ANTIOXIDANT PROPERTIES OF SELECTED EDIBLE MUSHROOM SPECIES
ANTIOKSIDATIVNE OSOBINE ODABRANIH JESTIVIH GLJIVA

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ABSTRACT

Mushrooms have a long tradition of use in many countries. They are food full of proteins, rich in vitamin B, rich in different minerals and have almost all essential amino acids. Mushrooms have been reported as useful in preventing diseases such are hypertension, hypercholesterolemia and cancer. Objective of this work was to evaluate the antioxidant properties of three edible mushroom species Lentinula edodes, Hericium erinaceus and Agrocybe aegerita. For determination of potential antioxidant activity content of antioxidant compounds, phenolics and flavonoids, and scavenging capacity on DPPH˙ radicals have been determined. Also the reducing power of obtained extracts have been investigated. The highest extraction yield has been achieved in L. edodes extraction (20.82%). Highest total phenolics (23.07 mg GAE/g) and total flavonoids (5.04 mg CE/g) content, as well as TF/TP ratio (21.85%) have been determined for A. aegerita dry extract. Radical scavenging activity was found to exhibit IC_{50} value for extract concentration of 0.198 mg/ml for H. erinaceus, 0.073 mg/ml for L. edodes and lower than 0.02 mg/ml for A. aegerita dry extract. All investigated mushroom dry extracts posses reductive capabilities.

Keywords: mushroom, antioxidant activity, antioxidant compounds, phenols, flavonoides

SAŽETAK

Gljive se od davnina koriste u mnogim zemljama. One su hrana sa visokim sadržajem proteina, bogate vitaminom B i različitim mineralima i sadrže gotovo sve esencijalne amino kiseline. Utvrđeno je da su korisne u prevenciji bolesti kao što su hipertenzija, hiperholisterolemija i kancer. Cilj ovog rada bio je određivanje antioksidativnog delovanje tri jestive gljive Lentinula edodes, Hericium erinaceus i Agrocybe aegerita. Radi utvrđivanja potencijalnog antioksidativnog delovanja određen je sadržaj antioksidativnih komponenata, fenola i flavonoida i antioksidativno delovanje na DPPH˙ radikale. Takođe ispitan je i reduktivni kapacitet dobijenih ekstrakata. Najveći sadržaj ukupnih fenola (23,07 mg GAE/g) i ukupnih flavonoida (5,04 mg CE/g), kao i odnos ukupnih flavonoida i ukupnih fenola (21,85%) određeni su za suvi ekstrakt gljive A. aegerita. Utvrđeno je da skevindžer aktivnost dostiže IC_{50} vrednost pri koncentraciji ekstrakta od 0,198 mg/ml za H. erinaceum, 0,073 mg/ml za L. edodes i koncentraciji nižoj od 0,02 mg/ml za suvi ekstrakt A. aegerita. Za sve ispitivane suve ekstrakte gljiva utvrđeno je da poseduju reduktivni kapacitet.

Ključne reči: gljive, antioksidativno delovanje, antioksidativne komponente, fenoli, flavonoidi

1. INTRODUCTION

Mushrooms have a long tradition of use in many Asian countries and have been used as a food and as a medicine. They are flavorful food, full of proteins, rich in vitamin B, rich in different minerals and have almost all essential amino acids. Mushrooms have been reported as useful in preventing diseases such are hypertension, hypercholesterolemia and cancer.

Today it is known that a wide variety of pathological damage, such are carcinogenesis and rheumatoid arthritis, can be caused by oxygen-derived free radicals. In living systems some free radical species, for example $^{\cdot}OH$ radical, can cause lipid peroxidation that is oxidative modification of low-density lipoproteins (LDLs). This process may play role in the development of arthrosclerosis. From all free radical species $^{\cdot}OH$ and $^{\cdot}O^{2-}$ radicals are found to be the main culprits in the damage that free radicals induce in biological systems. Almost all organisms are well protected against free radical damage by antioxidants and antioxidants systems such are enzymes, superoxide dismutase and catalase, and/or by compounds such are ascorbic acid, tocopherols and glutathione. In spite of that sometimes mechanism of antioxidant protection becomes unbalanced and then antioxidants take in by a food playing important role in reducing oxidative damage. Phenolic compounds, protein hydrolyzates and some amino acids, present in different food, were found to have antioxidant properties. Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups [2]. They were found to have excellent antioxidant activity in the inhibition of the mentioned LDL oxidation [11]. Today, in order to found dietary source full of antioxidants that can help to human body to reduce oxidative damage, different plant species have been explored: fruits, vegetables, herbs, seeds, etc.

Investigation of mushrooms and their extracts antioxidant activity and content of antioxidant compounds is very actual scientific subject [4, 12]. Objective of this work was to evaluate the antioxidant properties of three edible mushroom species Lentinula edodes, Hericium erinaceus and Agrocybe aegerita from Istra region, Croatia. This mushroom species are in use in Istra region as delicious food. Some of them are cultivated species today. As a mushrooms are considered to be a good source of proteins and phenolic compounds, such as variegatic acid and diboviquinone [4], for determination of antioxidant activity antioxidant compounds (phenolics and flavonoids) content, scavenging capacity on $DPPH^{\cdot}$ and reducing power have been investigated.

2. MATERIALS AND METHODS

2.1. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), butylated hydroxyanisole (BHA), Folin-Ciocalteu reagent, gallic acid and (±)-catechin, were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and reagents were of analytical reagent grade.

2.1. Plant material

Mushroom samples of L. edodes, H. erinaceus and A. aegerita were collected from Istra region, Croatia, in late summer of 2006. Identification and classification of this macrofungus was carried out. Mushrooms fruiting bodies were cleaned for removing any residual compost. Fresh and clean mushrooms were air-dried and then stored in air-tight plastic bags at the room temperature until use.

2.2. Sample preparation

All dried mushrooms samples were grounded in a blender before the extraction process. Mushroom sample (10.0 g) was extracted by 50% ethanol (50.0 ml). The extraction process was carried out using ultrasonic bath (Branson and SmithKline Company, B-220) at 45°C for 40 minutes. After filtration a volume of obtained liquid extract was measured and extraction solvent was removed by rotary evaporator (Devarot, Elektromedicina, Ljubljana) under vacuum. After that samples were dried at 60°C to the constant mass. In this way extraction yield was obtained. Dry extracts were placed into a glass bottles and stored at 4°C to prevent oxidative damage until analysis.

2.3. Antioxidant activity

2.3.1. Determination of antioxidant compounds

The content of total phenolic compounds in dry mushroom extracts was determined by Folin-Ciocalteu procedure [3.8], using gallic acid as a standard. Absorbance was measured at 765 nm. Content of total phenolic compounds has been expressed as mg of galic acid equivalent (GAE) per g of dry mushroom extract (mg GAE/g).

The total flavonoids content has been determined by aluminium chloride colorimetric assay [6], using catehin as a standard. It has been expressed as mg of catehin equivalents (CE) per g of dry mushroom extract (mg CE/g).

2.3.2. DPPH assay

The free radical scavenging activity of mushroom extracts was determined as described by Espin [1]. Briefly, the mushroom extract was mixed with methanol (96%) and 90 μM solution of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) to give final concentration 0.02, 0.05, 0.1 and 0.2 mg/ml of dry extract. After 60 min at room

Table 1. Extraction yield, total phenolics (TP) and flavonoids (TF) content

Mushroom species	Extraction yield (%, w/w)	TP (mg GAE/g)	TF (mg CE/g)	$\frac{TF}{TP}$ 100 (%)
L. edodes	20.82	11.70	1.98	16.96
H. erinaceus	11.34	7.80	1.61	20.62
A. aegerita	15.52	23.07	5.04	21.85

temperature, the absorbance was measured at 515 nm and converted into percentage of radical scavenging capacity. Radical scavenging capacity (%RSC) was calculated by following equation:

$$RSC(\%) = \frac{100 - (A_{sample} \times 100)}{A_{blank}}$$

where: A_{sample} is absorbance of sample solution and A_{blank} is absorbance of blank sample.

This activity has been also expressed as the inhibition concentration at RSC value of 50% (IC_{50}), i.e. the concentration of test solution required to give 50% of decrease in absorbance compared to the blank sample.

2.3.5. Determination of reducing power

The reducing power of mushroom extracts and ascorbic acid was determined by Oyaizu method [7]. Various concentration of mushroom extracts (0.2, 0.5, 1, 2 and 5 mg/ml) and ascorbic acid as standard antioxidant compound (0.025, 0.05, 0.1 and 0.2 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated for 20 minutes at 50°C. After incubation, 2.5 ml of 10% trichloroacetic acid solution was added to the mixture and mixture was centrifuged for 10 minutes (3,000 rpm). Obtained supernatant (2.5 ml) was mixed with bidestillated water (2.5 ml) and 0.1% $FeCl_3$ solution (0.5 ml). Absorbance of samples was measured at 700 nm. Higher absorbance of investigated indicates a higher reducing capability.

3. RESULTS AND DISCUSSION

3.1. Determination of antioxidant compounds

Numerous studies have showed that consumption of foods high in phenolic content can reduce risk of heart diseases by slowing the progression of atherosclerosis [5]. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g ingested daily from a diet rich in fruits and vegetables [10]. From all phenolic compounds flavonoids are considering as compounds with highest antioxidant activity due to their chemical structure. Extraction yield, total phenolics and total flavonoids, as well as total flavonoids/total phenols ratio (TF/TP) of three investigated mushroom species have been determined (Table 1).

The highest extraction yield has been achieved in a case of L. edodes extraction (20.82%). Highest total phenolics (23.07 mg GAE/g) and total flavonoids (5.04 mg CE/g) content, as well as TF/TP ratio (21.85%) has been determined for A. aegerita dry extract. This high content of total phenols and total flavonoids compounds could contribute to A. aegerita higher antioxidant activity in comparison to activity of other two investigated mushrooms.

3.2. DPPH assay

$DPPH^{\cdot}$ is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [9]. Decrease in absorbance of $DPPH^{\cdot}$ radical is caused by reaction between antioxidant molecules and the radical. This results in the scavenging of radical by hydrogen donation, what is visually noticeable as a discoloration from purple to yellow. This method is widely used to evaluate antioxidant activity because of relatively short time of analysis in comparison to some other methods. Scavenging effect of mushroom extracts on $DPPH^{\cdot}$ radicals increasing with increase of concentrations (Figure 1).

Radical scavenging capacity was found to exhibit 50% of inhibition value (IC_{50} value) for extract concentration of 0.198 mg/ml for H. erinaceus, 0.073 mg/ml for L. edodes and lower than 0.02 mg/ml for A. aegerita dry extract. With increasing of total phenols content IC_{50} value decrease, what indicate higher antioxidant activity. Highest antioxidant activity of dry A. aegerita extract is in correlation with its highest total phenols content in comparison to other two investigated. This mushroom extract could be considered as a product with high potential antioxidant activity.

3.3. Determination of reducing power

The reductive ability of mushroom extracts transformation $Fe^{3+} \rightarrow Fe^{2+}$, using the Oyiazu method [7], has been investigated. The reducing capacity of analyzed

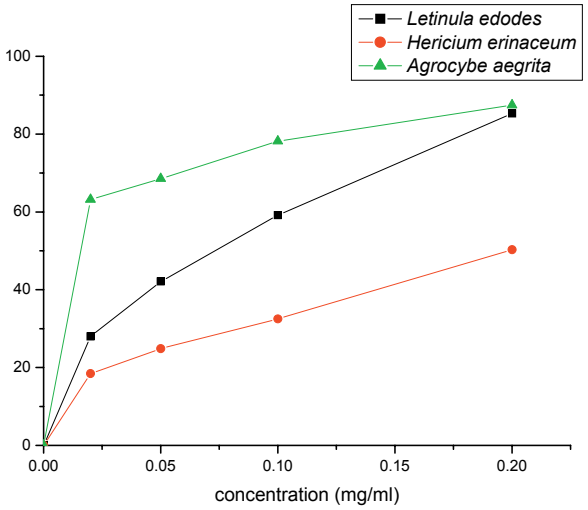

Figure 1. Radical scavenging capacity (%) of mushroom extracts at different extract concentration

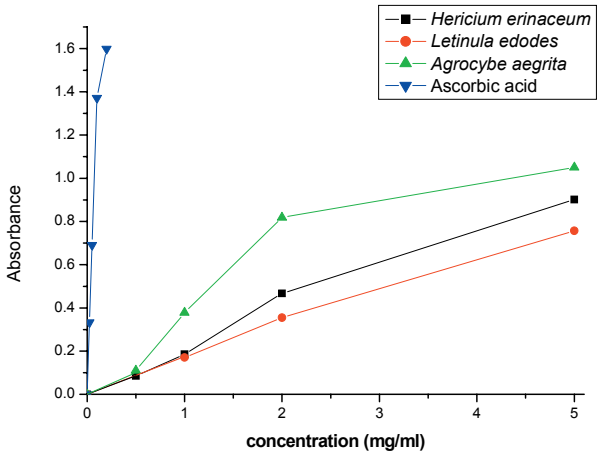

Figure 2. Reducing power of mushroom extracts and ascorbic acid at different concentration

mushroom extracts were compared to reductive capability of ascorbic acid, standard antioxidant compound.

All investigated mushroom dry extract posses reductive capabilities. It is clearly that ascorbic acid, as standard antioxidant, has a better reductive capability than investigated mushroom extracts. At the concentration of 1 mg/ml, absorbance of ascorbic acid cannot be measured because of high value what indicate very high reducing power, while absorbance of investigated extracts at the same concentration has been determined as 0.185, 0.171 and 0.378 for H. erinaceus, L. edodes and A. aegeita, respectively. Again, there have been detected the correlation between extract concentrations and reductive capabilities. The reducing power increases with the increasing of concentration.

Highest reductive capabilities have been determined for A. aegerita dry mushroom extract. H. erinaceus showed higher reductive capabilities than L. edodes extract. This is opposite than in the DPPH$^{\cdot}$ radical investigation case and could be the result of that that some other antioxidant compounds, not only total phenols, are responsible for reductive capabilities. From this it could be concluded that investigated mushroom extracts are primary antioxidant products, but they posses and reductive capabilities which are still much lower than of standard antioxidant compound ascorbic acid.

4. CONCLUSION

The results of this study indicated that investigated mushroom extracts possess antioxidant activity against various antioxidant systems. The antioxidant activity of mushroom extracts highly depends on extract concentration, i.e. concentration of active compounds. In all investigated mushroom extracts phenolic compounds have been detected, and all investigated possess antioxidant activity and reductive capability. Highest total phenols and flavonoides content, highest TF/TP ratio, lowest $IC_{50,}$ but also and highest reductive capabilities have been determined for A. aegerita mushroom extract. Because of well known nutrition value and determined potential antioxidant properties of all investigated mushrooms, L. edodes, H. erinaceus and especially A. aegerita, or their extracts, can be considerate for use as natural source of antioxidants or possible constituent in some food products or products of pharmaceutical industry.

5. REFERENCES

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