Comparative investigation of the chemical composition of fruiting body extracts of several mushrooms

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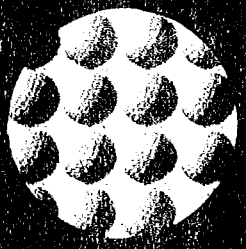

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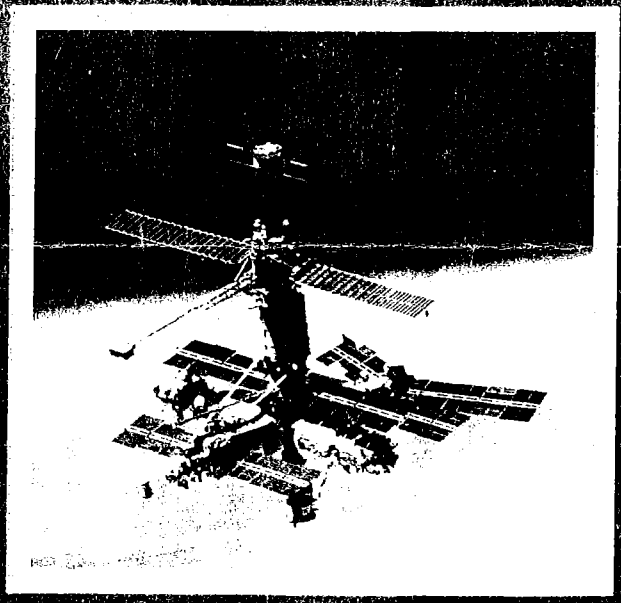

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Բնական Գիտությունների Ռուսաստանի Ակադեմիա, Բնության և Հասարակության մասին Գիտությունների Միջազգային Ակադեմիա (Հայկական [illegible])

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COMPARATIVE INVESTIGATION OF THE CHEMICAL COMPOSITION OF FRUITING BODY EXTRACTS OF SEVERAL MUSHROOMS

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ABSTRACT. - Aqueous and methanol extracts obtained from fruiting bodies of lignicolous basidiomycete mushrooms *Pholiota squarrosa, Pholiota gummosa, Kühneromyces mutabilis, Pleurotus ostreatus* and *Armillaria mellea* (Basidiomycetes) have been studied for content of free sugars. free amino acids, phenolic acids, fungal toxins, glycosides, alkaloids and nitrogen compounds. Thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and qualitative chemical reactions have been used. Phenolic acids (4-hydroxybenzoic, 4-hydroxycinnamic, 4-hydroxy-3-methoxybenzoic, and 3,4-dihydroxyphenylacetic), free amino acids (alanine, valine, serine, proline, methionine, asparagine), cortinarin A, steroid glycosides and choline were mostly observed within tested species. Free sugars, particularly glucose, arabinose, fructose, galactose, rhamnose, and sucrose have not been observed by our methods. The obtained data indicated the presence of biological active substances, such as phenolic acids. choline, and steroid glycosides in analysed mushroom samples.

Key words: *mushrooms; choline; cortinarin A; phenolic acids; glycosides; amino acids; TLC; HPLC.*

INTRODUCTION

Investigated species *Pholiota gummosa* (Lasch) Sing., *Ph. squarrosa* (Mull. : Fr.) Kumm., *Kühneromyces mutabilis* (Scop. : Fr.) Sing., *Armillaria mellea* (Vahl. : Fr.) Kumm., and *Pleurotus ostreatus* (Jacq. : Fr.) Kumm. are belong to wood-inhabiting widespread ecological group of mushrooms [8]. To the best of our knowledge, few data exist about the chemical constituents of the fruiting bodies (FB) of these mushrooms. The data concerning the chemical content of *A. mellea* and *P. ostreatus* mycelia has been reported [7, 10, 13. 14, 19, 20-22].

A data of comparative analyses of the chemical constituents of methanol and aqueous extracts obtained from the fruiting bodies of 5 mushroom species was presented.

MATERIALS AND METHODS

The five species were collected from France (Languedoc-Roussillon) and Germany (Bavaria) (Table 1). After the morphological identification of mushroom specimens, they were dried and preserved. The homogeneous fine powder was used in chemical analyses.

Table 1

The ecology and systematics of investigated mushrooms

Species	Family	Origination	Collection date
Pholiota gummosa	Strophariaceae	France	October, 1979
Pholiota squarrosa	"-"	"-"	October, 1980
Kühneromyces mutabilis	"-"	"-"	October, 1979
Armillaria mellea	Tricholomataceae	"-"	September,1982
Pleurotus ostreatus	Pleurotaceae	Germany	October, 1993

Preparation of mushroom extracts

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Methanol and aqueous extractions were carried out according to Badalyan *et al.* [4].

The residues of methanol extracts were used for TLC (free amino acids, free sugars, fungal toxins, alkaloids and nitrogen content compounds), HPLC (phenolic acids) and qualitative chemical methods (glycosides). Free sugars, alkaloids and nitrogen-content compounds of freeze-dried residues of the aqueous extracts were investigated by TLC, whereas glycosides by chemical reactions. Concentrations and Rf values of standard solutions were previously described [1, 4, 16, 17].

Qualitative estimation of free sugars

The method of TLC was used to analyze the aqueous and methanol extracts of five species on silica plate 60 F_{254} (Merck, ref. 5715) in the following solvent systems: ethanol-water (EW, 98:2, V/V); isopropanol-water (PW, 2:1, V/V), ethyl-acetate-methanol-chloroform-water (AMCW, 7:2:1:1, V/V) and *n*-butanol-acetic acid-water (BAW, 4:5:1). The TLC plates were sprayed by 2% benzidine reagent (0.5 g benzidine, 20 ml acetic acid, 80 ml ethanol) and aniline-phthaleine reagent (9.2 ml aniline, 16 g phthalic acid, 490 ml *n*-butanol, 490 ml ethyl ester, 20 ml water) at 110°C for 5 min. The TLC profiles of the extracts were compared with 0.1% sugar standard water solutions (W/V). The following colors and Rf values in BAW (4:5:1, V/V) solvent system were revealed: rhamnose (Rf=0.50, yellow-brownish), arabinose (Rf=0.31, reddish), fructose (Rf=0.29, yellow), glucose (Rf=0.24, brownish), galactose (Rf=0.23, brown), and sucrose (Rf=0.23, brown) [17].

The TLC analyses of methanol and aqueous extracts of tested mushrooms were performed using cellulose plates (Merck, ref. 5577) and in BAW solvent system (4:5:1, V/V) and developed with aniline phthaleine reagent.

Qualitative estimation of phenolic acids

Aliquots (1 ml) of the 20% (W/V) methanol extracts [1] were evaporated to dryness, then treated by 2 mL 5% NaOH in water (W/V) and extracted by diethylether (×3; 5 ml). The water solution was adjusted to pH 4-3 using 10% H_2SO_4 solution and partitioned with diethylether (×3; 5ml). The combined organic phases were dehydrated by Na_2SO_4 and evaporated to dryness. The residue was diluted by 0.5 ml 50% methanol solution for HPLC analysis.

Aliquot (3 µl) was injected into a Separon SGX - 7 µm, 150 mmЧ3.3 mm column on a HPP 400 chromatograph with a WCP 2563 detector. The mobile phase was methanol-isopropanol-heptan-ethyl acetate solution (1:3:97:99, V/V) at a flow rate of 0.2 ml/min. The phenolic acids were revealed by their fluorescence at 254 nm and compared with 0.1% detected standard methanol solutions (W/V): 4-hydroxybenzoic acid (HBA), 4-hydroxycinnamic acid (HCA), 3,4-dihydroxyphenylacetic acid (DPHA), and 4-hydroxy-3-methoxy-benzoic acid (HMBA). Their retention times (min) at 20-22°C were 16.0, 15.0, 14.6 and 13.6 for HMBA, DPHA, HCA and HBA, respectively.

Qualitative detection of alkaloids and nitrogen content compounds

The chloroform-ethanol solution (CE, 3:1, V/V) was used as a mobile phase [3] to test the methanol extracts on silica plates (Merck, ref. 5735). The TLC plates were sprayed by Dragendorff reagent according to Munier and Macheboeuf (Stahl, 1969). The Rf values, colors and concentrations of standard solutions (W/V) were the following: 0.25% choline-chloride in methanol-water (1:1) (Rf=0.70, red-brown), 0.2% betaine in methanol (Rf=0.56, orange), 0.1% citizine in methanol (Rf=0.55, brownish), and 0.2% berberine in methanol (Rf=0.49, brown).

Qualitative estimation of free amino acids

The composition of free amino acids was determined in methanol extracts on cellulose plates (Merck, ref. 5577) in *n*-butanol-acetic acid-water (BAW, 40:15:5, V/V) solvent system. TLC plates were sprayed by 2% ninhydrine reagent (Stahl, 1969). The following Rf values and colours of 0.5 % standard solutions in methanol-water (15:85) were observed: methionine (Rf=0.39, dark-violet), valine (Rf=0.32, bright violet), alanine (Rf=0.21, dark-violet), proline (Rf=0.18, yellow), serine (Rf=0.08, violet) and asparagine (Rf=0.06, violet).

Qualitative detection of glycosides

The methanol and aqueous extracts of mushroom specimens were investigated for glycosides by the following chemical reactions [2]: Keller-Killiany (for detection of sugar in glycoside molecules), Legal (for detection of five-part lactone ring in glycoside molecules) and Molich (for detection of steroid glycosides).

Qualitative estimation of other fungal metabolites: cortinarin A and orellanine

The methanolic extracts were analyzed for the presence of cortinarin A and orellanine [9, 15].

RESULTS AND DISCUSSION

Free sugar content

Free sugars in the methanolic and aqueous extracts of the five tested species were not detected. Therefore, further glycoside tests revealed the bounded forms of sugar molecules in the analyzed extracts.

Phenolic acid content

HPLC examination of the methanol extracts of all tested species showed the presence of 4-hydroxybenzoic acid in all specimens. This phenolic acid was previously detected by TLC analyses within *Cortinarius armillatus* [4] and three *Hypholoma* species [5].

The DPHA was detected within four tested specimens except *Ph. gummosa*. Meanwhile, the HMBA was observed within *A. mellea* and *Ph. gummosa*, whereas HCA - only within *Ph. gummosa*. Both *Pholiota* species were found different by their phenolic acid content.

According to the bibliographic data the mushroom derived phenolic compounds including phenolic acids possess antiseptic, antioxidant and antifungal effects [6, 11, 12].

Alkaloid and nitrogen content compounds

Choline chloride, as dark-brown spots was identified in the five samples of tested mushroom extracts. It is well known that choline plays an important role in lecithin metabolism and transmethylation reactions and can be used as nutritional and lipotropic agent. Citizine, berberine, betaine and other nitrogen content compounds were not detected within tested mushroom species.

Free amino acid content

According to Chivrina *et al.* (1969) glutaminic acid, asparagine and alanine are widely distributed in basidiomycete mushrooms. Alanine, serine and proline were detected in all extract specimens. Methionine and asparagine were not identified in *Ph. gummosa* and *Ph. squarrosa*, respectively. Valine was absent in both *Pholiota* species, and *P. ostreatus*.

Glycoside content

Glycosides are biologically active substances well studied within plants. However, there is not enough data concerning mushroom-origin glycosides, their physiological activity and applications in pharmacology [5, 18]. Meanwhile, these compounds are precursors of the D-vitamin and steroid hormone synthesis. They possess cytotoxic (cytotoxic steroids) and neurotoxic activities (neurotoxic glycosides).

High amounts of glycosides, detected by Keller-Killiany qualitative reactions, were found in methanol extracts *Ph. squarrosa, K. mutabilis,* and *A. mellea,* whereas in water extracts were detected only within *Ph. gummosa*. The lower amounts of methanol extracts were revealed within *P. ostreatus* and *Ph. gummosa* (Table 2). Five-lactone ring glycoside molecules, detected by Legal reaction were revealed within all tested samples. The presence of steroid glycosides was found only in *Ph. squarrosa* methanol extract by Molich reaction.

Table 2.

Glycoside content in fruiting body extracts of tested mushrooms

Species	Methanol extracts			Aqueous extracts
	Keller-Killiany-reaction	Legal reaction	Molich reaction	Keller-Killiany reaction
Ph. gummosa	+‡)	+++	-	+++
Ph. squarrosa	+++	+++	+++	+
K. mutabilis	+++	+++	-	-
A. mellea	+++	+++	-	-
P. ostreatus	+	+++	-	-

Notes: (-): absence; from (+) to (+++): presence

Other fungal metabolites content

Other fungal metabolites, such as cortinarin A were present in all mushroom extract samples, meanwhile orellanine - was not detected within any of them.

CONCLUSION

Data concerning the TLC, HPLC and qualitative chemical analyses of methanol and aqueous extracts of *Ph. gummosa, Ph. squarrosa, K. mutabilis, A. mellea* and *P. ostreatus* showed that these mushrooms contain biologically active substances. Phenolic acids, such as 4-hydroxybenzoic, 4-hydroxy-3-methoxybenzoic, 4-hydroxycinnamic, 3,4-dihydroxyphenylacetic, as well as nitrogen content compounds (choline-chloride), free

amino acids (alanine, asparagine, valine, serine, proline, and methionine); cortinarin A and glycosides were detected by our methods. The sugars (glucose, arabinose, fructose, galactose, rhamnose and sucrose) were mainly found in the bounded state.

Further investigations of the physiological and myco-pharmacological activities of screened mushrooms are in progress.

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Ամփոփում

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ՈՐՈՇ ՍՆԿԵՐԻ ՊՏՂԱՄԱՐՄԻՆՆԵՐԻ ԷՔՍՏՐԱԿՏՆԵՐԻ ՔԻՄԻԱԿԱՆ ԿԱԶՄԻ ՀԱՄԵՄԱՏԱԿԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒՄ

Ծառաբնակ բազիդիալ մակրոսկոպիկ սնկերի (*Pholiota squarrosa, Pholiota gummosa, Kȟhneromyces mutabilis, Pleurotus ostreatus* and *Armillaria mellea*) պտղամարմիններից ստացված ջրային և մեթանոլային էքստրակտները ուսումնասիրվել են նրանց մեջ ազատ շաքարների, ամինոթթուների, ֆենոլային թթուների, սնկային տոքսինների, գլիկոզիդների, ալկալոիդների և ազոտային միացությունների բացահայտման նպատակով: Կիրառվել են նրբաշերտ և բարձր էֆեկտիվության հեղուկ քրոմատոգրաֆիաների մեթոդները, ինչպես նաև որակական քիմիական ռեակցիաներ:

Ուսումնասիրված էքստրակտներում հիմնականում բացահայտվել են ֆենոլային թթուներ (4-հիդրօքսիբենզոական թթու, 4-հիդրօքսիդարչնաթթու, 4-հիդրօքսի-3-մեթօքսիբենզոական թթու և 3,4-դիհիդրօքսիֆենիլքացախաթթու), ազատ ամինոթթուներ (ալանին, վալին, սերին, պրոլին, մեթիոնին, ասպարագին), կորտինարին A, ստերոիդային գլիկոզիդներ և խոլին: Ազատ շաքարներից, մասնավորապես գլյուկոզան, արաբինոզան, ֆրուկտոզան, գալակտոզան, ռամնոզան և սախառոզան չեն բացահայտվել մեր կողմից կիրառված մեթոդներով:

Ստացված տվյալները մատնանշում են ուսումնասիրված տեսակների բաղադրության մեջ կենսաբանորեն ակտիվ միացությունների (ֆենոլային թթուների, խոլինի, ստերոիդային միացությունների) առկայությունը:

Резюме

С.М.БАДАЛЯН, В.А.МНАЦАКАНЯН, С.РАПИОР, Ж.Ж.СЕРРАНО И К.АНДАРИ

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ХИМИЧЕСКОГО СОСТАВА ЭКСТРАКТОВ ПЛОДОВЫХ ТЕЛ НЕКОТОРЫХ ГРИБОВ

Водные и метанольные экстракты полученные из плодовых тел базидиальных дереворазрушающих макромицетов (*Pholiota squarrosa, Pholiota gummosa, Kȟhneromyces mutabilis, Pleurotus ostreatus* and *Armillaria mellea*) были анализированы на наличие в них свободных сахаров, аминокислот, фенольных кислот, грибных токсинов, гликозидов, алкалоидов и азотсодержащих соединений с использованием методов тонкослоиной (ТСХ) и высокоэффективной жидкостнои (ВЭЖХ) хроматографии, а также качественных химических реакций.

В тестируемых грибных образцах в основном были обнаружены фенольные кислоты (4-гидроксибензоиная, 4-гидроксокоричная, 4-гидрокси-3-метоксибензойная и 3,4-дигидроксифенилуксусная кислоты), свободные аминокислоты (аланин, валин, серин, пролин, метионин, аспарагин), кортинарин А, стероидные гликозиды и холин. Свободные сахараё в частности глюкоза, арабиноза, фруктоза, галактоза, рамноза и сахарозаё не были обнаружены нащими методами.

Полученные результаты указывают на наличие биологически активных соединений (фенольных кислот, холина, стероидов) в экстрактах плодовых тел исследованных видов грибов.