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SECONDARY METABOLITES OF THE FUNGUS TRICHOLOMA CALIGATUM

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Abstract
In recent years, the demand for health care and food products has been on the rise. Mushrooms are gaining traction in an important product not only of the food industry, but also of the medical sector. Ancient and important fungus Tricholoma caligatum has long been used by humans as a medicinal substance. It presents various medicinal properties such as anti-cancer, immune boosting and antioxidant effects. In addition, it is considered a wholesome and healthy food product all over the world. A large number of secondary metabolites are distinguished from the Tricholoma species. It contains a number of terpenoids, alkaloids and steroids containing these secondary metabolites. This fungus is mainly known in Europe as Tricholoma matsutake. There are many different versions of this fungus. The distribution of the secondary metabolites of each version is different, as these vary with ecology and climate change. The changes can sometimes also be seen in the difference in smell and taste. In short, the Tricholoma caligatum group is in need of significant modern study and revision.
Keywords: Tricholoma caligatum, secondary metabolite, cultivated fluid, macroorganism, extract.

Introduction

Tricholoma caligatum is a mycorrhizal fungus with medicinal properties that can grow in a unique and incredibly natural way, which is rare in the world.Tricholoma is a fungus that contains thick-fleshed, white-spored gilled fungi that are found throughout the world, mainly in forests. These fungi are ectomicorrhizal fungi that live in symbiotic relationships with various types of coniferous or broadleaf trees. These fungi are predominantly eucharistic organisms, and there are over 40 species of the Tricholomataceae family. The most common species are East Asian Tricholoma matsutake, also known as matsutake, matsutake, or songi, and there is a collection of North American species Tricholoma magnivelare, also known as "ponderosa fungus", "American matsutake" or "pine fungus". Representatives of Tricholoma caligatum naturally grow differ from representatives of the brown spotted scrub that grow

locally by the whiteness of the paw. At first, they acquire a mild smell and taste, and then, as the litter matures, it becomes unpleasant smell. These plants are mainly a valuable food product in China and are widely used in the treatment of tumor diseases all over the world. The width of this mushroom pod is 4-11 cm. The color of their paw is white at a young age, and when it grows darker becomes even more yellowish or brown.

Review of literature on the topic

The fungi of Tricholoma caligatum are consumed all over the world as the most important healthy food of the 21st century. The fact that tricholoma species form a variety of secondary metabolites has been extensively studied by the scientific community. In 2018, Clericuzio and several other scientists summarized more than one structure, biosynthesis, and biological activity

They isolated 100 different secondary metabolites, isolated from the fruiting bodies of more than 24 species of Tricholoma species. Many scientists have identified a total of 101 compounds over a period of 5 years from 2018 to 2023, of which 46 are mostly newly discovered compounds. These compounds, i.e. secondary metabolites, are terpenoids, steroids and alkaloids that exhibit high chemical and biological activity. Tricholoma fungi is also popularized as the main raw material for the pharmaceutical and food industry. Tricholoma caligatum is characterized especially by the abundance of secondary metabolites, including polysaccharides, phenolics, and alkaloids with a high biological activity. [1,2]. These secondary metabolites are distinguished by not only confer unique biological activity on Tricholoma species, but also give them wide application possibilities. For example, the polysaccharides in Tricholoma caligatum have been found to have a bleach effect. On [3], tricholoma peptides show immunomodulatory activity [4] and tricholoma sterols show good anti-acetylcholinesterase (AchE) activity [5]. While the secondary metabolites of tricholoma have attracted interest from many, scientific research is still at a stage that requires further investigation. Studies show that Tricholoma species, including Tricholoma caligatum, contain many secondary metabolites, on which much scientific research is underway by researchers. The fact that Tricholoma species produce an unpleasant odor of its own accord was first discovered by German scientists in 1968, and they discovered that the main component responsible for the release of the bitter odor in Tricholoma is separated from fruit bodies, isolated using Thin Layer chromatography.[2] In 2018, Italian scientist Clericuzio and his colleagues discovered that Tricholoma They combined 112 secondary metabolites isolated from the fruit. [1]. Secondary metabolites of tricholoma,

Terpenoids are the largest and most diverse structured class of natural products among the secondary metabolites [6]. These compounds are based on isoprene units (C5 units) In the last five years, 73 terpenoid compounds from the Tricholoma species have been identified, including triterpenes, diterpenes, and less common sesquiterpenes and C17 compounds. Sesquiterpenes are noteworthy because they occur in large quantities in other fungi and are rare in the species Tricholoma.

Triterpenes and sterols are mainly considered to be the class of secondary metabolites, which are common in nature and are derived from the acyclic precursor squalene. [7]. The most

important sources of triterpenes and steroid compounds are fungi**.** These compounds have a variety of medicinal properties, in addition, they are crucial for novel types of medicines. A total of 64 steroid **or** triterpene **compounds have been found to be present** in Tricholosoma in the past five years.

Lanostanes are a class of tetracyclic triterpenes having a nuclear structure, consisting of 30 carbon atoms. [8]. Lanosterol is one of the most abundantly representative compounds for the synthesis of sterol compounds such as lanosterol, ergosterol, and stigmasterol, which play a crucial role in triterpene biosynthesis as an important intermediate. Ergosterol is an important component of the cell membranes of most fungi and serves as a precursor to vitamin D2 [9], which plays an important role in maintaining the fungus' vital activity. Stigmasterol has cholesterol-lowering, antioxidant, and potentially anti-cancer properties [10]. Zhang and a number of scientists have isolated 8 completely new lanostane-type triterpenes [11].

These compounds have a characteristic lanostan triterpene skeleton and contain a 3-hydroxy-3-methylglutaryl group in the ring [12]. This structural property is rare among other types of triterpenes. The structure of these compounds has been elucidated by the authors through spectroscopic analyses, including nuclear magnetic resonance (YAMR) and mass spectrometry (MS) and alkaline methanolysis methods.

There are several names (synonyms) of the fungus Tricholoma caligatum, including Agaricus caligtus, Armillaria caligata, Sphaerocephalus caligatus, Sphaerocephalus caligatum. The cap of the fungus Tricholoma caligatum is initially semicircular, quickly turning from convex to flat, reaching 12–15 cm in diameter, and covered with patches ranging from mallow to dark brown in color.[13] The petiole (stalk) is 4–12 cm long. The flesh of Tricholoma caligatum is thick and fibrous, with a fragrant pepper-like aroma. The color of the spare is white. [14][15][16][17][18] Tricholoma caligatum is a hardy southern species common in the forests of the Mediterranean. This species grows symbiotically with several coniferous shrubs, such as Pinus pinea, Pinus halepensis, Pinus nigra, Pinus brutia and Pinus pinaster, as well as evergreen oak, strawberry trees and terebinth shrubs. [19][20][21][22]. It occurs in autumn and winter in coastal and high forests.

Research Metadology. The fungi of Tricholoma caligatum produce a variety of secondary metabolites. Secondary metabolites of the fungus Tricholoma caligatum are mainly isolated from their fruit bodies. Secondary metabolites include **terpenoids**, **steroids, and** alkaloids **with a high bioactive** activity. Tricholoma mushrooms are distinguished for their wide use in the pharmaceutical and food industries. Tricholoma caligatum contains a large number of secondary metabolites, including **polysaccharides**, **phenolics**, and alkaloids **with high biological activity** [1,2]. These secondary metabolites confer the desired biological activity on the fungus Tricholoma caligatum, and this property holds true when they are applied on a large scale. For example, **polysaccharides** have been found to have bleaching effects, in addition, these substances have excellent immune system supports, antioxidant properties and anti-tumor anti-tumor properties. **Chitin and chitosan** are biologically active compounds that are used in some cases for medical and industrial purposes. **From phenolic compounds-** Flavonoids and phenolic acids- these substances also have antioxidant properties, which help

plant protection and strengthen the immune system. **Polyphenols** are substances that have antioxidant properties and reduce inflammation.

Figure 1. A natural breeding of the fungus Tricholoma caligatum in the Kashkadarya region.

Figure 2. The fruit and stem of the fungus Tricholoma caligatum are initially white in color and change to dark brown as they age.

The peak period of synthesis of secondary metabolites of the fungus Tricholoma caligatum coincides with the period of fruiting. However, when moisture, temperature, and adequate oxygen are the necessary factors for fruit formation, the synthesis of its secondary metabolites in the fungus is enhanced and they accumulate in large quantities [23]. These metabolites help the protective mechanisms of the fungus as well as enhance its biochemical activity.

Figure 3. Extraction and Separation Process by Column Chromatography of Tricholoma caligatum

Analysis of results. To determine the extraction efficacy for Tricholoma caligatum fungus by quantitative analysis. The fungus Tricholoma caligatum, which grows naturally in the Kashkadarya region, mainly after spring rainy weather, was picked and the amount of secondary metabolites in this fungus was determined and compared with the same fungus species grown in biohumus in 20 days at a temperature of 20-25 degrees Celsius in the laboratory of the Tashkent Institute of Microbiology. In this case, it was found that the secondary metabolites were equal to and abundant in the amount of secondary metabolites grown over a period of 14 days when quantitatively analyzed. Initially, in order to isolate the secondary metabolites, the biomass of Tricholoma caligatum was dried and ground in an environment not exposed to sunlight and sufficient air, and the composition of the biomass was studied using the following method. The harvested biomass was 85 grams. This biomass was 44 g when dried and crushed. Because methyl alcohol is expensive and toxic, ethyl alcohol was used for extraction purposes. Dried and grated Tricholoma caligatum fungal biomass was placed in a 1 liter bottom flat tube, 750 ml of ethyl alcohol was poured on it and the tube mouth was secured. The extraction process of lily biomass was repeated at least 6-10 times. The na'muna was collected in a special container and when the extract was dried with vacuum rotor equipment, an extraction sum of 3,448 g was obtained. When the remaining biomass was extracted with water and dried with vacuum rotor equipment, an extraction sum of 2 gr was formed. The total weight of the extraction sum was 5,448g.

Table 1. Results of a quantitative analysis of secondary metabolites of the fungus Tricholoma caligatum

Piglets' growth time (days)	Fungal biomass (gr)	Dried biomass of bark (gr)	Summary of Ethyl alcohol extraction (gr)	Aqueous Extraction sum (gr)
3	75	33	2,1	1,1
7	75	35	2,3	1,4
14	85	44	3,448	2
20	85	62	2,5	1,2

Table 2. Chemical Analysis of Tricholoma caligatum Extract

Extract	**Alkaloids**	**Tannins**	**Flavanoids**	**Saponins**	**Glycoside**
Ethyl Spirtli extract	+	-	-	+	+
Dry extract	+	-	-	+	+

Conclusion and Suggestions

In conclusion, it can be said that according to the results obtained, the extract from the 14-day harvest of the fungus Tricholoma caligatum showed a high extraction efficiency.A quantitative analysis of the secondary metabolites also revealed a high content of secondary metabolites in the litter extract collected at day 14. The highest activity of the synthesis of secondary

metabolites of the fungus Tricholoma caligatum coincides with the period of fruit body formation. For the formation of fruit there must be necessary living factors, including humidity, temperature, oxygen content, when the fungus synthesizes more of its secondary metabolites. These metabolites help the protective mechanisms of the fungus as well as enhance its biochemical activity. It was found that the process could be optimized using quantitative analyses. For example, ways to change suitable conditions (temperature, type of solvent or method) or save energy and time during the production process to increase extraction efficiency. These calculations are used to evaluate the effectiveness of the process and the amount of substance separated.

References

1. Clericuzio, M.; Mellerio, G.G.; Finzi, P.V.; Vidari, G. Secondary metabolites isolated from Tricholoma species (Basidiomycota,
Tricholomatacee): Sharh. Nat. Prod. Commun. 2018, 13, 1934578X1801300926. [CrossRef]
2. Hilber, O. Indole as a major component of some species of Tricholoma and Lepiota bucknallii odor. Z. Pilzkd. 1968, 34,
153–158.
3. Cheng, H.; Liu, Y.; Liu, H.; Liu, G.; U, C.; Li, L. Efficacy of Polysaccharide Separation and Purification as well as Whitewashing
Tricholoma matsutake Sing. China Surfaktan Deterg. Kosmet. 2013, 43, 134–138.
4. Lee, M.; Ge, Q.; Du, H.; Jiang, P.; Bao, Z.; Chen, D.; Lin, S. Potential mechanisms that mediate the protective effect of Tricholoma
Matsutake-derived peptides in reducing DSS-related colitis. J. Agric. Food Chemistry. 2021, 69, 5536-5546. [CrossRef]
5. Zhao, Z.-Z.; Chen, H.-P.; Wu, B.; Zhang, L.; Lee, Z.-H.; Feng, T.; Liu, J.-K. Matsutakone and Matsutoic Acid, Two (No) Steroids
Unusual skeletons from the fungus Tricholoma matsutake. J. Org. Chem. 2017, 82, 7974-7979. [CrossRef]
6. Ma, Y.; Zu, Y.; Huang, S.; Stephanopoulos, G. Universal and efficient platform engineering for terpenoid synthesis in yeast. Proc.
Natl. Acad. Sci. AQSh 2023, 120, e2207680120. [CrossRef]
7. Ma, Y.; Shang, Y.; Stephanopoulos, G. Engineering Peroximal Biosynthetic Pathways to Maximize Triterpene Output
Yarrowia lipolytica. Proc. Natl. Acad. Sci. AQSh 2024, 121, e2314798121. [CrossRef]
8. Gao, Y.; Wang, G.; Huang, H.; Gao, H.; Yao, X.; Hu, D. Biosynthesis of Fungal Triterpenoids and Steroids. Chin. J. Org. Chemistry. 2018,
38, 2335–2347. [CrossRef]
9. Jordá, T.; Puig, S. Regulation of ergosterol biosynthesis in Saccharomyces cerevisiae. Genes 2020, 11, 795. [CrossRef]
10. Zhang, X.; Wang, J.; Zhu, L.; Wang, X.; Meng, F.; Xia, L.; Zhang, H. Advances on the anti-tumor effects of stigmasterol and
mechanism of action. Forward. Oncol. 2022, 12, 1101289. [CrossRef]

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11. Zhang, S.-B.; Li, Z.-H.; Stadler, M.; Chen, H.-P.; Huang, Y.; Gan, X.-Q.; Feng, T.; Liu, J.-K. Tricholoma dan Lanostane triterpenoidlari
NO production inhibitor and pardinum with cytotoxic activity. Phytochemistry 2018, 152, 105-112. [CrossRef]
12. Zhong, S.; Lee, L.; Liang, N.; Zhang, L.; Xu, X.; Chen, S.; Yin, H. Acetaldehyde Dehydrogenase 2 regulates HMG-CoA reductase.
stability and cholesterol synthesis in the liver. Redox Biol. 2021, 41, 101919. [CrossRef]
13.Trudell, Steve; Ammirati, Joe (2009). Fungi of the Pacific Northwest. Timber Press Field Guides. Portland, OR: Timber Press. p. 101. ISBN 978-0-88192-935-5.
14. Galli R. (2003). I Tricholomi 2nd Edition. Med. Fit.
15. Riva A. (2003). Fungi Europaei 3: Tricholoma. Edizioni Candusso, Italiya.
16. Marchand, A. (1971). Mushrooms of the North and South. Volume 1. Perpignan. ISBN 8449906490
17. Courtecuisse, R. (1995). Collins Wild Guide: British and European Mushrooms. Harper-Collins.
18. Loizides M, Kyriakou T, Tziakouris A. (2011). Edible and toxic fungi of Cyprus (in Greek and English). Published by authors. pp. 270-71. ISBN 978-9963-7380-0-7.
19. Kytövuori, I. (1988). Tricholoma caligatum group in Europe and North Africa. Carstenia 28:65-77.
20. Loizides, M. (2011). Quercus alnifolia: The native of Cyprus golden oak and its fungus. Field Mycology 12(3): 81-88. doi:10.1016/j.fldmyc.2011.06.004.
21. Zervakis G., Dimou D., Balis C. (1998). Checklist of Greek macrofungi, including hosts and biogeographic distribution: I. Basidiomycotina. Mycotaxon 66: 273-336.
22. Malençon, G., Bartault, R. (1970). Upper fungal flora of Morocco. Tome I. Trav. Inst. Sci. Chérifien et Fac. sci. Rabat.
23.Taofiq O. et al. Contribution of phenolic acids to anti-inflammatory activity of fungi: screening in phenolic extracts, individual parent molecules, and synthesized glucuronated and methylated derivatives//Food Research International. – 2015. – T. 76. – C. 821-827.