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In vitro bioactive properties of some wild mushrooms collected from Kastamonu province

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Abstract

Aim of study: the protein and total phenolic contents, antioxidant and antimicrobial properties of some wild mushrooms (*Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers., *Hydnum rufescens* Pers. and *Macrolepiota procera* (Scop. ex Fr.) Sing.) collected from Kastamonu province, in Turkey was determined.

Area of study: Wild mushrooms were collected from Kastamonu province, Turkey.

Material and Methods: Dumas and Folin–Ciocalteu methods were used to determine the protein and the total phenolic contents, respectively. The antioxidant activities of the mushrooms were determined using the Ferric-reducing antioxidant power (FRAP). The antimicrobial activities were evaluated using the agar well diffusion method against the test organisms; *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Acinetobacter haemolyticus* ATCC 19002 and *Proteus mirabilis* ATCC 7002.

Main results: While the protein contents and the total polyphenol amounts of mushrooms were ranging between 9.94-27.46% and 0.592±0.004-1.624±0.026 mg GAE g^{-1}, respectively, the range of antioxidant activities were determined to range between 0.896±0.003-4.245±0.042 µmol $FeSO.7H_2O$ g^{-1}. The highest protein and the total phenolic content as well as the antioxidant properties were detected in *M. procera. H. rufescens* and *M. procera* mushrooms showed inhibitory effect against in vitro growth of *Klebsiella pneumoniae* and *Escherichia coli*.

Research highlights: Among the investigated mushrooms, *M. procera* showed the best analysis values in bioactive properties and so this mushroom can be evaluated for further studies

Keywords: Antioxidant, Antimicrobial, Mushroom, Protein, Total phenolic content

Kastamonu ilinden toplanan bazı yabani mantarların in vitro koşullar altında biyoaktif özellikleri

Özet

Çalışmanın amacı: Bu çalışmada Kastamonu ilinden toplanan *Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers., *Hydnum rufescens* Pers. ve *Macrolepiota procera* (Scop. ex Fr.) Sing. yabani mantarlarının protein ve toplam fenolik içerik miktarları ile antioksidan ve antimikrobiyal özellikleri belirlenmiştir.

Çalışma alanı: Yabani mantarlar Türkiye'nin Kastamonu ilinden toplanmıştır.

Materyal ve Yöntem: Protein içeriği; Dumas metodu, toplam fenol içeriği; Folin–Ciocalteu yöntemi, antioksidan özellik; demir indirgeyici güç (FRAP) metodu ve antimikrobiyal özellik ise *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Acinetobacter haemolyticus* ATCC 19002 ve *Proteus mirabilis* ATCC 7002'e karşı agar kuyucuk difüzyon yöntemi kullanılarak hesaplanmıştır.

Sonuçlar: Mantarların protein içerikleri %9.94 ile %27.46 arasında, toplam polifenol miktarları 0.592±0.004–1.624±0.026 mg GAE g^{-1} aralığında ve antioksidan özellikleri 0.896±0.003-4.245±0.042 µmol $FeSO.7H_2O$ g^{-1} aralığında değişmiştir. En yüksek protein miktarı, en yüksek toplam fenol miktarı ve en yüksek antioksidan özellik *M. procera*'da tespit edilmiştir. *M procera* ve *H. rufescens* mantarları *Klebsiella pneumoniae* ve *Escherichia coli'*e karşı inhibitor etki göstermiştir.

Araştırma vurguları: Çalışılan mantarlar arasında, *M. procera* mantarı biyoaktif özellikler yönünden en iyi değerleri göstermiştir dolayısıyla bu mantar ileriki çalışmalar için umut vaad edicidir.

Anahtar kelimeler: Antioksidan, Antimikrobiyal, Mantar, Protein, Toplam Fenol Miktarı

Introduction

For centuries, mushrooms consumption has been increasing and the mushroom industry is developing with it, all over the world. The mushroom industry generally classified in three basic categories: medicinal, edible and wild mushrooms (Chang, 2006). Wild mushrooms become more popular, due to not only their low fat and high protein contents (Barros et al., 2007a), but also for their chemical composition and biological properties (Barros et al., 2007b). In addition, mushrooms are natural alternatives reducing the protein and vitamin deficiency especially for vegetarians (Adejumo and Awosanya, 2005). Therefore, determining the protein content of especially wild mushrooms is important to know effect on human health. It has been reported that the protein in food intake plays a key role in body weight regulation (Westerterp-Plantenga, 2003).

Oxidative stress, known as basic source of unhealthy and shorter lifetime, occur during normal biochemical processes in the human body and rise with smoking, stress, using drugs and pollutants (Fang et al., 2002). These oxidative damages can be repaired by antioxidants (Turkoglu et al., 2006). The antioxidants are classified as natural and synthetic. However, the synthetic antioxidants are being restricted because of their inherent risk of carcinogenicity (Vidović et al., 2010). Therefore, natural agents containing antioxidant compounds, such as mushrooms have become more important as source of natural antioxidants, yet needs further investigations. Mushrooms contain secondary metabolites, such as terpenes, steroids, polyketides and phenolic compounds (Kim et al., 2008). Many phenolic compounds have been reported to have potential bioactive properties such as antioxidant activity (Attarat and Phermthai, 2014). Phenolics have been announced as one of the main groups of non-essential nutritional components that have been related to the inhibition of cancer and atherosclerosis. (Williams and Iatropulos, 1997). In addition, secondary metabolites which present in mushroom (extracellular secretions by the mycelium) can combat bacteria (Alves et al., 2012a; Alves et al., 2013) and viruses (Eo et al., 1999; Brandt and Piraino, 2000).

Drug resistance has increased with increasing disease and researchers have been forced to find new and natural antimicrobial agents. The search for natural antioxidant products has also been intensified because of the damage caused by artificial antioxidants. Thus, more research is needed on the bioactive properties of fungi thanks to their medicinal properties. Antioxidant properties of *Lactarius deterrimus, Suillus collitinus, Boletus edulis, Xerocomus chrysenteron* collected from Eskişehir (Sarıkürkçü et al., 2008) and antimicrobial activity of *Ramaria flava* collected from Kayseri (Gezer et al., 2006) had been investigated. Trace elements of *Boletus badius, Lactarius deliciosus, Hebeloma crustuliniforme, Lycoperdon perlatum, Boletus luridus, Lepiota cristata, Polyporus* (sp.), *Agaricus bisporus* mushroom samples from Kastamonu were investigated by Mendil et al., (2014). However, no research about in vitro bioactive properties of mushrooms are used in this study. The main objectives of this study were: to determine protein and total phenolic contents and antioxidant and antimicrobial properties of some wild mushrooms (*Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers., *Hydnum rufescens* Pers. *and Macrolepiota procera* (Scop. ex Fr.) Sing) collected from Kastamonu province, Turkey. Studies species are collected and consumed by local people. Also, these mushrooms are sold in bazaar and income is generated.

Materials and Methods

Material

Wild mushrooms were collected from Gökçe village, Doğanyurt district of Kastamonu province, located in the northwest of Turkey (Fig 1) in October of 2014. Information about the studied mushrooms were presented in Table 1. The forests of the region consist of beech, oak, chestnut, pine and fir trees. All mushrooms were collected in the same area and same time (10.10.2014).

The ecological and morphological and properties of mushrooms were noted and photographed in their natural habitats. Mushrooms were identified morphologically by Prof. Dr. Ertuğrul SESLİ

Table 1. Information about the studied mushrooms

No	Species of mushrooms	Habitat and Location	Edibility	n*
1	*Cantharellus cibarius* Fr.	On soil, Kastamonu	Edible	15
2	*Craterellus cornucopioides* (L.) Pers.	On soil, Kastamonu	Edible	9
3	*Hydnum rufescens* Pers.	On soil, Kastamonu	Edible	11
4	*Macrolepiota procera* (Scop. ex Fr.) Sing	On soil, Kastamonu	Edible	6

*n: Number of mushrooms used for analysis

Figure 1. Study area

Determination of protein content

All the parts of the mushrooms were used for analysis. Each mushroom was dried at 40˚C (Profilo, PFD1350W, Turkey) for 24 hours before analysis. Dried mushroom samples were crushed and powdered for passing a 40-mm mesh sieve. Protein contents of mushrooms were determined by Dumas method. Briefly, 0.500 - 0.700 mg dried mushroom samples were weighed and placed on 5 mm x 9 mm tin capsules. Capsules were placed into Costech ECS 4010 elemental analysis instrument and were burned. Ratio of carbon, hydrogen and nitrogen were determined using Costech ECS 4010 program. Protein contents were determined by multiplying carbon results with conversion factor (4.38) and the results were expressed as ratio (%) (Crisan and Sands, 1978).

Measurement of total phenolic content

Four grams of dried sample was extracted with 40 mL methanol by shaking at 150 rpm for 24 h then filtered through Whatman No. 4 filter paper. Extracts were stored at 4˚C for future use.

The total phenolic contents of the methanolic extracts were determined by Folin–Ciocalteu method using gallic acid standard (Slinkard and Singleton, 1977). The Folin assay was also based all phenolic contents including phenolic acids, flavonoids, and anthocyanins in the aquatic solution which gives a blue color complex whose maximum absorbance can be read at 760 nm. Briefly, 680 µL distilled water, 20 µL methanolic extract and 400 µL of 0.5 N Folin-Ciocalteu regents were mixed in a test tube, vortexed for 2 min, then 400 µL Na_2CO_3 10% was added and incubated for 2 hours at room temperature. Following the incubation, absorbance of the mixtures was measured at 760 nm on an ATI-Unicam UV-2 UV-VIS spectrophotometer (Cambridge, U.K.). The concentration of total phenolic compounds was calculated as mg gallic acid equivalents GAE g^{-1} of dry weight.

Determination of antioxidant activity

The antioxidant activity of the mushroom extracts was determined by Ferric- reducing antioxidant power (FRAP) method. The reducing ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for total antioxidant capacity assay (Benzie and Strain, 1996) with some modifications. Working FRAP reagent was prepared as required by mixing of 300 mM acetate buffer, pH 3.6 with 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. Three milliliters freshly prepared FRAP reagent and 100 µL of samples was mixed and incubated for 4 min at 37 °C and the absorbance was read at 593 nm against reagent blank containing distilled water. $FeSO_4 \cdot 7H_2O$ was used as positive control. The ferric-reducing antioxidant power of the antioxidants in the extracts was calculated by comparison with $FeSO_4 \cdot 7H_2O$ as µmol $FeSO_4 \cdot 7H_2O$ g^{-1} dry weight of mushrooms.

Antimicrobial activity testing

Mushroom extracts were tested for antimicrobial activity by agar-well diffusion method in accordance with the Clinical & Laboratory Standards Institute (CLSI). Tested microorganisms included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Acinetobacter haemolyticus* ATCC 19002, *Klebsiella pneumoniae* ATCC 13883, *Salmonella* were obtained from Karadeniz Technical University, Department of Medical Microbiology, Faculty of Medicine Trabzon, Turkey.

The bacterial colonies were suspended in 5 mL of sterile isotonic sodium chloride solution and turbidity was adjusted to 0.5 McFarland standards.

The microbial suspension was spread on Mueller Hinton agar using sterile cotton swabs. Then, wells in agar plates were made by using the wide end of a blunted sterile pasteur pipette. Each well was filled with 100 µL of mushroom extracts, positive controls (commercial Ampicillin, Gentamicin, Cefotaxime and Amphotericin B solutions) and negative control (methanol). The cultures were incubated at 37 ^{o}C for 24 hours. Activity was determined by visual inspection and measurement of the diameter of clear inhibition zones around the agar-wells.

Statistical analysis

Total phenolic content and antioxidant analyses were conducted in triplicates. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). Pearson correlation coefficient was used to determine the relationship between total phenolic content and the antioxidant activity in the same sample. Differences between means at 5% ($p < 0.05$) level were considered as significant. The level of statistical significance was realized using Duncan's multiple range test.

Results and Discussion

Protein content

Hydrogen, carbon, nitrogen ratios and protein contents of wild mushrooms were given in Table 2.

A protein estimate can be obtained from the sum of the amount of each amino acid expressed as percentage of dry or wet sample (Manzi et al., 1999). It was observed a linear relationship between the amount of protein and nitrogen ratio. In this study, nitrogen content of mushroom was ranged from 2.27 % to 6.27 % and are comparable with a previous study (3.47 to 7.93% (dry basis); (Manzi et al., 1999)).

Mushrooms are a great source in terms of protein and amino acids compared to foods of plant origin (Kurtzman et al., 1993). The highest protein content was determined in *Macrolepiota procera* (Scop. ex Fr.) Sing. with 27.46% while the lowest in *Craterellus cornucopioides* (L.) Pers. with 9.94%. In the literature; protein contents of some mushroom species have been reported as 1.5 - 7.9% (dry basis) (Manzi et al., 2004), and 15.19 - 34.73% (dry basis); (Manzi et al., 1999). The previous researchers informed that the protein content of mushrooms are influenced by many factors, such as the type of mushrooms, growth conditions, locations and nitrogen level and the (Al-Momany and Gücel, 2012; Yildiz et al., 2015).

Total phenolic content and antioxidant properties

The total phenolic content and antioxidant properties of mushrooms are reported in Table 3 and Fig 2. Table 3 also summarize the statistical test results.

It was reported that the antioxidant activity of plant materials is connected with the content of their phenolic compounds (Velioglu et al., 1998). The bioactivity of phenolic compounds may be related to their ability to chelate metals, inhibit scavenge free radicals and lipoxygenase (Decker, 1997). In the study, the total phenolic content ranged from 0.592 ± 0.004 to 1.624 ± 0.026 mg GAE g^{-1}. While the highest phenolic content was seen in *Macrolepiota procera* (Scop. ex Fr.) Sing, the lowest one was seen in *Cantharellus cibarius* Fr. Our results are lower than some wild mushrooms' content like *Morchella* species (12.36 - 25.38 mg GAE g^{-1}; (Gursoy et al., 2009)) Also, total phenolic content of mushrooms was found significantly different from each other by Duncan's multiple range

test. This difference can be related to different species of mushroom.

Ferric reducing antioxidant power (FRAP) assay treats the antioxidants in the samples as reductants in a redox-linked colorimetric reaction (Guo et al., 2003). The highest ferric reducing antioxidant power (4.245 ± 0.042 μmol $FeSO_4.7H_2O$ g^{-1}) was determined in *M. procera* extract. The lowest one (0.896 ± 0.003 μmol $FeSO_4.7H_2O$ g^{-1}) was seen in *Cantharellus cibarius* mushroom. When this study compared with other studies; average of chelating ability, FRAP activity (2.15925 μmol $FeSO_4.7H_2O$ g^{-1}) were found higher than some fresh wild edible mushrooms (*Lactarius deliciosus* (L.) Gray, *Lactarius sanguifluus* (Paulet) Fr., *Lactarius semisanguifluus* R. Heim & Leclair, *Russula delica* Fr., *Suillus bellinii* (Inzenga) Waltling) growing in the island of Lesvos, Greece (0.271 - 0.523 μmol Fe^{2+} g^{-1}, respectively) (Kalogeropoulos et al., 2013).

Antimicrobial activity

Antimicrobial test results of mushrooms and antibiotics were presented in Table 4.

In this study, *M. procera*. and *H. rufescens* showed inhibitory effect against *Klebsiella pneumoniae* and *Escherichia coli* (Table 4).

Table 2. Hydrogen (H), carbon (C), nitrogen (N) ratios and protein contents of mushrooms (dry weight)

Mushroom	H (%)	C (%)	N (%)	Protein (%)
Cantharellus cibarius	5.22	36.60	2.51	10.99
Craterellus cornucopioides	5.43	37.62	2.27	9.94
Hydnum rufescens	6.41	43.96	3.66	16.03
Macrolepiota procera	5.89	40.72	6.27	27.46

Table 3. Total phenolic content (TPC) and antioxidant properties (FRAP) of mushrooms

Mushroom	TPC (mg GAE g^{-1})		FRAP (μmol $FeSO_4 \cdot 7H_2O$ g^{-1})	
	$\bar{X}$ ± SD	H.G.	$\bar{X}$ ± SD	H.G.
Cantharellus cibarius	0.592±0.004	a	0.896±0.003	a
Craterellus cornucopioides	0.922±0.017	b	1.468±0.014	b
Hydnum rufescens	1.050±0.013	c	2.028±0.009	c
Macrolepiota procera	1.624±0.026	d	4.245±0.042	d

H.G.: Homogenity group means having the same superscript letter(s) are not significantly different ($p > 0.05$) by Duncan's multiple range test.

Table 4. Antimicrobial test results of mushrooms and antibiotics

Material	*S. aureus*	*E. coli*	*E. faecalis*	*C. albicans*	*K. pneumoniae*	*A. haemolyticus*	*S. typhimurium*	*P. mirabilis*
C. cibarius	-	-	-	-	-	-	-	-
C. cornucipiotes	-	-	-	-	-	-	-	-
H. rufescens	-	+	-	-	-	-	-	-
M. procera	-	+	-	-	-	-	-	-
Ampicillin	+	+	+	...*	…	…	+	…
Gentamicin	…	…	…	…	+	+	…	…
Amphotericin B	…	…	…	+	…	…	…	…
Cefotaxime	…	…	…	…	…	…	…	+
Methanol	-	-	-	-	-	-	-	-

(+)positive antimicrobial activity observed, (-)no antimicrobial activity observed*…: Not tested.

According to the literature, mushrooms such as *Russula delica* Fr. (Dülger et al., 1999) *Pleurotus sajor kaju* (Tambekar et al., 2006), *Lepista luscina, Leucopaxillus lepistoides* (Suay et al., 2000), *Laetiporus baudonii, Montagnea haussknechtii, Phellorinia herculea* (Al-Fatimi et al., 2005) and *Sarcodon imbricatus*, *Cantharellus cibarius, Agaricus arvensis* (Alves et al., 2012b) have antimicrobial properties.

Correlation between total phenolic content and antioxidant activity

Correlation between total phenolic content and antioxidant activity are reported in Table 5. The Pearson correlation tests revealed significant and positive correlation between total phenolic content and values of FRAP antioxidant properties for the mean of all species (Table 5).

Table 5. Correlation between total phenolic content and antioxidant activity

		TP	FRAP
TP	r value	1	0.985*
	p value		0.015
FRAP	r value	0.985*	1
	p value	0.015	

*. Correlation is significant at the 0.05 level (2-tailed).

There are a lot of linear correlation studies, which demonstrated a link between antioxidant activities and their total phenolic content in mushrooms (Barros et al., 2008; Gursoy et al. 2009; Babu and Rao, 2013).

Conclusion

In this study, the protein and total phenolic contents and antioxidant and antimicrobial properties of some wild mushrooms *C. cibarius*, *C. cornucopioides*, *H. rufescens* and *M. procera* collected from Kastamonu, Turkey were determined. *H. rufescens* and *M. procera* showed inhibitory effect against *Klebsiella pneumoniae* and *Escherichia coli*. The highest protein and total phenolic content and antioxidant properties was detected in *M. procera*. In comparation to the other mushroom species evaluated in this study, *M. procera* exhibited a good performance in many respects. Therefore; these type of mushrooms, which have high bioactive properties need to be examined more closely with more efficient methodologies for example to identify bioactive molecules with different instrumentals such as HPLC, GS-MS.

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