

Phenolic content and antioxidant activity of mushroom extracts from Bosnian market

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Abstract: Mushrooms are well balanced food that provides definite nutrition and health benefits for humans. Mushrooms are known to produce different kinds of bioactive compounds, generally linked with mycelial cell wall, that help in enhancing the capacity of immune system to fight against carcinogens. To consider the importance of polyphenolic compounds and its presence in many varieties of mushrooms, the total antioxidant activity of dry boletus mushroom, white and brown champignon, oyster mushroom and shiitake from bosnian markets was determined. Total phenolic content was estimated as Galic acid equivalents /g spectrophotometrically according to the *Folin-Ciocalteus* method. Total anthocyanine content was analysed by pH differential spectrophotometric method at 525 and 700 nm. The radical scavenging activity (RSA) of mushroom extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The analysis revealed that the total phenolic contents ranged from 4.94 mg GAEg⁻¹ in oyster mushroom to 35.56 mg GAEg⁻¹ in dry boletus mushroom. DPPH scavenging activity was the highest for brown champignon with value of 88.33 % and the lowest one was for oyster mushrooms with value of 43.88 %. The mushrooms examined in the present study could represent easily accessible sources of natural antioxidants.

INTRODUCTION

Mushrooms have been a part of human diet in many regions of the world for centuries due to organoleptic characteristic as well as the nutritional values (Wang and Xu, 2014). In nature, there are over 150 000 different types of mushrooms but only 10% is known and designated (Wasser, 2010). However, only about 2 000 species are grown and cultivated for nutritional purposes. The consumption of the mushrooms has even increased remarkably over the past few decades (Gan et al., 2013). Mushrooms are tasteful food, full of proteins, rich in vitamin B, rich in different minerals and have almost all essential amino acids (Mujić et al., 2011). Examination of antioxidant activity in mushroom extracts and content of antioxidant compounds is currently very interesting aim of research.. Mushrooms are found to be rich source of these antioxidants with immense antiradical activity (Valentão et al., 2005).

Phenolic acids were the major phenolic compounds reported in mushrooms.

The antioxidant activity of anthocyanins including the protection of low density lipoproteins (LDL) against oxidation, has been demonstrated in a number of different *in vitro* systems. Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano et al., 1989). In this study the radical scavenging activity (RSA) as well as the polyphenolic content and anthocyanins content of five edible mushrooms (*Boletus edulis*, *Agaricus bisporus*, *Agaricus bisporus var. Avellaneous*, *Pleurotus ostreatus*, *Lentinula edodes*) commercially available at Bosnian market was investigated. The amount of total phenol content was determined by *Folin-Ciocalteu* reagent method and total monomeric anthocyanin content was determined by pH differential spectrophotometric method.

MATERIAL AND METHODS

Plant material

Mushroom samples of boletus mushroom (*Boletus edulis*), champignon white (*Agaricus bisporus*), champignon brown (*Agaricus bisporus var. Avellaneus*), oyster mushroom (*Pleurotus ostreatus*), shiitake (*Lentinula edodes*) were collected in Bosnian market. Identification was done by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual (Moser, 1983; Bessette *et al.*, 2000; Uzelac, 2009). The investigated mushrooms are five of the most commercially cultivated ones available in Bosnia.

Chemicals and reagents

Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia), gallic acid (Fluka Chemica, Switzerland), anhydrous sodium carbonate (Kemika, Zagreb, Croatia) and methanol (Merck, Darmstadt, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, St. Louis, USA) and (Trolox) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma Aldrich, St. Louis, USA), hydrochloric acid (Sigma Aldrich, St. Louis, USA), potassium chloride (Fluka Chemica, Switzerland), acetic acid (Fluka Chemica, Switzerland), sodium acetate (Sigma Aldrich, St. Louis, USA) cyanidine-3-galactoside (Fluka Chemica, Switzerland) were used in this study. All the chemicals were of analytical grade purity.

Sample preparation

The fresh mushrooms 0.5 g were cut into small pieces, crushed in a mortar with pestle and consecutively extracted with 10 mL of 80 % ethanol. After maceration, extracts were put in centrifuge (Tehnica Železniki LC-320) at 4000 rpm for 20 min. and then the supernatant was separated. These obtained extracts were used for further investigations. Obtained extracts were stored in a refrigerator at 4°C until analysis.

Determination of total phenolic content

Total phenols (TP) were determined spectrophotometrically with *Folin-Ciocalteu* reagent (Waterhouse, 2002). The sample (2 mL) was dissolved in ethanol and mixed with 10 mL *Folin-Ciocalteu's* reagent diluted 1/10 with distilled water. After few minutes sodium carbonate (8 mL) was added to this solution. This solution was stored in dark place for two hours and after that, the absorbance was measured at 765 nm. A standard curve was prepared using gallic acid as standard with a concentration range from 100 to 500 µg/mL. Results are expressed in mg of gallic acid equivalents per gram (mg GAE g⁻¹) of mushrooms.

Determination of total monomeric anthocyanins

Total monomeric anthocyanins (TMA) content was quantified using a pH differential method (Giusti and Wrolstad, 2001). Samples were diluted in two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5) and then the absorbance was measured simultaneously at 525 nm and 700 nm, after 15 minutes of incubation at room temperature. Absorbance readings were made at room

temperature using distilled water as blank. A Spektronic Genesys TM2 UV-Vis spectrophotometer was used for determination. The content of total monomeric anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents (CGE) per gram of mushrooms. A molar extinction coefficient of cyanidin-3-glucoside of 26900 Lmol⁻¹cm⁻¹ and molar weight (MW) (449.2 g mol⁻¹) were used for calculations. The anthocyanin concentration was calculated according to the following equation:

$$\text{mg CGE/g} = \frac{A \times M_W \times D_f \times 1000}{\epsilon \times l}$$

where:

$$A = (A_{525} - A_{700})_{\text{pH } 1.0} - (A_{525} - A_{700})_{\text{pH } 4.5}$$

M_W- molecular weight of cyaniding-3-glucoside

D_f- dilution factor,

ε- molar absorbance

l - pathlength

DPPH Radical Scavenging Activity Assay

The mushroom extracts were mixed with methanol (96 %) and 63 µmol/L solution of DPPH. After 30 min. at room temperature, the absorbance was measured at 517 nm and converted into percentage of radical scavenging activity (Zeković *et al.*, 2010). The comparative analysis of samples was made by calculating DPPH scavenging activity which stands for the relative decrease of absorbance in the samples analysed. DPPH scavenging activity was calculated by using the following equation:

$$\text{DPPH scavenging activity} = 100 \times (A_c - A_s) / A_c$$

where:

A_c - absorbance of the control

A_s - absorbance of the sample.

RESULTS AND DISCUSSION

Results obtained for total phenolics, total monomeric anthocyanins and DPPH scavenging activity are presented in Table 1.

Table 1: Total phenolic content, total monomeric anthocyanins content and DPPH scavenging activity of dry boletus, champignon white, champignon brown, oyster and shiitake mushrooms

Name	TP (mg GAE /g)	TMA (mg CGE/g)	%RSA
dry boletus mushroom (<i>Boletus edulis</i>)	35.56	-	87.74
champignon white (<i>Agaricus bisporus</i>)	6.43	0.134	87.77
champignon brown (<i>Agaricus bisporus var. Avellaneus</i>)	7.66	-	88.33
oyster mushroom (<i>Pleurotus ostreatus</i>)	6.27	-	43.88
shiitake (<i>Lentinula edodes</i>)	4.94	0.134	71.85

TP- Total phenols; TMA - Total monomeric anthocyanins; RSA - Radical Scavenging Activity

It can be seen that total phenolics content ranged from 4.94 mg GAE g⁻¹ to 7.66 mg GAE g⁻¹ (fresh weight) and 35.56 mg GAE g⁻¹ (dry weight). According to review given by Mujić et al. (2011), content of total phenolics in mushrooms obtained in the range 7.8-23.07 mg GAE g⁻¹. 29.49 to 32.21 mg GAE g⁻¹ was determined by different investigators (Yildirim et al. 2012). On the other hand, it is difficult to compare our results with finding of other authors due to differences in extraction method applied, mode of expression of results (on dry or fresh basis of mushrooms), etc. For instance, Yildirim et al. (2012) used methanol to extract bioactive compounds from dry mushrooms. Ejelonu et al. (2013) extracted bioactive compounds from dry mushrooms with distilled water and obtained results in the range from 103.34 mg GAE g⁻¹ to 123.35 mg GAE g⁻¹. Furthermore, concentration of total phenols in medicinal mushrooms was between 4.45 mg GAE g⁻¹ to 14.44 mg GAE g⁻¹ (Abugri and McElhenney, 2013). Our results showed that the highest value of 7.66 mg GAE g⁻¹ was determined for fresh champignon brown and the highest value of 35.56 mg GAE g⁻¹ for dry boletus mushroom.

Total monomeric anthocyanins in investigated extracts were detected in champignon white and shiitake in an amount of 0.134 mg CGE g⁻¹ of fresh sample. Research results related to anthocyanin content in mushrooms are scarce.

Antioxidant activity of mushroom extracts investigated by DPPH method are presented in Table 1. The antioxidant activity of brown champignon extract exhibited a significant inhibition of DPPH with 88.33 %RSA and the lowest value determined for oyster mushrooms was 43.88 %RSA. Dry boletus, white and brown champignons showed a higher capacity of scavenging the DPPH radical than the extracts of oyster mushrooms and shiitake. These results can be considered as high antioxidant capacity, in the comparison with antioxidant capacity from various extracts of edible mushroom (*Pleurotus eous*) (Sudha et al., 2012).

Correlation between specific classes of bioactive compounds and antioxidant activity was also investigated. Obtained results are presented in Figure 1.

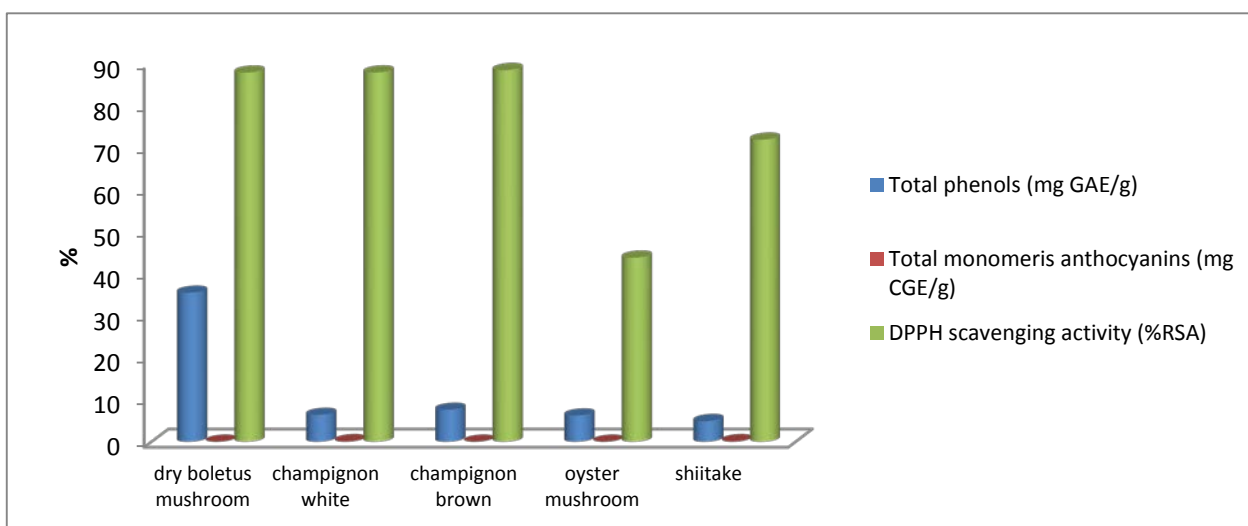


Figure 1. Correlation between DPPH scavenging activity, total phenolic content and total anthocyanin monomeric content

Maximum value of 88.33 %RSA was determined for champignon brown, and minimum value of 43.88 %RSA was determined for oyster mushroom, and was about 50% lower than those of champignons brown, and it was not in correlation with total phenolic content of investigated mushrooms.

CONCLUSIONS

The results of this study indicate that examined mushroom extracts possess good antioxidant activity. In all examined samples phenolic compounds have been detected, but monomeric anthocyanin compounds were detected only in champignon white and shiitake. Due to their high content of antioxidants, extracts of some mushrooms, especially champignon white and boletus, may be used as materials of dietary supplements. Their

rich antioxidant contents make the mushroom ideal nutritional supplement. Considering that mushrooms are of yellow, white, brown and dark hue, researchers have found that they are a good source of anthocyanins. Further studies are needed to identify which phenolic compounds are responsible for the antioxidant activity of the species, and assess the way in which the phenolic substances contribute to this activity. Additional *in vivo* antioxidant assays are necessary to confirm the potential use of these species in the treatment of different diseases. So we can conclude that those mushrooms represent a rich source of phenolic compounds and thereby might serve as possible nutraceutical food in human diet, and could help in the reducing the oxidative damage.

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Summary/Sažetak

Gljive su dobro izbalansirana hrana koja pruža određene prehrambene i zdravstvene pogodnosti za čovjeka. Gljive proizvode mnoge vrste bioaktivnih spojeva, uglavnom povezanih sa micelama ćelijskog zida, koji pomažu u jačanju sposobnosti imunološkog sistema da se bori protiv kancerogenih tvari. Da bi se razmotrila važnost polifenolnih supstanci i njihovo prisustvo u različitim vrstama gljiva, određena je ukupna antioksidativna aktivnost suhog vrgnja, bijelih i smeđih šampinjona i šitaki gljiva sa bosanskog tržišta. Sadržaj ukupnih fenola je izražen kao ekvivalent galne kiseline /g spektrofotometrijski metodom po Folin-Ciocalteu-u. Sadržaj ukupnih antocijanina je analiziran spektrofotometrijski pH-diferencijalnom metodom na valnim dužinama 525 i 700 nm. Antiradikalna aktivnost (RSA) ekstraktata gljiva je određena DPPH metodom. Analize su pokazale da se sadržaj ukupnih fenola kreće u rasponu od 4.94 mg GAE/g u bukovačama do 35.56 mg GAE/g u uzorku suhog vrgnja. Procenat inhibicije je bio najveći za smeđe šampinjone 88.33 %, a najmanji za bukovače 43.88 %. Gljive ispitane u ovoj studij predstavljaju lako pristupačan izvor antioksidanata.