Antimicrobial, Antioxidant Activities and Chemical Composition of *Lactarius deliciosus* (L.) Collected from Kastamonu Province of Turkey

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Abstract

Lactarius deliciosus (L.) is a well known mushroom which is widely used as a food in Kastamonu province of Turkey. In this sudy, the antimicrobial properties of acetone, ethanol, DMSO (dimethylsulfoxide) and water extracts from *L. deliciosus* (L.) were evaluated the effect against gram positive and gram negative bacteria and fungi. The highest inhibitory activity was determined against *P. aeruginosa* (30±0.0 mm, zone diameter) with acetone and ethanol extracts. On the other hand, the weakest inhibitory activity was determined against *S. aureus* and *C. albicans* (9±0.0 mm, zone diameter) with DMSO and distilled water extract of mushroom. Also, in this study, *L. deliciosus* (L.) was analyzed for proximate chemical constituents (moisture, fat, protein, ash and dry matter). Moisture, fat, protein, ash and dry matter levels of the mushroom were found as 8.75±0.72, 2.64±0.16, 75.25±0.15, 4.61±0.03, 89.96±0.24% mg/100g respectively. The quantitative estimation of the total phenolic contents and flavonoid quantification of *L. deliciosus* were determined by a colorimetric method. The total phenolic content and total flavonoid were found as 4,84±0,32 mgGAE/gextract and 2,76±0,03 mg/g. The antioxidative activity of the mushroom was elucidated by DPPH free radical scavenging capacity. As a result; DPPH scavenging activity of methanolic mushroom extract was found to be IC50: ›17. In this study showed that the *L. deliciosus* not only in terms of nutritional value is also noteworthy that the therapeutically.

Keywords*:* *L. deliciosus* (L.), antimicrobial, antioxidant, chemical composition

Kastamonu Bölgesinden Toplanan *Lactarius deliciosus* (L.)'ın Antimikrobiyal, Antioksidan Aktiviteleri ve Kimyasal Kompozisyonu

Özet

Lactarius deliciosus (L.) Kastamonu bölgesinde (Türkiye) yiyecek olarak tüketilen ve iyi bilinen bir mantardır. Bu çalışmada, *L. delicious*'un aseton, etanol, DMSO (dimetilsülfoksit) ve su ektraktlarının gram pozitif, gram negatif bakteriler ve fungusa karşı etkisi değerlendirilmiştir. En yüksek etki *P. aeruginosa* (30±0.0 mm zon çapı) üzerine aseton ve etanol ekstraktlarında tespit edilmiştir. Diğer yandan en zayıf aktivite *S. aureus* ve *C. albicans* (9±0.0 mm zon çapı) üzerine DMSO ve su ekstraktlarında tespit edilmiştir. L. deliciosus (L.) kimyasal içerik bakımından da incelenmiş ve nem, yağ, protein, kül ve kuru madde düzeyleri 8.75±0.72, 2.64±0.16, 75.25±0.15, 4.61±0.03, 89.96±0.24 % mg/100g olarak bulunmuştur. Bu mantarın toplam fenolik içeriği ve flavonoid miktarı kolorimetrik metodla belirlenmiştir. Toplam fenolik içeriği ve flavonoid miktarı 4,84±0,32 mgGAE/gextract ve 2,76±0,03 mg/g olarak bulunmuştur. Antioksidan aktivitesi DPPH serbest radikal süpürme kapasitesiyle açıklanmıştır. Mantarın metanol ekstraktının DPPH kapasitesi IC50: ›17 olarak tespit edilmiştir. Bu çalışmanın sonuçları, *L. deliciosus* 'ın sadece besin değeri açısından değil aynı zamanda terapötik açıdan da dikkat çekici olduğunu göstermiştir.

Anahtar Kelimeler: *L. deliciosus* (L.), antimikrobiyal, antioksidan, kimyasal kompozisyon

Introduction

Lactarius deliciosus (L.) is a well known mushroom which is widely used as a food in Kastamonu province of Turkey. Wild edible mushrooms have been part of the human diet for a long time since most of them have a very high protein content (Léon-Guzmán et al., 1997). Mushrooms have been used as food and food-flavoring material in soups and sauces for centuries due to their unique and subtle flavor (Sarıkürkçü et al., 2008). Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Researches have reported antimicrobial and antioxidant activites of several mushrooms (Yaltırak et al., 2009, Yamaç and Bilgili, 2006). Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for humans. The antioxidant capacity of the phenolic compounds, especially gallic acid, catechin, caffeic acid, rutin, quercetin, ellagic acid and *p*- coumaric acid in several models is well known. The quality of a nutraceutical is dependent on the chemical composition of the fruiting body, particularly in relation to the especially phenols content (Yaltırak et al., 2009). In recent years, several undesirable disorders have developed, due to the side effects of the use of synthetic antioxidants commonly used in the food and food-flavoring industries. This situation has forced scientists to search for new antioxidant substances from various plants which are good sources novel antioxidant agents (Sarıkürkçü et al., 2008).

L. deliciosus (L.), commonly known as the Saffron milk cap, red pine mushroom is one of the best known members of the large milk-cap genus *Lactarius* in the order *Russulales*. *L. deliciosus* grows under the acidic soil of conifers and forms a mycorrhizal relationship with its host tree. The saffron milk cap mushroom (*L. deliciosus*) has been eaten in Europe since Roman times and is still greatly appreciated in Europe, and in particular Portugal and Spain, for its mild, slightly bitter flavour (URL1, 2012). *Lactarius deliciosus* is used as food in Turkey.

The reason of this study is to research antimicrobial and antioxidant activities of the mushroom, that have not been reported enough in the literature although Anatolian people have been using them as food for a long time. In this work, *L. deliciosus* (L.) were used for quantitative estimation of the total phenolic contents as gallic acid equivalent (GAE) per gram dry weight and total flavonoid quantification were determined by a colorimetric method. The antioxidative activity of the extract was elucidated by DPPH free radical scavenging capacity of the extract. Also, the antimicrobial activities of *L. deliciosus* extracts have been investigated by disk diffusion.

Material and Methods

Mushroom

L. deliciosus (L.) gray used in this study was collected from Kastamonu province, in the north part of Turkey. The identification of macrofungi material was confirmed by Dr. Hakan Alli (Deptartment of Biology, Muğla Univ., Turkey). The mushroom was deposited for further reference at Fungarium of Mugla University.

Chemicals

Extraction Procedure

Collected mafrofungi material was dried in the shade and ground in a grinder. Approximately 50 g of the dried and powdered macrofungi materials were extracted with 96% extra pure ethanol, acetone and distilled water. All of the extracts were concentrated under vacum at 40 ºC by using rotary evaporator (Heidolph G1, Germany), preserved at $+4^0$C and resuspended in 1 gr/1ml DMSO (analytically pure dimethylsulfoxide) (Salman et al., 2008).

Microorganisms and Culture Conditions

Test bacteria used in this study were obtained from the culture collection of the Pharmaceutical Biotechnology Laboratuary at Erciyes University (Kayseri-TURKEY). *Staphylococus aureus* ATCC 25923, *Escherichia coli* ATCC 11230, *Pseudomonas*

aeruginosa ATCC 27853, *Klebsiella pneumoniae* ATCC 27736 and *Candida albicans* ATCC 10239 were used to screen antimicrobial activity of samples. Microorganisms were cultured at 37 °C in nutrient agar medium for bacteria, and at 30 °C in YEPD agar medium for fungi.

Antimicrobial Activity

Disc diffusion test was performed as described previously in BSAC (2003). The antimicrobial activity was performed using Mueller-Hinton Agar for bacteria and YEPD Agar for yeast. The cell culture suspension was adjusted by comparing against 0.4-0.5 Mc Farland scale standard. These suspensions (100 µl) of target strain were spread on the plates. For the investigation of the antibacterial and antifungal activity, all extract of *L. deliciosus* were weighed and dissolved with 70 and 96% ethanol to obtain 50 mg/ml and 200 mg/ml extract concentration. These solutions were filtered with a pore size of 0.2 µm. Each sample (100 µl) was filled into disc (6mm diameter) directly. The diameter of the inhibition zone (mm) was measured after overnight. All determinations were done triplicate (Mahasneh and Oqlah, 1999, Silici and Koç, 2006).

Chemical Composition

L. deliciosus was analyzed for chemical composition (moisture, fat, protein and ash) using the AOAC procedures. The crude protein content (Nx4.38) of the samples was estimated by the macro-Kjeldahl method according to Léon-Guzmán et al.(1997) The crude fat was determined by extracting a known weight of powdered mushroom sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 60 ±15 °C.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging activity

The ability of the extracts to scavenge DPPH• was determined by the method of Gyamfi, Yonamine and Aniya (1999). A 50 µL aliquot of each extract, in 50 mM Tris-HCl buffer (pH 7.4), was mixed with 450 µL of Tris-HCl buffer and 1.0 mL of 0.1 mM DPPH• in MeOH. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using equation 1. Estimated IC_{50} values are presented as the mean value of quadruplicate analyses.

Percentage Inhibition =

$$\left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] x100 \qquad \text{Eq. 1}$$

Total phenolics

Total phenols were estimated as gallic acid equivalents (GAE), per gram of extract (Singleton et al., 1999). To *ca.* 6.0 mL H_2O, 100 µL sample (conc. 4 mg/mL) were transferred into a 10.0 mL volumetric flask, to which 500 µL undiluted Folin-Ciocalteu reagent were subsequently added. After 1 min, 1.5 mL 20% (*w/v*) Na_2CO_3 were added and the volume was made up to 10.0 mL with H_2O. After 2 h incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the mean value of triplicate analyses.

Total flavonoids

Flavonoid contents in the extracts were determined by a colorimetric method described by Jia et al. (1999) with some modifications. The mushroom extract (250 μL) was mixed with 1.25 mL of distilled water and 75 μL of a 5% $NaNO_2$ solution. After 5 min, 150 μL of a 10% $AlCl_3 \cdot H_2O$ solution was added. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm. (+)-Catechin was used to calculate the standard curve (0.022–0.34 mM; Y , $0.9629X - 0.0002$; $R2$, 0.9999) and the results were expressed as milligrams of (+)-chatequin equivalents (CEs) per gram of extract.

Results and Discussıon

Antimicrobial activity of the extracts by the disc diffusion method

The antimicrobial effect of extract of *L. deliciosus* was tested against four species bacteria and one species fungus. In table 1, the inhibition zones of *L. deliciosus* (L.) which were obtained against all test bacteria were in the range of 9-30 mm. The antimicrobial properties of acetone, ethanol, DMSO and water extracts from *L. deliciosus* (L.) were evaluated against gram positive and gram negative bacteria and fungi. The highest inhibitory activity was determined against *P. aeruginosa* (30±0.0 mm inhibition zone diameter) with acetone and methanol extraction of *L. deliciosus* (L.). On the other hand, the weakest inhibitory activity was determined against *S. aureus* and *C. albicans* (9±0.0 mm inhibition zone diameter) with DMSO and distilled water extraction of mushroom.

According to literature, the methanol extract of *L. delicious* has determined to be particularly effective against the acid-fast *Mycobacterium* sp. and revealed antimicrobial activity against some gram (+) and gram (-) bacteria but showed no antagonistic effect against yeasts (Dülger et al., 2002).

Table 1**.** Antimicrobial activity of various extracts of *L. delicious*

Solvents	**Bacteria**				**Fungi**
	S.aureus	*E.coli*	*P.aeruginosa*	*K.pneumoniae*	*C.albicans*
Acetone	17±0.0	18±0.1	30±0.0	24±0.0	15±0.0
Ethanol	NI	NI	30±0.0	NI	NI
DMSO	10±0.0	13±0.0	NI	NI	9±0.0
dH_2O	15±0.0	9±0.0	NI	NI	18±0.1

NI: no inhibition
*Values are the means ±standart deviations of triplicate measurement

The saffron milk cap mushroom (*Lactarius deliciosus*) has been eaten in Europe since Roman times and is still greatly appreciated in Europe, and in particular Portugal and Spain, for its mild, slightly bitter flavour (URL1, 2012). *Lactarius deliciosus* is used as food in Turkey. The mushrooms are low in calories and fats as delicious foods, having rich source of vitamins, proteins and minerals, especially in potassium and phosphorus. Therefore, various mushrooms species have been the focus of researchers interest (Öztürk et al., 2014). Table 2 shows the proximate chemical composition of *L. deliciosus.* All the results obtained from this study are similar from values reported within Europe and Turkey (Konuk et al., 2006, Kabir and Kimura, 1989). In this study, biochemical analysis showed that the protein and fat composition of the *L. deliciosus* collected from Kastamonu province were higher than those in other studies (Konuk et al., 2006, Öztürk et al., 2014). As shown in the Table 2, the high protein (75%) and low fat content were found for *L. delicious*.

Table 2**.** Proximate chemical composition mg/100g (% dry weight) of *L. deliciosus*

Sample	**Moisture**	**Fat**	**Protein**	**Ash**	**T.Dry Mat.**
L. deliciosus	8.75±0.72	2.64±0.16	75.25±0.15	4.61±0.03	89.96±0.24

The major compounds of mushrooms are proteins and carbonhydrates. Total protein content, varying between 21-50% can be accepted high when compared with meat, milk, egg such as some commercially important fish species from the Black Sea region and some other mushroom species. Also, they are consumed for low-calorie diet because of their low crude fat (1,40-10,58%) content. In the previous reports, it is possible to see various fat content from 0,8% to 27,5% in dry mushrooms (Çolak et al., 2009).

Despite some similarities in the composition of mushroom samples, it is known that the chemical composition of mushrooms are affected by a number of factors, namely, mushroom strain/type, composition of growth media, time of harvest, management techniques, handling conditions and preparation of the substrates. This situation, they are diversity in antimicrobial activity of mushrooms at different cultivation status of same species. It can change the content and amount of active compounds according to growth media of mushroom. Therefore chemical contents and antimicrobial substances of mushroom species naturally grown in different geographic locations of world must be analyzed and comparison of this analysis is very important (Özen et al., 2011).

Mushrooms have recently become attractive as functional foods and a source of physiologically beneficial medicine. They contain rich source of phenolic compounds as phenolic acids, flavonoids, carotenoids, ά-tocopherol and ascorbic acid. Both fruiting body and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities. In present investigation, *L. deliciosus* were used for quantitative estimation of the total phenolic contents as gallic acid equivalent (GAE) per gram dry weight and total flavonoid quantification were determined by a colorimetric method. The antioxidative activity of the extract was elucidated by DPPH free radical scavenging capacity of the extract. As a result; DPPH scavenging activity of methanolic mushroom extract was found to be ›17. The total phenolic content and total flavonoid were found as 4,84±0,32 $mg_{GAE}/g_{extract}$ and 2,76±0,03 mg/g (Table 3). Öztürk et al. (2014) determined that the DPPH activity of *L. deliciosus* is <17.00 (6,43 mg/ml).

Table 3. Total phenolics, Total flavonoids and DPPH radical scavenging activities of mushroom extracts.

Sample	Total phenols[a] [$mg_{GAE}/g_{ext.}$]	Total Flavonoids [mg/g]	DPPH [IC_{50}, mg/mL]
L. deliciosus	4.84 ± 0.32[b]	2.76± 0.03	> 17.00

[a] Total phenols expressed as gallic acid equivs. milligrams of gallic acid per gram (dry weight) of extract. [b] mean ± standard deviation (n=3)

Conclusion

In recent years, several undesirable disorders have developed, due to the side-effects of the use of synthetic antioxidants commonly used in the food and food-flavoring industries. This situation has forced scientists to search for new antioxidant substances and antimicrobial substances from various mushrooms which are good sources of novel chemotherapeutic agents. Mushrooms have recently become attractive as functional foods and a source of physiologically beneficial medicine. In conclusion, the nutritional values of *L. delicious* and bioactivity properties of its extracts were remarkable. It is the first time that *Lactarius deliciosus* (L.) collected from Kastamonu province, in the north part of Turkey, wild edible mushroom was submitted in these study.

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