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THE ANTIOXIDANT ACTIVITY OF HONEY AND HONEY WITH ADDED MUSHROOM CORIOLUS VERSICOLOR

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Abstract

In this paper were analyzed samples of honey and honey with added mushroom *Coriolus versicolor*. *Coriolus versicolor* is one of the most important fungi used in traditional Chinese and Japanese medicine for centuries. It is also found in Serbia, where it is also known as the turkey tail fungus. Active components of the fungus *C. versicolor* include ß-glucan proteins which exhibit antiviral, antibacterial and antioxidant activity. Honey has been reported to contain about 200 substances and is considered to be an important part of traditional medicine. The aim was to determined antioxidant activity, tothal phenols content and reducing power in these two samples. The total phenols content was determined using modified Folin-Ciocalteu method and the antioxidant activity by the method of quenching stable free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The reducing power of honey sample and honey with added mushroom *Coriolus versicolor* sample were determined by the method of Oyaizu. Higher total phenols content (26,04 mg/100 g) and higher reducing activity it was found in the sample of honey with *Coriolus versicolor*. That indicate that this mushroom has significant antioxidant activity.

Keywords: *honey, Coriolus versicolor, antioxidant activity, phenol compounds*

Introduction

Coriolus versicolor is one of the most important fungi used in traditional Chinese and Japanese medicine for centuries. It grows in clusters on fallen branches and logs in deciduous forests throughout the world. It is also found in Serbia, where it is also known as the turkey tail fungus. *Coriolus versicolor* is inedible because it has a tough tissue, but its aqueous extract has been used in traditional Chinese medicine since ancient times (Mau et al., 2005). The top surface of the cap is velvet-like and occurs in multiple colors, such as green, dark green, grey, black, reddish, rust, brown. The flesh is white. The underside of the cap shows minute pores numbering 3-8\mm². Active components of the fungus *C. versicolor* include ß-glucan proteins which exhibit antiviral, antibacterial, antioxidant, antitumor and immune-stimulating properties and ergosterol (provitamin D2) which has anti-inflammatory effects on the upper respiratory, digestive and urinary tracts (Smith et al., 2002). Honey, a nectar collected from many plants and processed by honey bees (*Apis mellifera*), is one of the oldest and widely used food product (Savatovic et al., 2011). Honey has been reported to contain about 200 substances (complex mixture of sugars, but also small amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals), and is considered to be an important part of traditional medicine (White,1979; Ferreira et al., 2009). Overall, honey serves as a source of natural antioxidants (Ferreira et al., 2009; Al-Mamary et al., 2002; Aljadi and Kamaruddin, 2004). Many studies indicated that the antioxidant activity of honey varies widely, depending on the floral source (Effem, 1988). Honey sample was multifloral from village near Kraljevo. The purpose of the present study was to determine the the total phenolic and flavonoid contents,

their antioxidant activity by different tests, including the reducing power and 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging assay of honey samle and honey sample with added mushroom *Coriolus versicolor*.

Material and methods

In this study it was used mushroom *Coriolus versicolor* that is collected on mountain Vujan near Gornji Milanovac. The fungus was air dried in the dark under constant airflow conditions. The dried material was properly stored until was added in honey. Honey wich is usued for the experiment was multifloral honey that is processed by honey bees that collected nectar from plants in the village near Kraljevo.

Reducing power

The reducing power of honey sample and honey with added mushroom *Coriolus versicolor* sample were determined by the method of Oyaizu (Oyaizu, 1986). For this purpose, solution of honey and honey with added mushroom *Coriolus versicolor* (10/120 mg) in 1 ml of distilled water or 1 ml of distelled water (blank) was mixed with 1 ml of 1% potassium ferricyanide $K_3[Fe(CN)_6]$. The mixture was incubated at 50°C for 20 min and then rapidly cooled. Following this, 1 ml of trichloroacetc acid (10%) was added and the mixture was than centrifuged at 3000 rpm for 10 min. An aliquot (2 ml) ot the upper layer, mixed with 2 ml og distilled water and 0,4 ml of 0,1% $FeCl_3$ was left to stand for 10 min. The absorbance of the mixture was measured at 700 nm against the blank.

The effective concentration (EC_{50}), assigned at 0,5 value of absorption, was used to define specific reduction capability. Ascorbic acid (10-120 µg/ml) was used as positive control.

Radical scavenging activity and antioxidant content

The scavenging activity (SA) of honey sample and honey with added mushroom *Coriolus versicolor* sample for the DPPH radical was measured spectrophotometrically using the modified DPPH method (Meda et al.,, 2005). Honey sample and honey with added mushroom *Coriolus versicolor* sample were dissolved in methanol, and 1,5 ml of each sample or 1,5 ml of methanol (blank) was mixed with 3 ml of DPPH in methanol (0,135 µg/ml). The range of the investigated honey concentrations was 0,33-166,67 mg/ml. The mixture were left for 15 min at room temperature and then the absorbances was measured at 517 nm against reference mixtures that had been prepared in a similar manner, by replacing the DPPH solution with methanol. The capability to scavenge the DPPH radicals, DPPH scavenging activity (SA) was calculated using the following equation:

$$SA\ (\%)=100\cdot(A_0-A_1)/A_0$$

where A_0 is the absorbance of the blank and $A_1$is the absorbance of the sample.

The effective concentration (EC_{50}), defined as the concentration of honey required for 50% scavenging of DPPH radicals under experimental condition employed, was used to measure the free radical scavenging activity. Ascorbic acid (0,33-166, 67 µg/ml) was used as positive control.

The antioxidant content was evaluated as described by (Meda et al. 2005). Honey samples were dissolved in methanol (50 mg/ml) and 1,5 ml of each solution was mixed with 3 ml of a 0,135 µg/ml solution of DPPH in methanol. The blank for each sample consisted of 3 ml of a methanolic honey solution (50 mg/ml) with 6 ml of methanol. The mixture was left for 15 min at room temperature and the absorbances were measured at 517 nm. The antioxidant content expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 100 g of honey was determined using standard calibration curves for ascorbic acid (0-1,67 µg/ml).

Results and discussion

Reducing power

The reducing power of samples was measured by the method of Oyaizu (Oyaizu, 1986). In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of the antioxidant substances in samples. The reducing power may serve as a significant indicator of its potential antioxidant activity.

The EC_{50} values of reducing power of samples are shown in Table 1. The results for reducing power demonstrate the electron donor properties of honey and honey with added *Coriolus versicolor*, thereby neutralizing free radicals by forming stable products. The outcome of the reducing reaction is the termination of the radical chain reactions that may otherwise be very damaging.

Table 1. EC_{50} (mg/mL) values of honey and honey with added *Coriolus versicolor* and controls (quercetin and ascorbic acid) in the antioxidant activity evaluation asssay: reducing power

Sample	Reducing power
Honey with added *Coriolus versicolor*	2,0030± 0,6920
Honey	1,5620± 0,9950
Ascorbic acid	0,0154± 0,0007
Quercetin	0,0317± 0,00 14

The scavenging activity

The scavenging activity (SA) of honey sample and honey with added mushroom *Coriolus versicolor* sample for the DPPH radical was measured spectrophotometrically using the modified DPPH method. The DPPH free radical scavening activity of the samples increased with increase in concentration. The higher SA values were found in honey with added *Coriolus versicolor*. The results are shown in Table 2.

Table 2. SA values of the samples

Samples	SA values %
Honey	37,40
Honey with added *Coriolus versicolor*	53,82

The total phenolics contents

The total phenolic contents in samples were determined from the regression equation of gallic acid calibration curve, and expressed as mg of gallic acid equivalents per 100 g of honey and honey with added *Coriolus versicolor*. The total phenolics are shown in Table 3. The higher content of total phenolics was in honey with added *Coriolus versicolor*.

Table 3. Total phenolics

Samples	Phenolocs (mg gallic acid/100 g)
Honey with added *Coriolus versicolor*	26,04±1,71
Honey	22,94±4,81

Conclusion

In this study, total phenolic, antioxidant content and antioxidant activity of honey and honey with added mushroom *Coriolus versicolor* were determined.

The content of total phenolics (26,04±1,71) was higher in honey with added *Coriolus versicolor*.

Also, higher reducing power and DPPH free radical scavening activity showed the same sample.

Based on this results the conclusion is that the honey with added mushroom *Coriolus versicolor* has significant antioxidant activity. Also, based on this the conclusion is that the mushroom Coriolus versicolor has significant antioxidant activity, but honey with added *Coriolus versicolor* has bitter taste.

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