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The Antioxidant potential of ethanolic extract of edible mushroom *Lycoperdon molle* Pers. (Agaricomycetes)

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Abstract
Mushroom play an important role in the decay of organic cover in forest ecosystems. In addition, mushrooms which are forest products are consumed as food. This study aims to determine the antioxidant activity of ethanol (EtOH) extract from edible *Lycoperdon molle* Pers. mushroom collected from Antalya (Turkey). Total antioxidant status (TAS), total oxidant status (TOS) and the oxidative stress index (OSI) were analyzed using Rel Assay Diagnostics kits. In addition, free radical scavenging activity was determined using the DPPH method. As a result of the studies, TAS, TOS and OSI values of *L. molle* were 1.855±0.072, 2.201±0.085 and 0.119±0.008, respectively. The free radical scavenging activity of the fungus was at a normal level in comparison to the standards used, i.e. rosmarinic and caffeic acids. Consequently, it was determined that *L. molle* may be used as a source of natural antioxidants. As a result, it was determined that edible *L. molle*, which is one of the forest products, has antioxidant potential in addition to its nutritional properties.

Keywords: *Lycoperdon molle*, Antioxidant, Oxidant, DPPH, Oxidative stress.

Özet
Mantarlar orman ekosistemlerinde organic örtünün ayrıştırılmasında önemli rol oynarlar. Ayrıca orman ürünleri olan mantarlar gıda olarak tüketilmektedir Bu çalışmada Antalya (Turkey) ilinden toplanan yenilebilir *Lycoperdon molle* Pers. mantarının etanol (EtOH) ekstraktının antioksidan aktivitesinin belirlenmesi amaçlanmıştır. Mantar örneklerinin soxhlet cihazında özütleme işlemi yapılmıştır. Toplam antioksidan aktivitesi, toplam oksidan aktivitesi ve oksidatif stress indeksi Rel Assay Diagnostics kitleri kullanılarak analiz belirlenmiştir. Ayrıca serbest radikal süpürme aktivitesi DPPH metodu kullanılarak belirlenmiştir. Yapılan çalışmalar sonucunda *L. molle*'nin TAS değeri 1.855±0.072, TOS değeri 2.201±0.085 ve OSI değeri ise 0.119±0.008 olarak belirlenmiştir. Mantarın serbest radikal süpürme aktivitesinin ise kullanılan standartlar rosmarinik asit ve caffeic asite göre normal seviyelerde olduğu belirlenmiştir. Sonuç olarak *L. molle*'nin doğal antioksidan olarak kullanılabileceği belirlenmiştir. Sonuç olarak orman ürünlerinden olan yenilebilir *L. molle*'nin besin özelliklerine ek olarak antioksidan potansiyelinin olduğu belirlenmiştir.

Keywords: *Lycoperdon molle*, Antioxidant, Oxidant, DPPH, Oxidative stres.

Introduction

Mushrooms exhibit cosmopolitan distribution. Macrofungi belong to the order of Basidiomycetes or Ascomycetes and they can be found in soils rich in organic matter and

humus as well as various substrates such as in moist wood. Moreover, they can be found in animal dung after heavy rain or anywhere after sudden temperature changes occur and they then disappear after a short time, i.e. within several hours or at the end of the day (Girma and Tasisa 2018). The number of macrofungi species in nature is estimated to be between 53 and 110 thousand globally, however only a few are considered as nutrients and are able to be cultivated commercially (Mueller et al. 2007). Edible mushrooms have been globally consumed with increasing popularity due to their nutritional and medicinal value since Ancient Greek and Roman times (Udu-Ibiam et al. 2014). The fruiting bodies of fungi are consumed due to their variant textures and tastes. In addition, they are considerably important sources of dietary fiber, minerals, vitamins, water, proteins and carbohydrates (Kalac 2012, Yılmaz et al. 2016, Durmaz et al. 2018).

It has been found that mushrooms also have various pharmacological properties in addition to their nutritional value. Previous studies have reported that mushrooms had antioxidant, antimicrobial, anticancer, antiproliferative, DNA-protective, antiallergic, analgesic, antitumor, immunosuppressive, antiatherogenic, hypoglycemic, anti-inflammatory, hepatoprotective activities (Yang et al. 2008, Hetland et al. 2011, Patel and Goyal 2012, Ren et al. 2012, Li et al. 2013, Soares et al. 2013, Sun et al. 2014, Elsayed et al. 2014, Yıldız et al. 2015, Bal et al. 2017, Béni et al. 2018). Also, it has been found that *Lycoperdon* sp. mushrooms also have various pharmacological properties, in addition to their nutritional value. Previous studies have reported that mushrooms have antioxidant, antimicrobial, antiproliferative, antitumor, immunosuppressive and esterolytic activity (Colak et al. 2009, Shen et al. 2009, Sing et al. 2012, Novaković et al. 2015, Akpi et al. 2017).

Mushrooms are very valuable forest products. They are distributed in different forest ecosystems as saprotrophic, pathogenic and parasitic (Akata et al., 2018). In this context, they produce different levels of antioxidant and oxidant compounds depending on their habitats and capacities (Bal et al., 2019). In this study *L. molle* which is an edible species was used as material. In addition to the edible properties of the fungus, it was aimed to determine the presence of antioxidant potential. In addition, the oxidant level of the fungus was determined and the condition of the region where it was collected in terms of oxidant compound was determined.

In this context, this study aims to determine the antioxidant potential of the *L. molle,* an edible mushroom.

Material and Method

Mushroom Samples and Ethanolic Extract Preparation

L. molle were collected in Elmalı/Antalya, Turkey in 2018. Mushroom samples were collected from pine forest. Mushroom samples were introduced into the laboratory environment under suitable conditions. After identification of the mushroom samples, ethanol (EtOH) extraction of the mushrooms was carried out at 500C for nearly 6 hours using a Soxhlet extractor (Gerhardt EV 14). The extracts were concentrated using a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

TAS, TOS and OSI tests

The total antioxidant status (TAS) and total oxidant status (TOS) of the mushrooms were determined using Rel Assay kits (Rel Assay Diagnostics Kits, Turkey). Trolox was used as a calibrator in determining the TAS value and the results were expressed in mmol Trolox equiv./L (Erel 2004). Hydrogen peroxide was used as a calibrator in determining the TOS value and the results were expressed in μmol H_2O_2 equiv./L (Erel 2005). OSI values were calculated by dividing the obtained TOS value by the obtained TAS value. OSI (arbitrary unit: AU) was calculated according to the following formula and expressed in percentage terms (Erel 2005).

$$OSI = \frac{\text{TOS, } \mu\text{mol } H_2O_2 \text{equiv./L}}{\text{TAS, mmol Trolox equiv./L X 10}}$$

In this current study, six mushroom samples were obtained from the mushrooms and the measurements were repeated five times.

DPPH Free Radical Scavenging Activity Assay

The free radical scavenging activity of the mushrooms was determined using 1-diphenyl-2-picrylhydrazyl (DPPH). Stock solutions containing 1 mg/mL extract were prepared with DMSO. 50 μL solution was added to 160 μL 0.039% DPPH. The resulting solution was incubated in the dark at room temperature for 30 minutes. A reading for absorbance at 517 nm was obtained. The procedures were repeated individually for each concentration and sample (Shimada et al. 1992). In addition, caffeic and rosmarinic acids were used as reference antioxidants. Then, DPPH free radical scavenging percentages were calculated according to the formula:

Scavenging activity (%)= [(ADPPH-ASample)/(ADPPH)]x100.

Results and Discussion

Antioxidant activity

In addition to the benefits of oxygen for biological systems, it can also have side effects that are potentially harmful. Reactivity allows oxygen to take part in high-energy electron transfers and, therefore, supports a high amount of adenosine-5-triphosphate (ATP) formation via oxidative phosphorylation. Hence, it plays an important role in the development of multicellular organisms. Despite the benefits, oxygen also has the potential to harm many biological molecules such as proteins, lipids and DNA. Consequently, living organisms are always threatened by reactive oxygen species (ROS). This threat is generally balanced by the antioxidant protection system. However, this balance can be disrupted due to many environmental and inherent effects leading to oxidative stress (Burton and Jauniaux 2011; Sevindik et al., 2018).

The oxidative stress, which can manifest as a result of different environmental and inherent effects, is referred to as an imbalance between the production and elimination of reactive oxygen species (ROS) that cause multiple oxidative modifications of the basic and regulatory processes. Oxidative stress can increase due to increased levels of ROS, drug metabolism, over-expression of enzymes producing ROS or ionizing radiation as well as antioxidant enzyme deficiency (Gospodaryov and Lushchak 2012). Oxidative stress is associated with cellular

aging, acute and chronic kidney disease, neurodegenerative diseases, macular degeneration, biliary diseases, cancer and various acute and chronic pathological processes in addition to cardiovascular risk factors (obesity, diabetes, hypertension and atherosclerosis) (Burton et al. 2010; Chandrasekaran et al. 2017, Liguori et al. 2018; Sevindik, 2018).

In synthesis, given the close relationship between oxidative stress, inflammation, and aging, the oxidation-inflammatory theory of aging or oxi-inflamm-aging has been proposed: aging is a loss of homeostasis due to chronic oxidative stress that affects especially the regulatory systems, such as the nervous, endocrine, and immune systems. The consequent activation of the immune system induces an inflammatory state that creates a vicious circle in which chronic oxidative stress and inflammation feed each other and consequently, increases the age-related morbidity and mortality (De la Fuente and Miquel 2009, Liguori et al. 2018). Living organisms developed antioxidant defense systems in order to protect themselves from the negative effects of oxidative stress. These systems include some antioxidants produced in the body (endogenous) and some antioxidants derived from the diet (exogenous) (Rahman et al. 2012). It is of utmost importance to take antioxidant supplements in order to reduce oxidative stress when endogenous antioxidants are insufficient.

In this study, the antioxidant capacity of an edible mushroom, *L. molle*, was investigated. As a result, TAS, TOS and OSI values of *L. molle* were determined to be 1.855±0.072 mmol/L, 2.201±0.085 μmol/L and 0.119±0.008, respectively. There is no previous study investigating the oxidative stress status of *L. molle*. According to previous studies, the TAS values of other medicinal mushrooms, such as *Auricularia auricula* and *Trametes versicolor* were found to be 1.010 and 0.820 mmol/L, TOS values of the same species were found to be 23.910 and 17.760 μmol/L, whereby OSI values 2.367 and 2.166, respectively (Akgül et al. 2017). Moreover, TAS, TOS and OSI values for *Fomitopsis pinicola* were reported to be 1.44, 14.21 and 0.99, respectively (Sevindik et al. 2017). In comparison to these studies, *L. molle* was found to have a higher TAS value compared to *A. auricula, T. versicolor* and *F. pinicola* . Moreover, *L. molle* had lower TOS and OSI values compared to *A. auricula, T. versicolor* and *F. pinicola* species. It is thought that the differences in TAS, TOS and OSI values stem from the antioxidant production capacity, oxidant production capacity and environmental conditions of habitat of the fungus, as well as from their substrate.

Table1. DPPH scavenging activity of *L. molle*

	Mushroom extract concentrations			
	0.25 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL
Caffeic acid	8.62±0.91	21.34±0.66	38.39±0.66	59.47±0.05
Rosmarinic acid	6.03±0.15	7.00±0.41	35.09±0.10	61.91±8.77
L. molle	25.15±1.86	34.48±3.75	43.05±1.27	51.72±3.25

In addition, it was found that the DPPH activity of *L. mole's* fruiting bodies extract at 0.25, 0.5, 1 and 2 mg/mL concentrations ranged between 25.15% and 51.72 % (Table 1). Caffeic and rosmarinic acids as standards displayed 59.47±0.05% and 61.91±8.77% activity, respectively at a 2 mg/mL extract concentration. It was observed that the crude extract of the *L. molle* fruiting bodies exhibited activity similar to the standards used. Previous studies have reported that

methanol, ethanol and acetone extracts of *L. molle* displayed high DPPH free radical scavenging activity (Barros et al. 2008, Singh et al. 2012). This current study also indicated that *L. molle's* fruiting bodies extract possesses DPPH free radical scavenging activity which has demonstrated the antioxidant potential of the studied fungus.

Wild edible and medicinal mushrooms represent important forest products world-wide (Bonet et al., 2008; Bal et al., 2019). The material used in our study, *L. molle* is quite common and edible mushroom According to the results of the study, *L. molle* has antioxidant potential. Mushrooms, which are among forest products, are very important in determining new antioxidant sources. In this study, it was determined that *L. molle* has an important place in the forest ecosystem.

Conclusion

In this study, the antioxidant status of the edible mushroom *L. molle* was determined. This fungus displayed a high TAS value and a good DPPH free radical scavenging activity. *L. molle* may be a source of antioxidant compounds. Mushrooms are not only a source of income for collectors and tourism businesses, but can also provide economic incentives for forest owners. Therefore, it can improve forest management.

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