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ANTIOXIDANT ACTIVITY OF HONEY MUSHROOMS (*ARMILLARIA MELLEA*)

Strapáč, I.[1], Baranová, M.[2]
Smrčová, M.[1], Bedlovičová, Z.[1]

[1]Department of Chemistry, Biochemistry and Biophysics, Institute of Pharmaceutical Chemistry
[2]Department of Food Hygiene and Technology, Institute of Milk Hygiene and Technology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

imrich.strapac@uvlf.sk

ABSTRACT

Mushrooms *Basidiomycota* have long been appreciated for their taste, flavour, desirable aroma, texture, nutraceutical and medicinal attributes. Honey mushrooms (*Armillaria mellea*) are edible mushroom generally used in traditional oriental medicine. The aim of this study was to examine extracts from the fruiting bodies of these mushrooms. The content of the components with antioxidant properties, such as total phenols, total flavonoids, β-carotene, lycopene and β-glucans were determined by spectrophotometric methods. The results obtained showed that the water extracts contained higher levels of total phenols and total flavonoids (367.1 and 548.5 mg.dm^{-3} gallic acid equivalent — GAE, respectively) in comparison with methanol extracts (108.2 and 113.4 mg.dm^{-3} GAE, respectively). Very low contents of β-carotene and lycopene were determined in the methanol extract (0.756 mg.g^{-1} dry weight and 0.05 mg.g^{-1} dry weight, respectively). Methanol extracts from the fruiting bodies of Honey mushrooms (*Armillaria mellea*) inhibited the uptake activity of 2,2-diphenylpicrylhydrazyl (DPPH) free radicals by 45 %. The IC50 (mg of compound, that inhibit 50 % of DPPH radicals) of methanol extract was below 10 mg.cm^{-3} (6.448 mg.cm^{-3}), suggesting a high antioxidant potential of fruiting bodies of the Honey mushrooms *Armillaria mellea*.

Key words: antioxidant property; *Armillaria mellea*; Honey mushrooms; polyphenols; total flavonoids

INTRODUCTION

Honey mushrooms (*Armillaria mellea*) are an autumn mushroom growing in our latitudes. They grow in tufts on the living and dead trees, causing significant damage in forestry. They are an excellent edible mushroom which can be culinary processed in different ways. They can be dried or pickled. Some people are allergic to Honey mushrooms (*Armillaria mellea*) and for some consumers *Armillaria mellea* are difficult to digest or slightly toxic. They are collected as young fruiting bodies. The stems are often ligneous, therefore, they are not consumed. These mushrooms

contain minerals, healthy fibre, vitamins and are low in fat. They have been traditionally used in alternative medicine in many countries, mainly in Asia, for their antimicrobial and anti-carcinogenic effects [8] and the content of health-promoting substances such as: polysaccharides, sterols, sphingolipids, fatty acids, sesquiterpenoids, indole compounds, peptides, and for their enzymes involved in the immunostimulatory and immunomodulatory responses [9, 13]. They participate in the protection of the brain [20] and bone marrow cells [4]. Honey mushrooms (*Armillaria mellea*) contain large amounts of biologically active substances. From arylesters of sesquiterpenes, there are included: melleolid, armillarin, armillaridin, arnamial, armillaric acid (exhibit antimicrobial activity against gram-positive bacteria), as well as judeol, melleolid, 4-o-methylmelleolid and meleollids B–D, K, L and M [12], [16]. Aerobic organisms need oxygen for their life cycle. In cells, the oxidation-reduction reactions result in the formation of: reactive oxygen species, free oxygen radicals, such as superoxide $O_2^{\bullet -}$, hydroxyl radical $HO^{\bullet}$, peroxide radical $ROO^{\bullet}$, alkoxy radical $RO^{\bullet}$, hydroperoxy radical $HO_2^{\bullet}$, hydrogen peroxide H_2O_2, nitric oxide NO and hypochlorous acid (HOCl). Reactive oxygen species are generally considered to be by-products of damaged cells. It is now known that these oxygen species are the essential mediators of cellular signalling and regulation [14]. A disturbance of the balance between the need for oxygen and an excess of free radicals in cells leads to oxidative stress and violation of cell membranes, which may cause damage to the body. Uncontrolled production of reactive oxygen species is the trigger for many diseases, such as: cancer, atherosclerosis, liver damage, degenerative processes associated with lipid peroxidation of cell walls and inhibition of protein synthesis, and many other diseases [5, 6, 18]. Almost all organisms are protected against reactive oxygen species by enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase. To reduce the oxidative damage to an organism caused by oxygen free radicals, synthetic antioxidants are now used such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tercbutylhydroquinone (TBHQ) [2, 18]. Currently, there is a search for the source of natural antioxidants which are less toxic than the synthetic antioxidants. One of the sources of antioxidants is the edible fungi *Basidiomycota*.

The aim of this study was to determine, in the fruiting bodies of Honey mushrooms (*Armillaria mellea*), the content of some substances which are supposed to have antioxidant properties.

MATERIALS AND METHODS

For our analysis were used 1 kg of freshly harvested Honey mushroom (*Armillaria mellea*) fruiting bodies with a moisture of 87.24 %, collected in the autumn of 2014 in an area of Bankov near Košice, in the Slovak Republic. The mushrooms were dried at 60 °C to a constant weight and then homogenized into a fine powder.

The analysis was carried out using different extracts. Water and methanol extracts were prepared by the extraction of 100 mg samples in 2 cm^3 of water or methanol for 24 hours with occasional vigorous stirring at 8 °C in a refrigerator. After returning to room temperature, the extraction mixtures were filtered through filter paper (Whatman No. 4) and the filtrates were used for the determinations of the total phenolic compounds and total flavonoids and the uptake activity of metanolic extracts of fungi. For the determination of lycopene and β-carotene, we prepared the extract from 100 mg of dried powdered Honey mushroom fruiting bodies using a solvent mixture of acetone and n-hexane at a ratio of 4 : 6 (v/v) [19].

The total phenolic compounds were determined by a micromethod, using Folin-Ciocalteu reagent (Sigma, USA) according to the method described by Waterhouse [21]. The determination of the total flavonoids was carried out according to the method published by Konczak [10]. The determination of the uptake activity of methanolic extracts of the fungi was carried out based on the methodology described by Tsai et al. [17]. Beta-glucans were isolated according to the patent No. 285 062 of 2006 [19]. The content of Beta-glucans (lycopene and β-carotene) was determined by a spectrophotometric method of Nagata and Yamashita [7] and Dasgupta [1].

All chemicals and water were of an analytical grade and p. a. purity. For the determination of the total phenolic compounds, the calibration of signals was performed using gallic acid (Fisher Scientific, UK). Ascorbic acid (LACH NER Ltd., Czech Republic) was used for the determination of the inhibitory activity of methanolic extracts of mushroom against 2,2-diphenylpicrylhydrazyl (DPPH) radicals (Sigma, USA). Spectrophotometric measurements were performed by a UV VIS spectrophotometer (Biochrom Li-

Table 1. Mean content of the total phenolic compounds, total flavonoids, β-carotene and lycopene in the extracts of Armillaria mellea

Armillaria mellea	mg.dm^{-3} GAE	SD	mg.g^{-1} GAE DW	µg.mg^{-1} GAE DME
Total phenolic compounds				
Methanol extract	108.2	0.005	2.2	6.1
Water extract	367.1	0.007	7.3	ND
Total flavonoids				
Methanol extract	113.4	0.007	2.3	6.4
Water extract	548.5	0.012	11.0	ND
	mg.100 cm^{-3}		**mg.g^{-1} DW**	
B-glucans acetone + n-hexane 4:6 (v/v)				
β-carotene	0.0076	0.0001	0.756	
lycopene	0.0005	0.0001	0.05	

SD — standard deviation; ND — not detected;
GAE — gallic acid equivalent; DW — dry weight; DME – dry methanol extract

Table 2. Uptake activity of Armillaria mellea methanol extract against DPPH free radicals

Measured quantities	% of inhibition	mg.dm^{-3} AAE	mg.g^{-1} AAE DW	µg.mg^{-1} AAE DME	IC50 mg.cm^{-3}
Methanol extract	45	53.21	1.06	3.02	6.448

AAE — ascorbic acid equivalent; DW — dry weight; DME — dry methanol extract
IC50 = mg of compound, that inhibit 50 % of DPPH radicals

bra S12, England). The wavelengths used for measurements are stated in the cited methods.

Results are reported as means of three measurements with the corresponding SD.

RESULTS AND DISCUSSION

It was found that 1 ml of methanol extract contained 17.6 mg of methanol extractable compounds, which is equivalent to 352 mg of methanol extractable compounds in 1 g of dried fungi. The mean contents ± SD of the total phenols (TP), total flavonoids (TF), β-carotene and lycopene are listed in Table 1. The antioxidant activity of methanol extracts of Honey mushrooms (*Armillaria mellea*) against DPPH free radicals is presented in Table 2.

Honey mushrooms (*Armillaria mellea*) contain phenolic compounds and flavonoids. From Table 1 it is evident, that the water is a better extracting agent for these compounds. In comparison with methanol, 3.4-times higher amounts of total phenolic compounds were extracted by water. Water extracted 4.8-times higher amounts of total

flavonoids than methanol. Similar results were obtained by Lung and Chang [5], who extracted almost identical compounds from Honey mushroom (*Armillaria mellea*) tissue cultures by hot water and methanol. The yield of extraction by hot water was considerably higher than that by methanol. The antioxidant activity of extracts correlated with the amount of the respective antioxidants in the extracts [3, 6]. Phenolic acids, lignans and flavonoids with -OH and -COOH functional groups are better extracted by polar solvents [22]. When comparing the content of the total phenolic compounds and flavonoids in methanol extracts converted to gallic acid equivalent (GAE), we obtained almost identical results for 1 g of dry mushroom (2.2 and 2.3 GAE.g-1 DW) and for 1 mg of dry methanol extract (6.1 and 6.4 μg.mg-1GAE DME). These results indicate that the contents of total flavonoids and phenolic compounds extracted by methanol were quite similar.

The water extracts showed greater differences, so we can conclude that the other investigated substances are extracted only by water and not by methanol. The samples of Honey mushrooms (*Armillaria mellea*) showed a low content of β-carotene, which also contributed to the antioxidant activity of fungi. The lycopene exhibited an even lower content (50 mg.g^{-1} DM). From 100 g samples, we isolated only 1.13 g of β-glucans, which was a low yield compared to 7.6 g per 100 g of edible portion as stated by Muszyńska [13]. It may be caused by differences in laboratory isolation compared to the conditions specified in Patent No. 285 062.2006 [19]. β-glucans and chitins affect the immune system, reduce blood pressure and blood glucose, and have an antibacterial, antiviral and anti-inflammatory effect [15]. An important indicator of antioxidant activity was an uptake activity of DPPH free radicals. In our experiments the methanol extract of Honey mushrooms (*Armillaria mellea*) showed 45 % inhibition of DPPH free radicals, equivalent to 53 mg of ascorbic acid per 1 dm^{-3} solution and 1.06 mg of ascorbic acid per 1 g of dry Honey mushrooms (*Armillaria mellea*) and it is equivalent to 3.02 μg of ascorbic acid per 1 mg of methanol extractable substances. Lung and Chang [5] detected 83.2 % inhibition of DPPH radicals in a methanol extract of Honey mushroom (*Armillaria mellea*) tissue culture using a concentration of 10 mg of methanol extract per 1 ml of solution. The methanol extracts of different species of fungi have different inhibitory activity against DPPH radicals as declared by Mau [11], who determined for methanol extracts of mycelia, 10 mg.ml^{-1} an inhibitory activity equal to 78.8 % for Parasol mushrooms (*Termitomyces albuminosus*), 79.4 % for Maitake mushrooms (*Grifola frondasa*) and 94.1 % for Morel mushrooms (*Morchella esculenta*).

An important indicator of the antioxidant activity was the IC50 value with the unit mg.cm^{-3}, which determines how many mg of extractable compounds in 1 ml solution inhibit 50 % of the DPPH radicals. It was stated that extracts possessed good antioxidant properties when their IC50 value was below 10 mg.cm^{-3} [11]. The IC50 equal to 6.448 mg.cm^{-3}, which was established in our study for the methanol extract of Honey mushrooms (*Armillaria mellea*) was in compliance with this requirement which indicates that Honey mushrooms (*Armillaria mellea*) exhibits good antioxidant properties.

CONCLUSIONS

The present study characterizes the fruiting bodies of Honey mushrooms (*Armillaria mellea*) collected in the Slovak Republic in terms of the content of total phenols, total flavonoids, β-carotene, lycopene and β-glucans; the compounds which are believed to have antioxidant properties. The results obtained by analysing methanol and water extracts confirmed the presence of these substances in the fruiting bodies of Honey mushrooms (*Armillaria mellea*). The methanol extracts showed inhibitory activity against DPPH free radicals. The IC50 of methanol extract was below 10 mg.cm^{-3}, confirming a high antioxidant potential of Honey mushroom (*Armillaria mellea*) fruiting bodies. This knowledge makes these mushrooms a functional food with the possibility of using their antioxidant potential in the pharmaceutical industry.

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