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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM PLEUROTUS OSTREATUS

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Abstract

In this study, the ethyl acetate extract of *Pleurotus ostreatus* mushroom was analyzed using gas chromatography–mass spectrometry (GC–MS, EI ionization). The main objective of the study was to identify lipophilic secondary metabolites in the mushroom and evaluate their structural properties. According to the results of GC–MS analysis, three main bioactive components were identified in the extract – ethyl linoleate, ergosta-3,5,7,22-tetraene (22E) and ergosta-5,7,22-trien-3-ol acetate, and their molecular ions and fragmentation patterns were confirmed to match the database. Ethyl linoleate was characterized by the McLafferty rearrangement characteristic of fatty acid ethyl esters, while sterol derivatives were identified by fragment ions characteristic of the ergosterol skeleton. The results obtained show that the ethyl acetate fraction of *P. ostreatus* is rich in bioactive sterols and fatty acid esters, allowing this mushroom to be evaluated as a promising raw material for the development of pharmaceutical, nutraceutical, and functional food products.

Keywords: *Pleurotus ostreatus*, GC–MS, ethyl linoleate, ergosterol derivatives, secondary metabolites

Introduction

In recent years, the *Pleurotus ostreatus* mushroom has been widely studied as a natural resource with high nutritional and pharmacological value. While its primary metabolites (amino acids, vitamins, lipids, and carbohydrates) enhance its nutritional value, secondary metabolites (phenolic compounds, terpenoids, flavonoids, and polysaccharides) determine the pharmacological properties of the mushroom, including antioxidant, antibacterial, and antifungal activities (Zhang et al., 2020; Patel et al., 2019). Recently iso-

lated terpenoid compounds, such as pleurotusin A and pleurotusin B, have been shown to have potent antioxidant and antibacterial properties (Singh et al., 2022). Metabolism studies have shown that genes in *P. ostreatus* cells are associated with regulators responsible for polysaccharide synthesis and cellulose degradation (Chen et al., 2022). In addition, salicylic acid activates stress response mechanisms and stimulates the synthesis of antibiotics, polyketones, and other bioactive substances (Kumar et al., 2021). Heat stress also significantly alters fungal metabolism.

Studies have shown that heat stress increases the content of amino acids, lipids, vitamins, and carbohydrates in the mycelium, and also stimulates the synthesis of metabolites associated with stress tolerance (Wang & Zhang, 2021). At the same time, heat leads to an increase in the amount of more than 130 metabolites in mushrooms, including substances that stimulate the growth of beneficial microorganisms such as *Trichoderma asperellum* (Patel et al., 2020). The co-cultivation approach is also an effective strategy for enhancing the production of metabolites. As a result of co-culturing *P. ostreatus* and *Trametes robiniophila*, silenced genes are activated and new sesterterpenes are synthesized, which show strong activity against pathogenic fungi (Li et al., 2023). This approach is promising for the development of new drugs and the identification of bioactive compounds. HPLC and mass spectrometry methods have created the possibility of comprehensive qualitative and quantitative analysis of *P. ostreatus* metabolites. The use of different solvents allows for the simultaneous detection of metabolites and the assessment of their pharmacological and nutraceutu-

tical properties (Zhou et al., 2020; Chen et al., 2022). At the same time, salicylic acid, as a signaling molecule, activates genes, stimulates the biosynthesis of secondary metabolites, and increases the stress resistance of the fungus (Kumar et al., 2021). As a result, the data presented in the literature scientifically substantiate the biological activity, metabolite synthesis, and stress adaptation mechanisms of the *Pleurotus ostreatus* mushroom, and also indicate the prospects for their application in the fields of cosmetology, pharmaceuticals, and nutraceuticals.

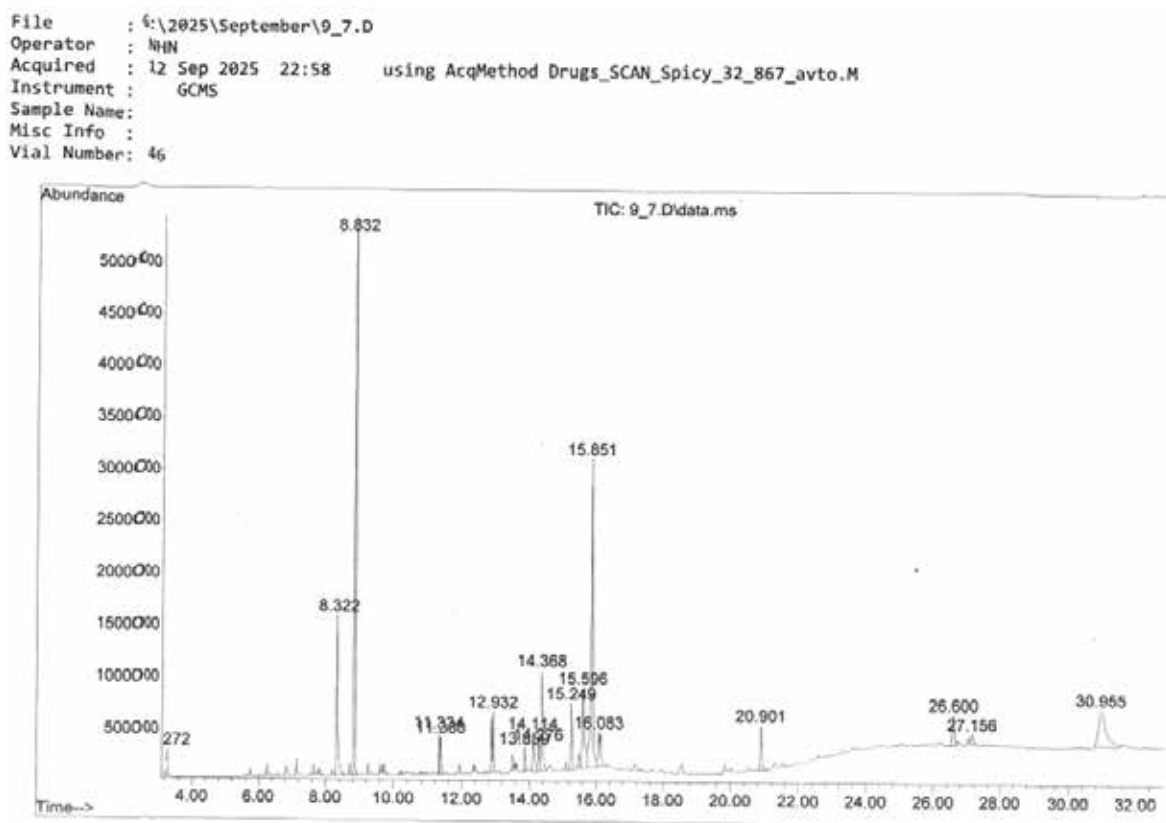
Sample preparation

Pleurotus ostreatus was grown and harvested in the field of the Department of Microbiology of the State University of Antioch for 15 days. The resulting crop was dried and extracted with ethyl acetate.

Results and Discussion

The ethyl acetate fraction of *Pleurotus ostreatus* was analyzed by GC–MS (EI, 70 eV) and the extract was found to contain secondary metabolites of mainly lipophilic nature.

Figure 1. GC–MS/EI–MS analysis of *P. ostreatus* sample (solvent ethyl acetate)



Intense peaks were observed in the chromatogram between 18.5–24.1 minutes, which were confirmed by spectral analysis to be related to ethyl esters of fatty acids and sterol derivatives. The molecular ion of ethyl linoleate was observed at m/z 308 with low intensity, which is typical of fatty acid esters and is explained by the easy cleavage of the carbon chain. The basic peak observed at m/z 87 in the spectrum was formed as a result of McLafferty rearrangement and reliably confirms the presence of an ester functional group. Also, the fragment ions at m/z 55, 81 and 95 correspond to allyl and dienyl cations associated with double bonds in the linoleate chain. These results indicate the presence of ethyl linoleate in the *P. ostrea-*

tus extract and its possible biological role in lipid metabolism. Sterol fraction analysis revealed a molecular ion of m/z 378 for ergosta-3,5,7,22-tetraene, and a sequential fragmentation characteristic of the sterol nucleus (m/z 109, 157, 211, 253, and 335). These fragments are characteristic of the ergosterol skeleton and are consistent with the data reported in the literature. Although a relatively low match (7.62%) with the database was recorded for ergosta-5,7,22-trien-3-ol acetate, the characteristic distribution of fragment ions indicates the presence of an acetated sterol. This is explained by the partial decomposition of sterol derivatives during the extraction process or the separation of the acetate group during ionization.

Figure 2. *Linoleic acid ethyl ester*

Hit 1 : Linoleic acid ethyl ester

C₂₀H₃₆O₂; MF: 939; RMF: 950; Prob 61.9%; CAS: 544-35-4; Lib: replib; ID: 9397.

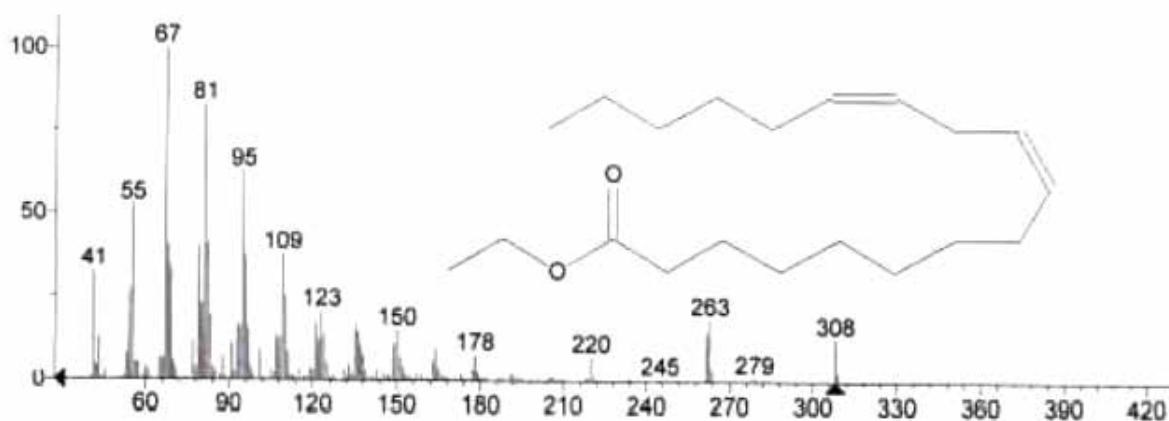
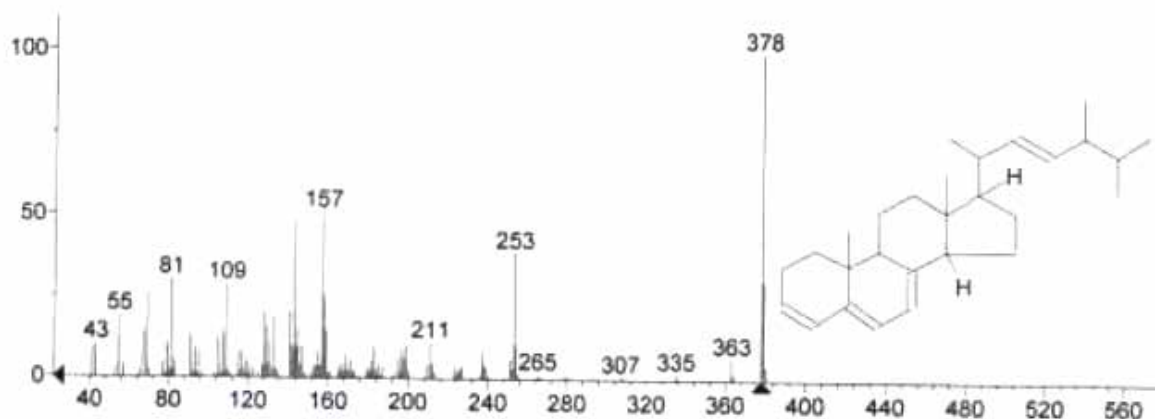


Figure 3. *Ergosta-3,5,7,22-tetraene*

Hit 1 : Ergosta-3,5,7,22-tetraene, (22E)-

C₂₈H₄₂; MF: 599; RMF: 819; Prob 39.3%; CAS: 75678-55-6; Lib: mainlib; ID: 260895.



The results obtained are consistent with the data on secondary metabolites of *P. ostreatus* presented in the literature and confirm that this fungus is an important source of lipophilic bioactive compounds, in particular sterols and fatty acid esters. The ethyl acetate solvent has a medium polarity and

allowed for the efficient extraction of metabolites of sterol and lipid nature.

An extract from the ethyl acetate fraction of *Pleurotus ostreatus* was analyzed by GC–MS and three major bioactive metabolites were identified: ethyl linoleate, Ergosta-3,5,7,22-tetraene (22E), and Ergosta-5,7,22-trien-3-ol acetate.

Figure 4. *Ergosta-5,7,22-trien-3-ol acetate*

Hit 2 : Ergosta-5,7,22-trien-3-ol, acetate

C₃₀H₄₆O₂; MF: 544; RMF: 780; Prob 7.62%; Lib: mainlib; ID: 260933.

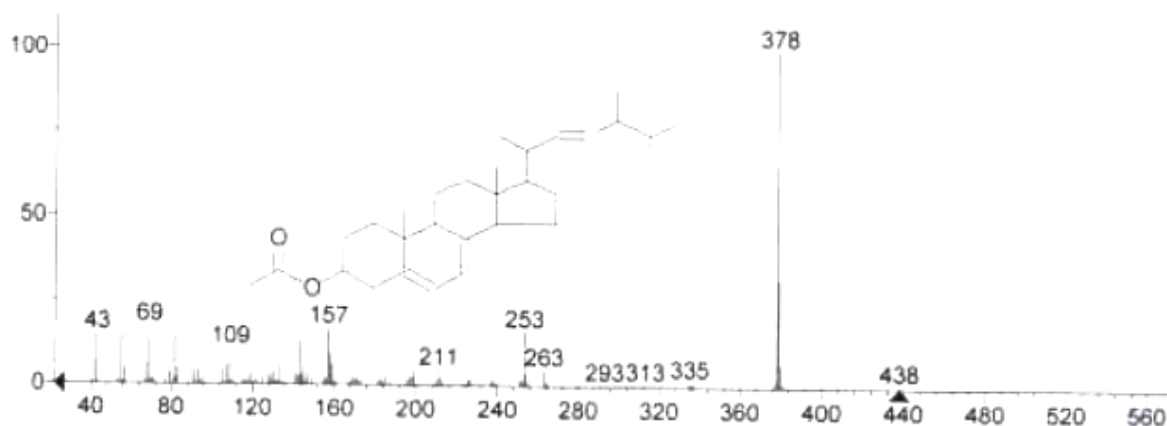


Table 1.

Metabolite	Chemical formula	Molecular mass (Da)	RT (min)	Molecular ion (m/z)
Ethyl linoleate	C ₂₀ H ₃₆ O ₂	308	18.5	308
Ergosta-3,5,7,22-tetraen	C ₂₈ H ₄₂	378	23.2	378
Ergosta-5,7,22-trien-3-ol acetate	C ₃₀ H ₄₆ O ₂	378	24.1	378

Conclusion

In this study, the ethyl acetate extract of *Pleurotus ostreatus* mushroom was analyzed for the first time in a comprehensive manner using the GC–MS method. As a result of the analysis, the extract contained ethyl linoleate and two bioactive sterol derivatives belonging to the ergosterol skeleton – ergosta-3,5,7,22-tetraene and ergosta-5,7,22-trien-3-ol acetate. The spectral fragmentation patterns reliably confirmed

the structural properties of the identified compounds. The obtained data indicate that *P. ostreatus* is rich in lipophilic secondary metabolites, scientifically justifying its prospects for the production of pharmacological, nutraceutical and functional food products. In the future, it is advisable to conduct quantitative analysis of these metabolites, evaluate their biological activity, and study their biosynthesis under the influence of stress factors.

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