ORIGINAL ARTICLE

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND METALS CONTENTS OF *LACTARIUS SALMONICOLOR* (R. HEIM & LECLAIR)

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Abstract

The mushroom *Lactarius salmonicolor* (R. Heim & Leclair) was investigated for its antioxidant potential, total phenolic and metal contents. The antioxidant activity was studied by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals scavenging, reducing power, ferrous ion chelating and 15-lipoxygenase inhibition assays. The total phenolic content was evaluated using the Folin-Ciocalteu method. The metals content was analysed by inductively coupled plasma mass spectrometry (ICP-MS). The ethanolic extract, having a total phenolic content of 11.47 ± 0.19 mg/g, showed a high 15-lipoxygenase inhibitory activity being only 3.5 times less active than (+)-catechin (EC_{50} = 226.67 ± 0.95 and 64.17 ± 0.15 μg/mL, respectively). The concentrations of As, Cd and Hg in the fruiting bodies were found to be 0.3 ± 0.0, 0.05 ± 0.00 and 0.14 ± 0.00 mg/kg, respectively. With respect to these metals, the consumption of 300 g fresh or 30 g dry fruiting bodies daily has no health risk for the consumer. *L. salmonicolor* fruiting bodies proved to be an important source of Ca (3607.98 ± 1.15 mg/kg), Mg (934.67 ± 1.07 mg/kg), Se (1.49 ± 0.11 mg/kg) and Zn (152.53 ± 1.05 mg/kg). The results are promising regarding a possible use of *L. salmonicolor* ethanolic extract in the food supplements industry.

Rezumat

Studiul a evaluat activitatea antioxidantă, conținutul în polifenoli și metale al ciupercii *Lactarius salmonicolor* (R. Heim & Leclair). Activitatea antioxidantă a fost studiată prin determinarea capacității de *scavenger* față de radicalii 2,2-Diphenyl-1-picrylhydrazyl (DPPH) și 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), capacității reducătoare, capacității de chelatare a ionilor feroși și capacității de inhibare a 15-lipoxigenazei. Conținutul în polifenoli totali a fost determinat prin metoda Folin-Ciocâlteu. Conținutul în metale a fost analizat prin spectrometrie de masă cuplată inductiv cu plasmă (*ICP-MS*). Extractul etanolic, cu un conținut în polifenoli totali de 11,47 ± 0,19 mg/g, a prezentat o capacitate ridicată de inhibare a 15-lipoxigenazei, de numai 3,5 ori mai mică decât cea a (+)-catehinei (CE_{50} = 226,67 ± 0,95 și respectiv, 64,17 ± 0,15 μg/mL). În corpul fructifer al ciupercii au fost determinate concentrații de As, Cd și Hg de 0,3 ± 0,0, 0,05 ± 0,00 și respectiv, 0,14 ± 0,00 mg/kg. În ceea ce privește aceste metale, consumul zilnic a 300 g ciuperci proaspete sau 30 g ciuperci uscate nu reprezintă un risc pentru sănătatea consumatorului. Ciuperca *L. salmonicolor* s-a dovedit a fi și o importantă sursă de Ca (3607,98 ± 1,15 mg/kg), Mg (934,67 ± 1,07 mg/kg), Se (1,49 ± 0,11 mg/kg) și Zn (152,53 ± 1,05 mg/kg). Aceste rezultate sunt promițătoare în vederea unei posibile utilizări a extractului etanolic din *L. salmonicolor* în industria suplimentelor alimentare.

Keywords: *Lactarius salmonicolor*, 15-lipoxygenase, metal content

Introduction

Edible mushrooms have played an important role not only in the human diet but also in the traditional medicine, especially in the Asian countries. Recent studies have shown antioxidant, immune-modulating, antitumor, antithrombotic, cholesterol-lowering, antibacterial and antiviral effects for different mushroom extracts/constituents [3, 12].

Lactarius salmonicolor (R. Heim & Leclair) (*Russullaceae*) is a wild, edible mushroom that grows in the coniferous woods. Phytochemicals such as carbohydrates (mannitol, trehalose) [1, 9], fatty acids (oleic, linoleic, palmitic and stearic acids) [1, 5], phenolics (*p*-hydroxybenzoic and cinnamic acids) [20], tocopherols (α- and β-tocopherol) [8] and 7-acetyl-4-methylazulene-1-carboxylic acid [2] have been identified in *L. salmonicolor* fruiting bodies collected from different regions in Turkey, Greece and Portugal. In literature there are few studies on the antioxidant activity of *L. salmonicolor* extracts, reporting

mainly on the free radical scavenging effects [1, 2, 8]. Several metals (Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Zn, Mg, Na) have been quantified in *L. salmonicolor* fruiting bodies of Polish, Greek and Turkish origin [13, 14, 18]. The aim of the present study was to evaluate the antioxidant activity of an ethanolic extract obtained from *L. salmonicolor* fruiting bodies, with reference to its phenolic content. The contents of 18 metals in the fruiting bodies were also determined. To the best of our knowledge, this is the first study on the antioxidant activity and metals content of *L. salmonicolor* growing in Durau area (Ceahlau Mountain, North East Romania). In addition, this is the first investigation on the ferrous ion chelating and 15-lipoxygenase inhibitory effects of *L. salmonicolor*.

Materials and Methods

All chemicals and reagents were of analytical grade.

Mushroom Material

Samples of *L. salmonicolor* were collected in Durau area (Ceahlau Mountain, North East Romania) in September 2010. The mushroom material was identified by Prof. dr. Catalin Tanase ("*Alexandru Ioan Cuza*" University, Iasi, Romania). The fruiting bodies were cleaned without washing and air dried in shade. A voucher specimen was deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, "*Grigore T. Popa*" University of Medicine and Pharmacy, Iasi, Romania.

Extraction

Dried and powdered mushroom material (20 g) was extracted two times with 200 mL of 96% ethanol at room temperature for 3 h. The combined ethanolic extracts were evaporated to dryness at 40°C under reduced pressure and kept at -18°C until further studies.

Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assessed according to the method described by Wangensteen et al. [22] except that the reaction time was 60 min. ± Catechin was used as positive control.

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity was evaluated according to the method of Re et al. [16]. The scavenging activity was determined after 6 min. reaction time. ± Catechin was the positive control.

Reducing power assay was performed using the method described by Ferreira et al. [7]. ± Catechin was used as positive control.

Ferrous ion chelating activity was determined according to the method described by Venditti et al. [21] except that ethanol was used instead of acetate buffer. Ethylenediaminetetraacetic acid (EDTA) was the positive control.

15-Lipoxygenase inhibitory activity was evaluated as previously described [4, 22] except that the enzyme and the extract were incubated for 5 min. at 25°C. ± Catechin was used as positive control.

Total Phenolic Content

The total phenolic content was determined by Folin-Ciocalteu method as previously described [17, 22].

Metals Content

After dehydration (105°C for 24 h), the mushroom sample (1 g) was digested as described by Ouzoumi et al. [14] with minor changes. The metals content was analysed by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700x ICP-MS system. Metranal® 3 Strawberry leaf (Analytika, Prague, Czech Republic) was used as quality control material. Metal concentrations were determined on a dry weight basis.

All assays were performed in triplicate. The results were expressed as mean value ± standard deviation.

Results and Discussion

The ethanolic extract obtained from the fruiting bodies of *L. salmonicolor* was investigated for its antioxidant activity and total phenolic content.

Antioxidant Activity

The antioxidant activity of *L. salmonicolor* ethanolic extract was evaluated by free radical scavenging, reducing power, ferrous ion chelating and 15-lipoxygenase inhibition assays.

DPPH radical scavenging assay is commonly used to assess the free radical scavenging potential. *L. salmonicolor* ethanolic extract scavenged the DPPH radical in a dose-dependent manner showing 73.84 ± 0.27% scavenging activity at the highest concentration tested (1666 µg/mL). In our study, the EC_{50} value of the ethanolic extract (959.3 ± 3.8 µg/mL) was considerably higher than the one determined for ± catechin (5.3 ± 0.0 µg/mL) (Table I). Different DPPH scavenging activities have been reported for the methanolic extracts from *L. salmonicolor* fruiting bodies collected from various regions in Greece, Turkey and Portugal. Athanasakis et al. reported a DPPH scavenging activity of 36.7% at 3 mg/mL [2]. Akata et al. found an activity of 94.17 ± 0.31% at 2.9 mg/mL [1] while Heleno et al. determined an EC_{50} value of 7.8 ± 0.52 mg/mL [8].

ABTS radical cation scavenging assay is another method widely used to evaluate the free radical scavenging potential of antioxidants. *L. salmonicolor* ethanolic extract proved to be an efficient scavenger of ABTS radical cation. Its scavenging activity increased from 12.39 ± 0.33% at 62.5 µg/mL to 85.78 ± 0.57% at 500 µg/mL.

According to the EC_{50} values, the scavenging activity of *L. salmonicolor* ethanolic extract was lower than that of ± catechin (271.6 ± 1.4 *vs*.1.07 ± 0.06 µg/mL) (Table I).

The reducing power assay evaluates the electron-donating capacity of antioxidants. *L. salmonicolor* ethanolic extract reduced the ferric ions in a dose-dependent manner; its reducing power ranged from 0.19 ± 0.00 at 133.34 µg/mL to 0.54 ± 0.00 at 600 µg/mL. The reducing power of the ethanolic extract (EC_{50} = 552.71 ± 2.93 µg/mL) was lower than that of ± catechin (EC_{50} = 3.99 ± 0.05 µg/mL) (Table I). In the reducing power assay, Heleno et al. reported an EC_{50} value of 2.39 mg/mL for the methanolic extract from *L. salmonicolor* collected in Portugal [8].

Ferrous ion chelation is an important mechanism of reducing oxidative stress. Ferrous ion chelating capacity of *L. salmonicolor* ethanolic extract increased with the concentration. At 36 µg/mL, the extract showed 21.56 ± 0.30% chelating activity; the capacity to chelate ferrous ions increased to 82.07 ± 0.50% at 576 µg/mL. *L. salmonicolor* ethanolic extract showed a good chelating ability (EC_{50} = 111.37 ± 0.85 µg/mL) but lower than the activity of EDTA (EC_{50} = 6.23 ± 0.05 µg/mL) (Table I).

15-Lipoxygenase catalyses the peroxidation of polyunsaturated fatty acids such as linoleic and arachidonic acids thus being involved in the physiopathology and progression of cardiovascular diseases (atherosclerosis), pulmonary diseases (bronchial asthma) and different types of cancer (colorectal and prostate cancers) [11]. Inhibition of the soybean 15-lipoxygenase is a good indicator for the mammalian 15-lipoxygenase inhibitory capacity. *L. salmonicolor* ethanolic extract inhibited 15-lipoxygenase dose-dependently; its activity increased from 10.82 ± 0.48% at 117 µg/mL to 92.35 ± 0.61% at 500 µg/mL. It is worthy to note that *L. salmonicolor* ethanolic extract showed a remarkable 15-lipoxygenase inhibitory activity being only 3.5 times less active than the positive control, ± catechin (EC_{50} = 226.67 ± 0.95 and 64.17 ± 0.15 µg/mL, respectively) (Table I).

Table I

EC_{50} values (µg/mL) of *L. salmonicolor* ethanolic extract in different antioxidant assays

Extract / Positive control	DPPH radical scavenging activity	ABTS radical cation scavenging activity	Reducing power	Ferrous ion chelating activity	15-Lipoxygenase inhibition
L. salmonicolor ethanolic extract	959.3 ± 3.8	271.6 ± 1.4	552.71 ± 2.93	111.37 ± 0.85	226.67 ± 0.95
± Catechin	5.3 ± 0.0	1.07 ± 0.06	3.99 ± 0.05	n.d.	64.17 ± 0.15
EDTA	n.d.	n.d.	n.d.	6.23±0.05	n.d.

n.d. – not determined

Total Phenolic Content

In the present study, a total phenolic content of 11.48 ± 0.19 mg/g was determined in the ethanolic extract from *L. salmonicolor* fruiting bodies. The value obtained in this study is higher than the ones reported previously in literature for the methanolic extracts from *L. salmonicolor* collected in Bragança, North East Portugal (4.14 ± 0.26 mg/g) [8] and Greece (6.8 mg/g) [2].

Metals content

Wild mushrooms can accumulate variable amounts of toxic metals depending on the mushroom species and environmental factors. The consumption of toxic metals accumulated mushrooms represents a serious risk for the consumer health [10]. In this respect, the fruiting bodies of *L. salmonicolor* were analysed for metals content. The results are given in Table II.

In 2011 FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) established the maximum intake values for As, Cd, Cu, Fe, Hg and Zn (Table II) [6]. Considering a 300 g portion of fresh mushrooms (30 g dry weight)/day for a 60 kg consumer [19] and the metal concentrations in *L. salmonicolor* fruiting bodies, the daily metal intakes were calculated. As can be seen from Table II, the intake of As, Cd, Cu, Fe, Hg and Zn by consumption of 30 g of dry fruiting bodies of *L. salmonicolor*/day does not exceed the maximum intake values given by FAO/WHO (2011). It is noteworthy that *L. salmonicolor* fruiting bodies under study are an important source of Ca, Mg, Se and Zn. These elements are nutritionally valuable as they are essential for the bone formation, skeletal growth (Ca) and function of many enzymes involved in the antioxidant defence (Se), cell division and metabolism (Mg, Zn) [15].

The literature data regarding the metal contents in the fruiting bodies of *L. salmonicolor* from different regions in Poland, Greece and Turkey are systematized in Table III. Except for Zn and Mn, the levels of other metals determined in our study were comparable to or lower than those reported previously in *L. salmonicolor* fruiting bodies.

Table II

Metals content in *L. salmonicolor* fruiting bodies and daily metals intakes by a 60 kg consumer

Metal	Content (mg/kg)	Daily intake (mg/day)	Maximum intake (FAO/WHO, 2011)
Ag	1.05 ± 0.01	0.03 ± 0.00	-
As	0.3 ± 0.0	0.009 ± 0.000	2 – 7 µg/kg body weight per day 0.12 – 0.42 mg/day
B	16.83 ± 0.30	0.50 ± 0.01	-
Bi	0.02 ± 0.00	0.0005 ± 0.0000	-
Cd	0.05 ± 0.00	0.001 ± 0.000	25 µg/kg body weight per month 0.05 mg/day
Co	0.04 ± 0.00	1.32 ± 0.03	-
Cu	14.61 ± 0.31	0.44 ± 0.01	0.05 – 0.5 mg/kg body weight per day 3 – 30 mg/day
Fe	137.87 ± 1.12	4.14 ± 0.03	0.8 mg/kg body weight per day 48 mg/day
Hg	0.14 ± 0.00	0.004 ± 0.000	4 µg/kg body weight per week 0.03 mg/day
Li	0.01 ± 0.00	0.0004 ± 0.0000	-
Mn	36.35 ± 1.02	1.09 ± 0.03	-
Ni	0.86 ± 0.00	0.03 ± 0.00	-
Pb	0.18 ± 0.01	0.006 ± 0.000	-
Se	1.49 ± 0.11	0.04 ± 0.00	-
Sr	2.59 ± 0.06	0.08 ± 0.00	-
Zn	152.53 ± 1.05	4.58 ± 0.03	0.3 – 1 mg/kg body weight per day 18 – 60 mg/day
Ca	3607.98 ± 1.15	108.24 ± 0.03	-
Mg	934.67 ± 1.07	28.04 ± 0.03	-

Table III

Literature data on the metal contents in *L. salmonicolor* fruiting bodies

Region of collection	Metal	Content (mg/kg)	Reference
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece	Cd	0.09 ± 0.01	[14]
Soguksu National Park, Ankara, Turkey		1.62 ± 0.01	[18]
selected woods throughout Poland	Co	8.44 ± 0.03	[13]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece		0.20 ± 0.01	[14]
Soguksu National Park, Ankara, Turkey		n.d.	[18]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece	Cu	6.15 ± 0.30	[13]
Soguksu National Park, Ankara, Turkey		16 ± 0.1	[18]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece	Fe	239 ± 12.2	[14]
Soguksu National Park, Ankara, Turkey		272 ± 4	[18]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece	Mn	20.8 ± 1.1	[14]
Soguksu National Park, Ankara, Turkey		24 ± 0.1	[18]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece	Mg	855 ± 41.9	[14]
selected woods throughout Poland	Ni	5.01 ± 0.03	[13]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece		1.61 ± 0.08	[14]
Soguksu National Park, Ankara, Turkey		2.1 ± 0.0	[18]
selected woods throughout Poland	Pb	1.15 ± 0.01	[13]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece		n.d.	[14]
Soguksu National Park, Ankara, Turkey		1.3 ± 0.1	[18]
Epirus (Ioannina) and West Macedonia (Grevena, Kastoria), Greece	Zn	94.5 ± 3.9	[14]
Soguksu National Park, Ankara, Turkey		87 ± 0.2	[18]

n.d. = not detected

Conclusions

Our study showed a significant 15-lipoxygenase inhibitory activity for the ethanolic extract from *L. salmonicolor* fruiting bodies, besides ferrous ion chelating and free radical scavenging effects. These results suggest a possible use of the ethanolic extract as ingredient in food supplements. In addition, *L. salmonicolor* proved to be an important source of functional minerals. The contents of heavy metals were low and acceptable to human consumption thus suggesting that the sampling area was not polluted.

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