoic Acid(Z)-*	$C_{18}H_{34}O_2$	*282.5 g/mol*	Anticancer, Antitumor, Antihistaminic, HMG-COA inhibitor	[34]
29.	10.510	115471	0.04	*2-Ethylhexyl 2-Ethylhexanoate*	$C_{16}H_{32}O_2$	*256.42 g/mol*	Calcium-Antagonist	[34]

30.	10.670	1418128	0.45	*Octadecanoic Acid,Ethyl Ester*	$C_{20}H_{40}O_2$	*312.5 g/mol*	Anticancer, Antitumor, Antihistaminic	[34,53]
31.	10.746	548201	0.17	*Heptadecanoic Acid,Ethyl Ester*	$C_{19}H_{38}O_2$	*298.5 g/mol*	Antiinflammatory, Anticancer	[34]
32.	10.793	199744	0.06	*(Z)-Ethyl Heptadec-9-Enoate*	$C_{19}H_{36}O_2$	*296.5 g/mol*	Anticancer, Antitumor, Antihistaminic, Antiinflammatory, HMG COA Inhibitor	[34]
33.	10.872	2915285	0.92	*Ethyl 9-Hexadecenoate*	$C_{18}H_{34}O_2$	*282.5 g/mol*	T-cell stimulant, anticancer, antitumor, cosmetic, collagen-sparing	[34]
34.	10.968	824530	0.26	*Docosanoic Acid,Ethyl Ester*	$C_{24}H_{48}O_2$	*368.6 g/mol*	Hallucinogenic, antitumor	[34,62]
35.	11.056	344522	0.11	*6-OctadecenoicAcid , Methyl Ester,(Z)-*	$C_{19}H_{36}O_2$	*296.5 g/mol*	Hallucinogenic, antitumor. Anti-inflammatory, Anticancer	[34]
36.	11.147	253439	0.08	*Hexadecyl NonylEther*	$C_{25}H_{52}O$	*368.7 g/mol*	Cosmetic, antiinflammatory	[34]
37.	11.250	1632881	0.52	*Tetratriacontane*	$C_{34}H_{70}$	*478.9 g/mol*	Anticancer, Antitumor, Anti-5-HT	[34]
38.	11.514	43819798	13.85	*Oleic Acid*	$C_{18}H_{34}O_2$	*282.5 g/mol*	Anticancer, Antihistaminic	[34]
39.	11.621	25017623	7.91	*Linoleic Acid EthylEster*	$C_{20}H_{36}O_2$	*308.5 g/mol*	Antihistaminic, Antiinflammatory, Antileukotriene-D4 ,Supports heart health	[34,41]

40.	11.665	10565173	3.34	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5 g/mol	HMG-CoA Inhibitor, Improve Cerebral Hypoxia, Suppress HMG-CoA Reductase Activity	[34]
41.	11.901	20387310	6.45	Octadecanoic Acid,Ethyl Ester	$C_{20}H_{40}O_2$	312.5 g/mol	Antioxidant, Antihistaminic Anti-cancer	[34,53]
42.	12.044	19800	0.01	Sulfurous Acid,Octadecyl 2-PropylEster	$C_{21}H_{44}O_3S$	376.6 g/mol	Not reported	[34]
43.	12.112	160853	0.05	2-Tetradecanol	$C_{14}H_{30}O$	214.39 g/mol	Antibacterial	[34]
44.	12.650	874177	0.28	Ethyl 9-Hexadecenoate	$C_{18}H_{34}O_2$	282.5 g/mol	T-cell stimulant, anticancer, antitumor, cosmetic, collagen-sparing	[34]
45.	12.766	1544969	0.49	Glycidyl Palmitate	$C_{19}H_{36}O_3$	312.5 g/mol	Not reported	[34]
46.	12.850	354024	0.11	Tetradecanoic Acid	$C_{14}H_{28}O_2$	228.37 g/mol	Antioxidant	[34,43]
47.	12.945	493150	0.16	DodecanoylChlori de	$C_{12}H_{23}ClO$	218.76 g/mol	Antimicrobial	[34]
48.	12.995	677205	0.21	2-Methylhexacosane	$C_{27}H_{56}$	380.7 g/mol	Anticancer, antitumor	[34]
49.	13.213	1396255	0.44	9-Octadecenamide	$C_{18}H_{35}NO$	281.5 g/mol	Antioxidant	[34]
50.	13.447	13672	0.00	3,7-Undecanedione,6, 6,10-Trimethyl-	$C_{14}H_{26}O_2$	226.35 g/mol	Not reported	[34]
51.	13.511	672460	0.21	1-Docosanol, MethylEther	$C_{23}H_{48}O$	340.6 g/mol	Not reported	[34]

52.	13.768	296811	0.09	Benzeneethanamine,N,.Alpha.-Dimethyl-N	$C_{13}H_{18}ClN$	223.74 g/mol	Not reported	[34]
53.	14.057	3110741	0.98	Cis-9-HexadecenoicAci d, Heptyl Ester	$C_{23}H_{44}O_2$	352.6 g/mol	Antiinflammatory	[34,37]
54.	14.171	6367798	2.01	Hexadecanoic Acid,2-Hydroxy-1-(Hydroxymethyl) Ethyl Ester	$C_{19}H_{38}O_4$	330.5 g/mol	Antiinflammatory	[34,36]
55.	14.845	703793	0.22	Hexadecanoic Acid,1(Hydroxymethyl)-1,2-Ethanediyl Ester	$C_{35}H_{68}O_5$	568.9 g/mol	Antiinflammatory	[34,36]
56.	11.901	23619843	7.47	9-Octadecenoic Acid(Z)-, 2,3-Dihydroxypropyl Ester	$C_{21}H_{40}O_4$	356.5 g/mol	Anticancer, Antitumor, Antihistaminic, HMG-COA inhibitor	[34,39]
57.	15.756	1477673	0.47	Octadecanoic Acid,2,3-Dihydroxypropyl Ester	$C_{21}H_{42}O_4$	358.6 g/mol	Antimicrobial	[34,52]
58.	16.394	986624	0.31	(E)-9-OctadecenoicAcid Ethyl Ester	$C_{20}H_{38}O_2$	310.5 g/mol	Antiinflammatory	[34,51]
59.	18.845	362774	0.11	Ethyl Docosanoate	$C_{24}H_{48}O_2$	368.6 g/mol	Not reported	[34,40]
60.	21.206	692412	0.22	Alpha. -TocopherylAcetate	$C_{29}H_{50}O_2$	430.7 g/mol	Not reported	[34]
61.	21.385	1686255	0.53	Ergosta-4,7,22-Trien-3. Beta. -Ol	$C_{28}H_{44}O$	396.6 g/mol	Antiinflammatory	[34,52]

62.	22.827	3244927	1.03	Ergosta-4,7,22-Trien-3. Beta. -Ol	$C_{28}H_{44}O$	396.6 g/mol	Antiinflammatory	[34,52,75]

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