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MASS-CHROMATOGRAPHIC ANALYSIS OF METABOLITES OF THE FUNGUS TRICHOLOMA CALIGATUM

Ruziyeva Zarnigor [1], Kamolov Luqmon[1], Gulboyeva Dilafruz[1], Hasanova Madina[1], Meyliyeva Muxlisa [2]

[1] Faculty of Chemistry and Biology, Karshi State University, Uzbekistan, Karshi

[2] Faculty of Medicine, Turon University, Uzbekistan, Karshi

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Abstract

In recent years, macrofungi are famous for their unique nutrient retention, moreover, for the production of biologically active substances such as microfungi. In our study, we aimed to study the biologically active substances of the fungus Tricholoma caliogatum and in this study, the insoluble residue GC–MS (gas chromatography — mass spectrometry) was analyzed in organic solvents (hexane, chloromine, methanol) that separated during the last steps of the alcohol (ethanol) extract process of the fungus Tricholoma caligatum. According to the analysis results, the residual revealed the following major components: 1-(3,5-dimethyl-1-adamantanoyl)semicarbazide, Amphetamine-3-methyl cyclobutanol, N-Acetyl-2-methylamphetamine, Amphetamine-3-methyl acetate, N-Acetyl-4-methylamphetamine, Rhodopin, Octadecanoic acid, 2-hydroxy-1,3-propanedilyl di-ester, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanyyl ester and other lipid and carotenoid derivatives of high molecular weight. The different natures of the compounds identified (amine/alkaloid-like, carotenoid pigments, fatty acid esters, and semicarbazide derivatives) indicate that the residue is a complex organic polymer-and-conjugate mixture.

Keywords: *Tricholoma caligatum; alcohol extract; GC–MS; a secondary metabolite; lipid esters; Chromatographic*

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In recent years, the secondary metabolites in macrofungs have been studied extensively as they are considered as an important resource in pharmaceuticals, food industry, and biotechnology. Fungi belonging to the conslog Tricholoma, in particular Tricholoma caligatum, are remarkable for the richness of their chemical components. (Erol et al., 2020) have isolated substances such as lascivol, trametenolic acid, ergosterol, and ergosterol peroxide from the fruit body of T. caligatum. The anti-inflammatory effects of these compounds and the possibility of suppressing the NF-κB signaling pathway have been established. This case forms the basis for considering T. caligatum as a source of pharmacologically active molecules. (Taşkın et al., 2019) compared the volatile substances of T. caligatum and T. anatolicum species, noting that terpenes are higher in T. caligatum and alcohol and esters are relatively low. These findings explain the odor and taste profile unique to this species. In a study conducted by Gillardoni et al., 2023, novel triterpenoid structures from the genus Tricholoma were identified, and their structure was confirmed using NMR and MS techniques. This result further enriches the diversity of metabolites in the constellation Tricholoma. Zhao et al., 2024) analyzed studies between 2018 and 2023 and summarized that more than 100 different metabolites from Tricholoma species had been identified. Among them, steroids, terpenoids, phenolics, polysaccharides and alkaloids indicate the chemical diversity of the species of Tricholoma. (Stojek et al., 2024) analyzed the fatty acid content in wild mushrooms and noted that linoleic, oleic, and palmitic acids are found as the main components in the genus Tricholoma. These lipids are significant in cell membrane stability and biological activity. Research by Yu et al., 2023 shows that Tricholoma species has a balanced amino acid and fatty acid profile. It is one of the factors that increase the nutritional value of piglets. (Broser et al., 2023) Having discovered the presence of rhodopsin-cyclase enzymes in the type Tricholoma, they have shown that it is important in the synthesis of pigment and light-sensing molecules. Studies (led by Qin and Pandey., 2023–2024) highlight the presence of melanin and other pigment polymers in the genus Tricholoma, which play a key role in protecting fungi from external factors. Saiz-Jimenez et al. 2021) have shown that it is possible to detect the phenolic and aromatic components via pyrolytic analysis of melanin. Such a method is important when chemically characterizing insoluble residues. Gutierrez-Reyes et al., 2025) have provided methods for characterizing biomass-derived substances based on MS. This methodology is useful for the detection of high molecular components in Tricholoma extracts. Sun et al., 2022) gave a general analysis of the polysaccharides of edible fungi, highlighting the importance of β-glucan and chitin for the cell wall in Tricholoma species. (Clericuzio et al., 2018) Metabolites isolated from the Tricholoma type have been reported to show pharmacological activity. Among them, the important role of terpenoids and steroids has been noted. Roziyeva et al., 2025), while describing novel metabolites from T. caligatum, confirming their antimicrobial and antioxidant activity. And additional research will further deepen the diversity of the species Tricholoma. (Inside vs. Others, 2023) The Tricholoma section revealed structural differences in the genomes of Caligata, indicating the evolutionary position of T. caligatum. S. et al. (2018) has investigated the antioxidant activity and safety of T. equestre based on the phenolic/flavonoid content. (Benazza-Bouregba et al., 2016) A new find of T. caligatum from North Africa has been confirmed via molecular markers. Metabolome and transcriptome analyses in 2022–2023 have expanded knowledge of the cell wall and metabolite biosynthesis of T. matsutake. (Gamboa-Becerra et al., 2024) described metabolites from T. mesoamericanum and evaluated its antioxidant and nutritional value. In general, the available literature confirms the chemical diversity of the species Tricholoma caligatum and indicates that studying it as a source of bioactive compounds is of scientific and practical importance.

Research methodology

Sample Preparation

Fruit bodies of the fungus Tricholoma caligatum were selected as the object of research. The sample was first thoroughly washed with distilled water, cleaned of surface contamination, and dried at 45–50 °C. The dried material was ground into a fine powder in a lab grinder and prepared for storage in an airtight container.

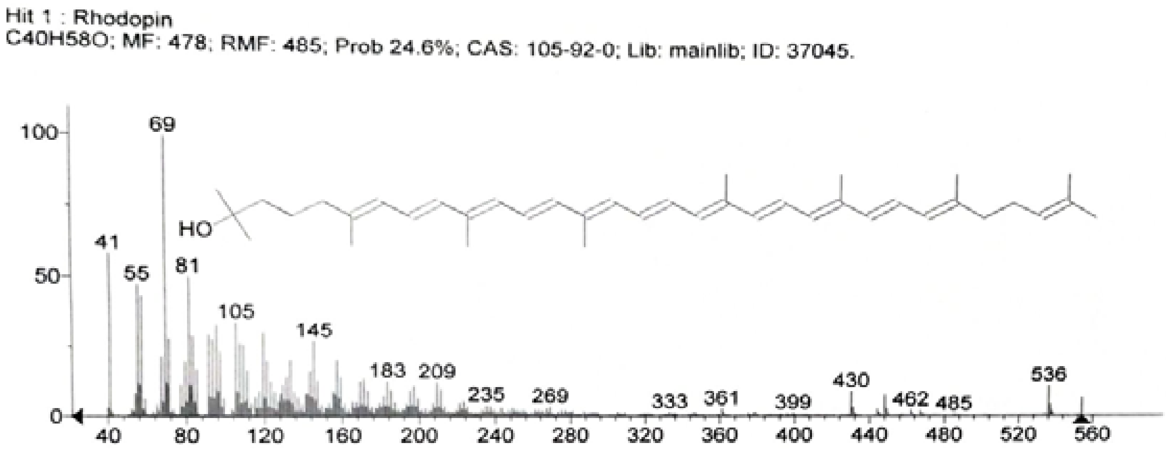
Extraction Process

The extraction process was carried out using an alcoholic solvent. The cousin powder was extracted in 98% ethanol solution for 8 h in the Soxhlet extraction apparatus. The extraction process has been carried out minimum 6-7 times. In the solvent, the substance was washed out, after which the solvent was removed by a rotary evaporator. This extraction separated the stained at the beginning and white at the end of the process, the residual part, insoluble in any organic solvent.

Conclusion and discussion

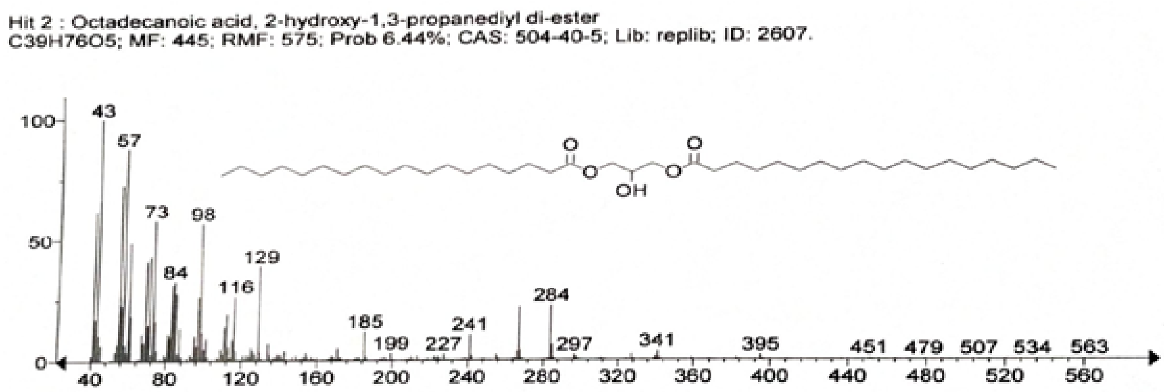
The bleached substance in the last stage of alcohol extract of the litter biomass obtained by the above method was attracted to the chromatographic separation process. The dissociating whitish substance (insoluble in hexane, chloroform, ethyl acetate, and even methanol) at the last step of alcohol extraction of Tricholoma caligatum was analyzed using the GC–MS method, mass chromatogram analysis revealed peaks consistent with a number of important compounds. The chromatographic profile of this extract showed additional cleavage and differentiation of metabolites, and among those dissociated substances the biological importance of rhodopin is great.

Figure 1. *Gas-liquid chromatogram of extract (Rhodopin) in an alcohol-solvent system*



The volatile metabolites detected by GLX (gas-liquid chromatography) chromatogram of the resulting extract were identified through the MC database. The substance belongs to the group of carotenoids. Carotenoids are pigments found in many organisms, and they play photoprotection, light harvesting, and antioxidant roles. Rhodopin (1,2-dihydro-ψ, ψ-caroten-1-ol) is a C40H58O-formulated oxycarotene and is also found in purple bacteria (e.g. Rhodopseudomonas acidophila, Rhodomicrobium vannielii), and several other microorganisms.

Figure 2. *Gas-liquid chromatogram of extract (Octadecanoic acid, 2-hydroxy-1,3-propanedilyl di-ester (C39H76O5)) in an alcohol-solvent system*



The 2nd substance is Octadecanoic acid, 2-hydroxy-1,3-propanedilyl di-ester (C39H76O5), better known as glyceryl distearate (GDS), among the mono- and di-esters of natural fatty acids. This substance is used as an excipient component in many pharmaceutical products. The main composition is stearic acid, which in the body is hydrolyzed and broken down into stearic acid and glycerin. Glyceryl distearate is not an active substance as a drug, but its pharmacological importance is manifested in its helpful properties: Improve the bioavailability (absorption of drugs). Being a carrier of fat-soluble vitamins (A, D, E, K) and lipophilic drugs. Application in fat-based adjuvant systems for antigens and vaccines. Its use as a transport system for lipid-based drugs. Glyceryl distearate is recognized by FDA and European pharmacopoeia as a GRAS (Generic Safe Substance). Its toxic effect has not been determined and is widely used in medicines, cosmetics. The biological activity of most of the decaying substances is considered to be high.

Conclusion

As a result of GC–MS analysis, the substances identified and their biological activity were evaluated as: 3-Methylamphetamine – has stimulant effect on the central nervous system, increases attention, but has toxic effect. N-Acetyl-2-methylamphetamine – is a metabolite of amphetamine, which forms in the body during the process of suppressing the action of the drug. Amphetamine is a powerful psychostimulant that increases alertness, but can cause addiction. Benzeneethanol,α-(1-aminoethyl)-, hydrochloride–is a derivative of phenethylamine, generally showing psychotropic property by influencing neurotransmitters. D-Mannitol – used for medicinal purposes; As an osmotic diuretic, it reduces swelling, stimulates kidney function. 1-Octadecanamine, N-methyl – a long-chain amine, may exhibit antimicrobial properties by interacting with biological membranes and lipid layers. 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol – a compound of steroidal nature, is among the derivatives of cholesterol, is involved in hormonal and metabolic processes. Cyclotrisiloxane, hexamethyl – generally not biologically active; these substances are mainly technical mixtures and come from the GC-MS hardware. In general, among the substances identified: - Biologically active amines (1–4) are mostly psychotropic and have stimulant effects. Mannitol (5) is used in medicine as a diuretic and detoxifier.- Lipid/steroid derivatives (6–7) play an important role in metabolic and hormonal processes. Siloxane (8) is a technical mixture and is considered to be lower in biological value.

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Contact: roziyevazarnigor90@gmail.com