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Chromato-mass-spectrometry of the analysis of the sum of the common mushrooms

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Abstract. There are conflicting views among researchers on the precise beginning of champignon cultivation. While some contend that its cultivation began in France, others assert that it originated in Italy around a thousand years ago. French farmers discovered in the 17th century that champignons thrived not just on manure-fertilized lawns but also in dark, damp quarters, a method that is still used today. Champignon was cultivated on dedicated farms on royal estates when European kings ruled the continent because it was a costly and uncommon delicacy. The objective of this study was to analyze the composition of the common mushroom using mass spectrometry with a Chromatek-Crystal 5000 spectrometer. The study aimed to extract and quantitatively determine the compounds present in the common fungus. A technology for obtaining the drug was developed through experiments, which involved extracting plant materials with absolute alcohol. The methodology employed can be used to further explore the properties of the common mushroom, and contribute to the development of effective pharmaceutical products. **Keywords.** Common fungus, Chromatek-Crystal 5000 spectrometer, mass spectrometric analysis, quantitative determination, extraction of plant materials, drug preparation, 1% KOH solution, 5% potash solution.

1 Introduction

The Agaricus species, commonly referred to as champignon, is a globally distributed and extensively cultivated mushroom. It thrives in various regions spanning Europe, Africa, Asia, and the Americas. The name champignon, derived from the French language, translates to mushroom in English [1-7].

The exact origin of champignon cultivation remains uncertain, as opinions among scholars differ. Some argue that its cultivation dates back to Italy approximately one thousand years ago, while others suggest that France may be its place of origin [4-9]. In the 17th century, French farmers observed that champignons flourished not only in manure-fertilized lawns, but also in dark, humid rooms - a technique still utilized to this day. During the time of European monarchs, champignon was considered an expensive and rare delicacy, leading to its cultivation on special farms located on royal estates. Agaricus, also known as champignon, belongs to the Agaricaceae family within the Agaricales order. The fruit bodies

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of this species vary in size, ranging from 3 cm (Agaricus comtulus) to 25 cm (Agaricus arvensis) [1-3, 12].

The aim of this study was to investigate the process of extracting analysis amounts of a prevalent fungus from finely dissected samples, and to develop a quantitative determination method for these compounds. Through experimentation, a drug production method was devised that involves the extraction of plant materials with absolute alcohol, followed by the thickening of the resulting extract, dilution with water, and extraction of the common mushroom [8-12]. Further processing involved treatment with a 5% solution of potash, and a 1% KOH solution of ordinary mushrooms, followed by acidification with sulfuric acid, and extraction with chloroform. The resulting chloroform solution was then evaporated, and common mushrooms were subject to chromatographic purification via a column. [8-13]

2 Materials and methods

In order to obtain an extract for analysis, a representative sample of the raw material was first crushed to a specific particle size. The crushed material was then placed in a filter paper and loaded into the Soxhlet apparatus for extraction, which was carried out using 100% alcohol for a duration of 2.5 hours in a boiling water bath [1-4, 9-11]. The resulting alcoholic extract was subsequently concentrated on a rotary evaporator, and the resulting concentrate was then quantitatively transferred to a volumetric flask, where the volume was adjusted using the same alcohol to reach the mark. The solution was thoroughly mixed prior to analysis. Spectral data was collected using a "chromatek crystal" spectrophotometer equipped with a mass spectrometric detector, which was produced in Russia in the year 2022 [1-5, 9].

An analytical sample of the raw material was comminuted to a particle size that conformed to the requirements specified in GOST 214-83, utilizing a sieve with openings of 2 mm in diameter. Approximately 2.5 g of the crushed raw material, which had been precisely weighed, were placed onto filter paper and lowered into the extractor of the Soxhlet apparatus, whose working volume was roughly 200 ml. The extractor was then filled with 180 ml of 100% alcohol and extracted for 2.5 h (6 cycles) in a boiling water bath. The alcoholic extract was subsequently concentrated to a volume of 15 ml on a rotary evaporator, quantitatively transferred to a 25 ml volumetric flask, brought up to the mark with the same alcohol, and thoroughly mixed.

The obtained sample was analyzed using a Chromatek Crystal spectrometer equipped with a mass spectrometric detector manufactured in Russia in 2022. Spectral data was collected and recorded for further analysis.

3 Results and discussion

The primary constituents of champignon mushrooms are ethers belonging to various types, including but not limited to But-1-ene-3-yne-1-ethoxy, Cyclohexane, ethoxy, and 2,7-Octadiene, 1-butoxy [4-7] (Fig. 1).

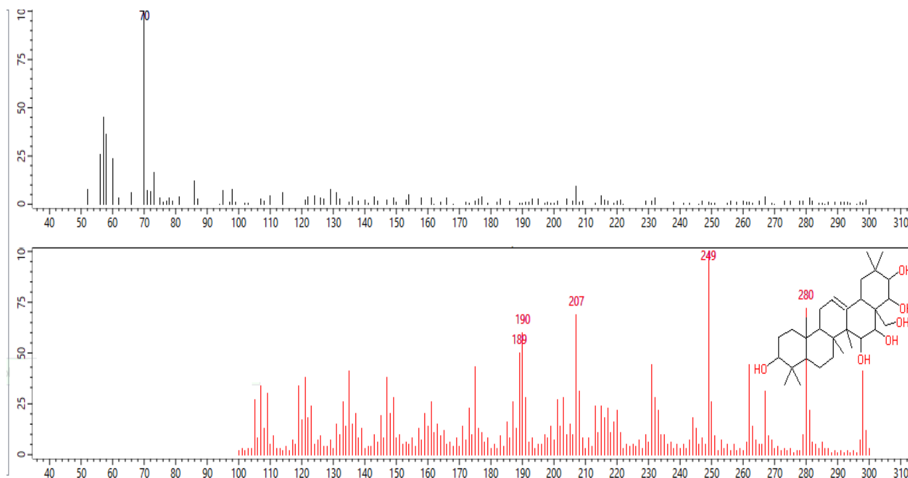

Fig. 1. Mass spectrum champignon extract.

The structural organization of the ethanol extract of diverse sources relies primarily on compounds such as Hexanoic acid, 2-oxo-, methyl ester, 2-methylbutanoic anhydride, 2-butenedioic acid, 2-methyl-, (E)-, pentanedioic acid, 2,2-dimethyl-, bis(1-methylpropyl) ether, and carbamic acids. Other key constituents include 2-(dimethylamino)ethyl ether, phthalic acid, and bis(2-pentyl) ether [4-7, 11] (Fig. 2 and 3).

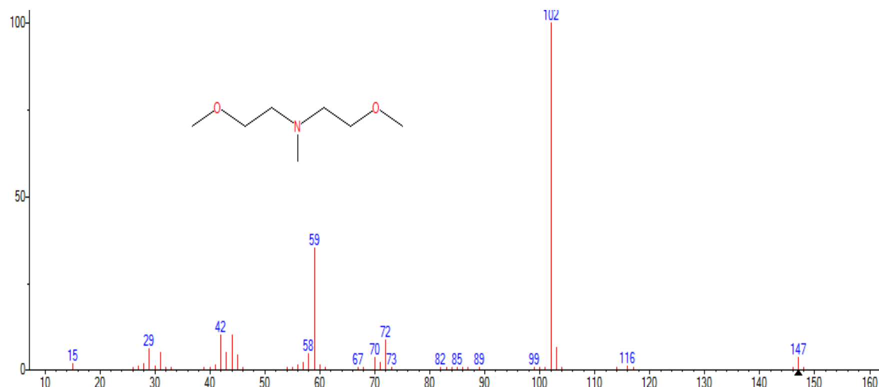

Fig. 2. 2.7-octadiene diester.

It is required to add the mycelium of the fungus to it in order to grow the mycelium. Mycelium is grown industrially in laboratories under sterile conditions.

To cultivate mushrooms over an area of 1 square meter, 500 g of compost mycelium or 400 g of champignon grain spores are required. The process involves introducing the "seeds" into the substrate, where the mycelium will grow. Properly prepared compost should exhibit slight resilience when pressed [1-3]. Depressions, spaced 20 centimeters apart, are made at a depth of 5 centimeters and filled with a small amount of compost mycelium. Alternatively, if mushroom spores are used, they can simply be scattered on the surface. Over time, the mycelium will spread along the substrate, forming visible threads [4-8, 11].

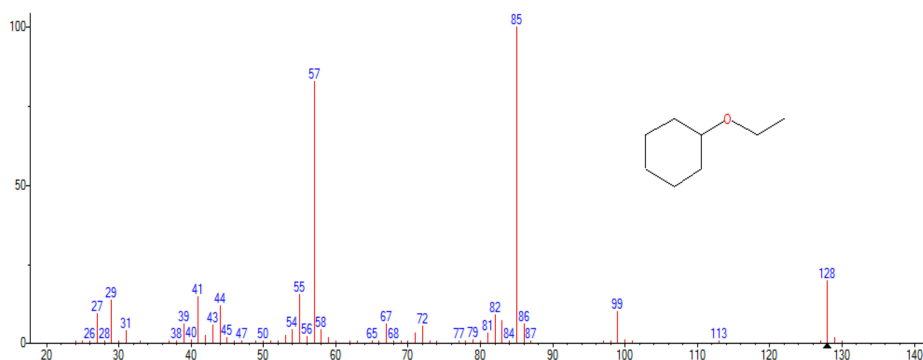

Fig. 3. But-1-en-3-in-1-ethoxy.

The period of mycelial growth until the appearance of the fruiting bodies is known as the incubation period. During this phase, it is crucial to maintain a high level of humidity, typically within the range of 70-95%, to prevent desiccation of the substrate. This can be achieved by covering the substrate with a porous material such as paper or cloth and periodically spraying it with water [4-8]. The optimal temperature for mycelial growth ranges from 20 to 27 degrees Celsius. After about 10-12 days, the mycelium will start spreading actively, and the surface of the substrate should then be covered with a 3–4-centimeter layer of a specific earth mixture consisting of manure (5 parts), limestone (1 part), and soil (4 parts). It is imperative to maintain the moisture level by regular spraying with water [3-7, 13].

Following the incubation period, it is recommended to lower the temperature in the cellar to 12–17 degrees or move the substrate boxes to the forcing room, as previously described. This marks the onset of the mushroom growth period. The first crop can be harvested after 3-4 months. It is advisable to harvest the mushrooms before they become overripe. The optimal time for harvesting is when the lower part of the cap is still covered with a white film and the brown plates are not yet visible [5-7, 9-12]. It is important to carefully remove each mushroom by unscrewing it from its place, rather than cutting it off (Fig. 4).

Fig. 4. Mushroom mycelium.

It was found that the instrumental method involved the use of a split-flow injection mode with an analysis time of 20 minutes and no purge or stabilization time required. The column thermostat temperature is set to 40°C initially, followed by a ramp up to 250 °C over 30

minutes, and then held at 250°C for another 30 minutes. The carrier gas flow is constant at 1,000 ml/min, with a split flow division of 20 and a pressure of 10.174 kPa. There is no prestart, but waste flow is set at 20 ml/min and membrane blowing is set at 3 ml/min. Valve 1 controls the input port for sample injection.

The method involves the use of an electron ionization ion source with an ion source temperature of 200°C and a transfer line temperature of 250°C. There is no calibration gas used during the analysis, and data is logged using the centroid method. The mass spectrometer detector (MSD) is operated for 38 minutes with the cathode turned on and an emission current of 20. The gain is set to 300000 and there is only one segment with one scan of the mass range between 50 to 300. The scan duration is 0.2 minutes. The prestart is turned off, and there is no switching time for the MSD. The settings for the current are adjusted according to the desired voltage (DV). The sample collection mode is set to simple, with a volume of 1 and vial number 3. The washing mode is set to vial, and the vial insertion depth and immersion depth in the evaporator were 28 and 29, respectively. There are no drain vials used during the analysis, and the flushing breakdown occurs after every three samples. It was reported that the drain speed was set to 1, and the sample volume was 5 μl. Sampling was done at a sample rate of 1, using six pumps with no delay between each sample. The sample volume for injection into the evaporator was 1 μl, with no warm-up time before or after injection. The input speed was set to 9 (Fig. 5).

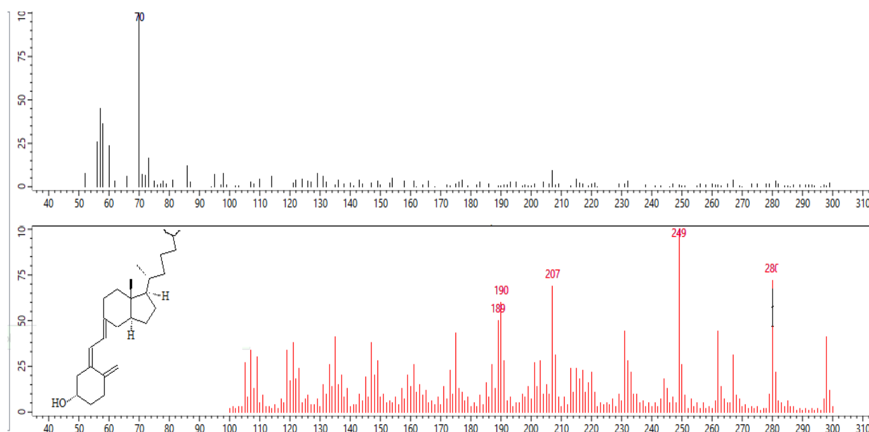

Fig. 5. Vitamin D spectrum.

4 Conclusion

Chromatography-mass spectrometry was used to study the organic matter of the champignon mushroom. 11 individual compounds were identified and their quantitative content, mass spectra, and structural formulas were determined. The compounds contain fragments of furan, pyran, bi- and tricyclanes, arenes, and functional groups such as aldehyde, ketone, alcohol, and ether. Nitrogen- and sulfur-containing compounds were also found. Steroid compounds were identified as derivatives of cyclopentanoperhydrophenanthrene. The study concludes that furan, pyran derivatives, and nitrogen- and sulfur-containing structures play a significant role in shaping the direction of the pharmacological action of drugs derived from champignon mushroom.

To cultivate champignon mushrooms, it is recommended to install a hygrometer and thermometer to monitor humidity and temperature levels. Heating and cooling methods

should also be considered. Before planting, the growing area should be disinfected and treated with copper sulphate, but this does not guarantee protection against bacteria and molds. Regular monitoring of mushroom health is necessary. Chromatography-mass spectrometry was used to study the chemical composition of the organic matter of the champignon mushroom, revealing that more vitamin D is formed under ultraviolet light than infrared light.

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