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Investigation of Oxidant and Antioxidant Status of Edible Mushroom *Clavariadelphus truncatus*

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Abstract: In the present study, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of *Clavariadelphus truncatus* Donk mushroom ethanol extracts that were collected in Kütahya province (Turkey) were determined. Rel Assay Diagnostics kits were used to determine TAS, TOS and OSI values. It was determined that the TAS value of the mushroom was 2.415 ± 0.176 mmol/L, the TOS value was 3.367 ± 0.155 μ mol/L and the OSI was 0.140 ± 0.005 . The study findings demonstrated that the region of growth of the mushroom exhibited adequate oxidative stress levels. It was also thought that the mushroom might be a natural antioxidant source due to its antioxidant potential.

Keywords: *Clavariadelphus truncatus*, edible mushroom, antioxidant, oxidant, oxidative stress

Yenilebilir Mantar *Clavariadelphus truncatus*'un Oksidan ve Antioksidan Durumunun Araştırılması

Öz: Bu çalışmada Kütahya (Turkey) ilinden toplanan *Clavariadelphus truncatus* Donk mantarının etanol özütlerinin toplam antioksidan seviyesi (TAS), toplam oksidan seviyesi (TOS) ve oksidatif stres indeksi (OSI) belirlenmiştir. TAS, TOS ve OSI değerleri Rel Assay Diagnostics kitler kullanılarak belirlenmiştir. Yaptığımız çalışma sonucunda mantarın TAS değeri 2.415 ± 0.176 mmol/L, TOS değeri 3.367 ± 0.155 μ mol/L ve OSI değeri 0.140 ± 0.005 olarak belirlenmiştir. Elde edilen bu sonuçlar mantarın toplandığı bölgenin yetişmesi açısından uygun oksidatif stres seviyelerinde olduğunu göstermektedir. Ayrıca tespit edilen antioksidan potansiyelinden dolayı mantarın doğal antioksidan kaynağı olabileceği düşünülmektedir.

Anahtar kelimeler: *Clavariadelphus truncatus*, yenilebilir mantar, antioksidan, oksidan, oksidatif stres

Introduction

Reactive oxygen species (ROS) are produced by the cellular metabolism of living organisms. Oxidative stress results from the imbalance between the ability of living organism to repair metabolic disorders that could be induced by reactive oxygen species in biological systems. They play a role in physiological cell processes at low to moderate ROS concentrations. However, high levels of reactive oxygen species have adverse effects on cell components such as lipids, proteins and DNA. Reactive oxygen species such as O₂⁻ (superoxide radical), OH (hydroxyl radical) and H₂O₂ (hydrogen peroxide) can lead

to adverse effects such as DNA breakdown and deterioration of cellular signaling mechanisms (Aprioku 2013; Ozougwu 2016; Thapa and Carroll 2017; Korkmaz et al., 2018).

As a result of the oxidative stress induced by reactive oxygen species, serious health problems such as cancer, Parkinson's and Alzheimer's diseases, cardiovascular diseases, chronic fatigue, depression could be observed in humans (Sarrafchi et al., 2015; Ozougwu 2016). Antioxidant compounds produced by living organisms play an important role in inhibition of oxidative stress. In cases where these antioxidants produced by the



living organism are inadequate, intake of supplementary antioxidant sources are very important in preventing oxidative stress-induced diseases (Dias et al., 2013; Riaz et al., 2018). Since ancient times, people have searched for nutrients and mushrooms became significant natural nutrients. They are rich in protein and amino acids with a low-calorie content in addition to their texture and taste and thus, they are considered to have high nutritious value (Yılmaz et al., 2016). Along with their nutritive properties, mushrooms have also been reported to possess several medicinal properties such as antioxidant, antimicrobial, antifungal, antibacterial, anticancer, anti-inflammatory properties (Imtiajet al., 2007; Khan et al., 2010; Smiderle et al., 2014; Akgül et al., 2016; Sevindik et al., 2017). Therefore, it was considered that the mushrooms, a significant natural source, can be used as a natural antioxidant sources in reducing oxidative stress. The present study aimed to determine total antioxidant status, total oxidant status and oxidative stress index of *C. truncatus* mushroom collected in Kütahya province (Turkey). Thus, antioxidant capacity of *C. truncatus*

mushroom was determined in the present study to assess its use as a natural antioxidant source.

Material and Method

The *C. truncatus* samples were collected in *Fagus orientalis* Lipsky and *Pinus nigra* L. mixed forest in the province of Kütahya/Turkey (Domaniç district, Çatalaliç location) (Figure 1). Morphological (shape, color, size) and ecological properties of the samples were recorded at the field. Microscopic characteristics of the samples that were transported to the laboratory under adequate conditions were determined by light microscopy using a 3% KOH solution (Leica DM750). The samples were morphologically identified with reference to the studies by Breitenbach and Kränzlin (1986), and Dähncke (2006). After identification of the mushroom samples, ethanol (EtOH) extracts were obtained with a Soxhlet extractor (Gerhardt EV 14). The obtained extracts were concentrated using a rotary evaporator (Heidolph Laborator 4000 Rotary Evaporator).



Figure 1. *Clavariadelphus truncatus* Donk

Determination of TAS, TOS and OSI

To determine mushroom TAS and TOS values, Rel Assay brand commercial kits (Assay Kit Rel Diagnostics, Turkey) were used. Trolox was used as calibrator for the TAS, and the findings were expressed in mmol Trolox equiv./L. Hydrogen peroxide was used as the calibrator for the TOS and the findings were reported in $\mu\text{mol H}_2\text{O}_2$ equiv./L (Erel, 2004, 2005). When the OSI value (Arbitrary unit: AU) that indicates the tolerance level of the oxidant compounds by the antioxidant compounds was calculated, TAS and TOS units were equalized, and the TOS values were divided by the TAS values. Thus, percentage

oxidative stress index value for the mushroom was determined (Erel, 2005).

Results and Discussion

TAS, TOS and OSI

Literature review did not reveal any previous studies that were conducted to determine the oxidative stress status of the *C. truncatus* mushroom. In the present study, the TAS and TOS values of *C. truncatus* mushroom were determined and OSI of the mushroom was determined based on the TAS and TOS values. The findings are presented in Table 1.

Table 1. TAS, TOS and OSI values of *C. truncatus*

	TAS (mmol/L)	TOS ($\mu\text{mol/L}$)	OSI
<i>C. truncatus</i>	2.415 \pm 0.176	3.367 \pm 0.155	0.140 \pm 0.005

Mushrooms include numerous and various types of reduced molecules such as phenolic compounds that contain electron-donor properties with antioxidant effect. In addition, they have the potential to contain several antioxidant enzymes and reduced coenzymes. They are rich in C and E vitamins that have high antioxidant effects and could include several elements with redox potential in their metabolism. Thus, these natural products potentially have strong antioxidant properties (Kalač 2013; Selamoglu et al., 2016). The analysis and evaluation of TAS as a marker of the system that reflects all these enzymatic and non-enzymatic molecules that the mushrooms potentially produce and maintain became significant for the identification and determination of new antioxidant natural resources. Little information is available in the literature on the TAS, TOS and OSI values of mushrooms. In previous studies, it was reported that the TAS value of *Pleurotus eryngii* (DC.) Quél. was 1.93 and the TAS value of *Auricularia polytricha* (Mont.) Sacc. was 0.93 (Yıldırım et al., 2012; Avcı et al., 2016). It was also reported that TAS values of *Tricholoma terreum* (Schaeff.) P. Kumm. and *Coprinus micaceus* (Bull.) Fr. were 0.38 and 0.46, TOS values were 16.76 and 16.87, and OSI values were 4.41 and 3.67, respectively. It was also determined that the TAS value of *Auricularia auricula* (L.) Underw. was 1.010, the TOS value was 23.910, and the OSI was 2.367, while the TAS value of *Cyclocybe cylindracea* (DC.) Vizzini & Angelini was 4.325, the TOS value was 21.109 and the OSI was 0.488, the TAS value of *Sarcosphaera coronaria* (Jacq.) J. Schröt. was 1.066, the TOS value was 41.672 and the OSI was 3.909, the TAS value of *Omphalotus olearius* (DC.) Singer was 2.827 mmol/L, TOS value was 14.210 $\mu\text{mol/L}$ and OSI was 0.503 (Akgül et al., 2016; Sevindik et al., 2017; Akgül et al., 2017; Sevindik et al., 2018ab). In this study, the TAS value of *C. truncatus* was determined as 2.415 \pm 0.176. When compared to previous study findings, it was determined that *C. truncatus* had a lower TAS value when compared to *O. olearius* and *C. cylindracea* mushrooms in the present study. It was also found that *C. truncatus* had higher TAS values when compared to *C. micaceus*, *T. terreum*, *A. polytricha*, *P. eryngii*, *S. coronaria* and *A. auricula* mushrooms. The differences in the findings were due to the antioxidant production capacities of different mushroom species. These results could be due to especially the endogenous or exogenous effects on the organism and resulting differences in the number and diversity of phenolic compounds related to the synthesis and release of secondary metabolites by the defense mechanism of the organism, differences in the vitamin levels with antioxidant

effects, and the changes in the levels of enzymatic/non-enzymatic antioxidant molecules.

In this study, the TOS value of *C. truncatus* was determined as 3.367 \pm 0.155. Analysis of the TOS values demonstrated that the TOS value of *C. truncatus*, used in the present study, was lower when compared to *C. micaceus*, *T. terreum*, *O. olearius*, *C. cylindracea*, *S. coronaria* and *A. auricula*. It was considered that the main reason for these differences between the TOS values was due to the differences between the regions where the mushrooms were collected and the oxidant compound production and accumulation capacities of the mushrooms and the differences in metabolic processes between different mushroom species. It is suggested that mushrooms with high TOS value or any natural product collected in these regions should be consumed with care. It is considered that agents with high TOS values exhibit these biochemical data due the impact of environmental and metabolic factors and these outcomes activate the defense mechanisms, particularly by inducing the production of certain free radicals such as reactive oxygen species for protection against endogenous harmful factors in the environment. The induction of the production of endogenous oxidant molecules by the defense mechanisms of organisms such as mushrooms provides protection against several environmental pollutants. Thus, the fact that *C. truncatus* had low TOS value suggested that there was no adversity related to the consumption of the mushroom in terms of oxidant compounds.

In this study, the OSI value of *C. truncatus* was determined as 0.140 \pm 0.005. It was found that *C. truncatus* OSI value was lower when compared to *C. micaceus*, *T. terreum*, *O. olearius*, *C. cylindracea*, *S. coronaria* and *A. auricula*. This finding demonstrated that oxidant compounds produced by *C. truncatus* were more resistant to endogenous antioxidant compounds when compared to the other mushrooms. *C. truncatus* antioxidant system was more potent and effective, resulting in low OSI levels. The oxidative stress, induced by oxidant molecules, was able to be prevented and removed by TAS, which covers the whole enzymatic and non-enzymatic systems, resulting in low OSI levels.

Conclusion

Total antioxidant status, total oxidant status and oxidative stress level of *C. truncatus* mushroom collected in Kütahya (Turkey) were determined for the first time in the present study. It was thought that the mushroom might



be consumed as a natural antioxidant product due to its antioxidant potential and the low oxidant and oxidative stress status.

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