CHEMICAL CONTENTS IDENTIFICATION ON GC-MS FROM SELECTED SPECIES OF MACROMYCETES

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ABSTRACT. Edible (Xerula radicata), inedible (Russula foetens), semi-edible (Lactarius piperatus) and poisonous mushrooms (Amanita phalloides) were analyzed on gas chromatography-mass spectrometry for its chemical contents. Analysis performed on GC-MS shown a high content of phenolic compounds in these basidiomycete species. Differences between chemical contents with 45 % were observed between young and mature species. Content of selected phallotoxins was determined in Amanita phalloides species.

Keywords: volatile compounds, organic fractions, basidiomycetes, GC-MS

INTRODUCTION

Mushrooms in the division of basidiomycota are worldwide distributed. Four species such as Xerula radicata, Russula foetens, Lactarius piperatus and Amanita phalloides, found in Faget forest, were studied for chemical compounds content.

Edible basidiomycetes such as Xerula radicata, commonly known as the rooting shaker or beech rooter is an edible basidiomycete fungus of the genus Xerula. As many researches shown, *Xerula radicata* is appreciated due to its medicinal properties as antihypertensive effects due to contain oudenone ((S)-2-[4,5-dyhydro-5-propyl-2-(3H)-furylidene]), an antihypertensive molecule. This molecule is a fungal metabolite and was reported as a strong inhibitor of catecholamine biosynthesis, inhibiting the enzymes phenylalanine and tyrosine hydroxylase (1). Different study has shown that the physiological effect of this enzyme inhibition is the reduction of blood pressure (2). Also Xerula radicata has been shown to contain an antibiotic named oudemansin X (E-β-methoxyacrylate oudemansin X, antifungal metametabolite), which lacked antibacterial activity against various organisms tested, but showed a good antifungal activity (3). *Lactarius piperatus* also known as the peppery milk cap is a semi-edible

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(depending on regions from world, for example in Romania this mushroom is consumed by many people) basidiomycete fungus of the genus Lactarius. It's known also to have some antioxidant activity, based on its chemical content (4). *Russula foetens* named also as Fetid russula is an inedible fungus of genus Russula, but recent medical studies shown that due to its polysaccharides content present antitumoral effects (5, 6, 7). As poisonous mushroom, Amanita Phalloides was studied, known also with Death cap name. These species of Amanita genus is a seriously poisonous basodiomycetes fungus, its toxicities is thanks to presence of two main group of toxins such as amatoxins and the phallotoxins, both multicyclic peptides, spread throughout the mushroom tissue (8).

RESULTS AND DISCUSSION

The volatile fractions were obtained from the fresh mushrooms fruiting bodies and analyzed as described in experimental section. The results obtained for the four species studied are presented in table 1.

Table 1. Volatile compounds present in the four basidiomycetes studied (Percent of the total ion current, GC-MS*,**)

Compounds	Xerula r. (%)	Lactarius p. (%)	Russula f. (%)	Amanita ph. (%)
Phenol	0.8	0.9	1.1	2.9
Hexanal	0.5	-	0.4	0.8
2.4-decadienal	-	-	0.2	0.5
2-undecanone	0.1	0.1	0.2	0.2
Acetic acid	1.2	1.1	1.2	2.1
Hydroxy acetic acid	-	-	-	0.1
Hexadecanoic acid	1.1	0.8	0.7	1.3
Hydroxyquinone monopryl ether	0.5	0.4	1.2	2.8
Acetic acid phenyl ether	0.1	0.1	0.5	0.3
Hexadecanoic acid ethyl ester	1.5	1.8	0.9	2.2

*) The ion current generated depends on the characteristics of the compound concerned and is not a true quantification.

**) All matching (as percentages) of mass spectrometry data with literature data were in the range of 83 – 98 %.

The most founded compounds in all mushroom samples were the phenolic compounds. These results in quietly normal, based on the several researches reported in the last decades. They also suggested that the production of phenolic compounds originates from an evolutionary ancestral biochemical shift. Some research sustain that these phenolic compounds may be involved in a chemical mechanism against insects and microorganisms (9).

After the comparison of chemical contents results in each parts of the analyzed mushroom it was observed that in the cap and flesh higher value with 20 – 30 % were determined than in the stem of the same basidiomycete's species. Also for volatile compounds the levels were higher in the young mushrooms species with 15 % than in the mature mushrooms (for the same species), but the content of some organic compounds (such as urea, tyrosine, proline, glycine, resorcinol and catechol) were lower with almost 45 % than in the mature mushrooms as shown in table 2.

Table 2. The main organic fraction constituents in xerula radicata and Lactarius piperatus basidiomycetes as a comparation between young and mature mushrooms (Percent of the total ion current, GC-MS*,**)

Compounds	**Xerula r.**		**Lactarius p**	
	Young sp.	***Mature sp.***	***Young sp.***	***Mature sp.***
Urea	3.5	4.9	3.3	5.0
Tyrosine	-	-	-	0.1
Proline	0.3	0.7	0.2	0.5
Glycine	-	0.2	-	-
Resorcinol	0.2	0.3	-	0.2
Catechol	0.3	1.2	0.1	0.4
Valine	-	0.3	-	0.2
Leucine	0.1	0.4	-	-

*) The ion current generated depends on the characteristics of the compound concerned and is not a true quantification.

**) All matching (as percentages) of mass spectrometry data with literature data were in the range of 83 – 98 %.

In Amanita phalloides were also identified α-amanitin, β-amanitin, amanin and amanullin in an average of 0.5 % in mature species. Also compounds such as phalloidin, phalloicin and prophalloin belonging to phallotoxin class, characteristic for these species was determinate in an average of 0.8 % in mature mushrooms.

CONCLUSIONS

The results obtained add new stocks to the knowledge on the chemical composition of the selected species mushrooms belonging basodiomycetes division studied in this work. These dates help to provide further explanation for their reported toxicity or positive medical effects in some health anomaly.

EXPERIMENTAL SECTION

Collection of samples: The four basidiomycetes were collected from Faget forest, near Cluj-Napoca, Romania in June 2009.

Extraction: The fresh fruiting bodies of basidiomycetes were separated as: cap, gills, flesh and stem. All of these parts were cut again in very small species as possible, and from them 10 g from every group was put to leach with trichloromethane for 1 hour in order to establish volatile compounds content for each species of basidiomycetes. Also the same think was done but with ethanol and for 24 hour in order to establish the organic compounds content in each mushroom species. After soak they were extracted with headspace heated ultrasound apparatus (Elmasonic S 10 H) for 1 hour for separate the volatile compounds. The solid phase microextractiom technique was performed on each sample in order to analyze the organic compounds contents from mushroom samples.

Analysis of volatile organic compounds: They were analyzed by gas chromatography with electron capture detector (ECD) and flame ionization detector (FID) with Trace GC Ultra apparatus and DSQ II quadupole mass spectrometry. TR-V1 cyanopropylphenyl polysiloxane based phase capillary column (30 m $\times$ 0.53 mm ID $\times$ 3.0 μm) was used to perform all analysis. The FID detector temperature was set at 300 °C and the ECD detector at 250 °C. The mixture was heated at 80 °C for 30 minutes. The temperature program was programmed from 40 – 220 °C at a rate of 7 °C min^{-1}, and nitrogen was used as carrier gas.

Analysis of organic compounds: They were analyzed on gas chromatography coupled to an guadrupole mass spectrometer (DSQ II – FOCUS GC) with columns Thermo TR-WaxMS (30 m $\times$ 0.25 mm ID $\times$ 0.25 μm). The ion source was set at 270 °C and the ionization voltage at 70 eV. The column temperature program was set from 40 °C with 5 °C min^{-1} to 100 °C, with 3 minutes hold time and after that continued with 10 °C min^{-1} to 280 °C and maintained at this temperature for 7 minutes. Helium was used as carrier gas.

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