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# Wild Edible Mushroom *Cantharellus cibarius* as a Natural Antioxidant Food

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ABSTRACT

In this study, DPPH free radical activity, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of wild edible mushroom *Cantharellus cibarius* Fr. collected from Antalya (Turkey) province were determined. Ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) extracts of *C. cibarius* mushroom were obtained using soxhlet apparatus. TAS, TOS and OSI values were determined by using Rel Assay kits. Free radical scavenging activity was determined using DPPH method. As a result of the studies, TAS value of the mushroom was found as 5.268±0.059 mmol/L, TOS value was 6.380±0.256 µmol/L and OSI value was 0.121±0.005. DPPH free radical activity was determined as EtOH extract 70.52±0.50, MeOH extract 64.34±1.54 and DCM extract 61.72±0.59 in 1 mg/mL extract concentration of mushroom. As a result, edible *C. cibarius* mushroom could be a natural antioxidant source.

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# Doğal bir Antioksidan Gıda Olarak Yenilebilir Yabani Mantar *Cantharellus cibarius*

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ÖZ

Bu çalışmada Antalya (Türkiye) ilinden toplanan yenilebilir yabani mantar *Cantharellus cibarius* Fr. mantarının DPPH serbest radikal aktivitesi, toplam antioksidan seviyesi (TAS), toplam oksidan seviyesi (TOS) ve oksidatif stres indeksi (OSI) belirlenmiştir. *C. cibarius* mantarının etanol (EtOH), metanol (MeOH) ve diklorometan (DCM) ekstraktları soxhlet aparatı kullanılarak elde edilmiştir. TAS, TOS ve OSI değerleri Rel Assay kitleri kullanılarak belirlenmiştir. Serbest radikal süpürme aktivitesi DPPH metodu kullanılarak tespit edilmiştir. Yapılan çalışmalar sonucunda mantarın TAS değeri 5,268±0,059 mmol/L, TOS değeri 6,380±0,256 µmol/L ve OSI değeri 0,121±0,005 olarak belirlenmiştir. 1 mg/mL özüt konsantrasyonunda DPPH serbest radikal aktivitesinin ise EtOH özütü 70,52±0,50, MeOH özütü 64,34±1,54 ve DCM özütü 61,72±0,59 olarak belirlenmiştir. Sonuç olarak yenilebilir *C. cibarius* mantarının doğal bir antioksidan kaynağı olabileceği belirlenmiştir.

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## Introduction

It is widely accepted that many natural foods taken by diet can reduce the risk of oxidative stress-related disease. There are many food components with antioxidant properties, such as α-tocopherol, γ-tocopherol, tocotrienol, ascorbic acid, β-carotene, and other substances like ubiquinol, and phenolic compounds. Antioxidants derived from the diet can be effective in different ways. It protects the cell from oxidative damage by preventing the increase of free radicals. After damage formation, antioxidants can stabilize free radical levels. Thus, it can relieve some of the symptoms caused by oxidative stress by preventing further damage (Traber and Atkinson, 2007; Urquiza-Martínez and Fenton Navarro, 2016). Determination of the nutrients that contribute to the antioxidant defense system is very important. In this study the antioxidant potential of wild edible mushroom *C. cibarius* was emphasized.

Mushrooms are high nutritional values found in many kitchen of the world. Mushrooms are considered nutraceutical foods in addition to their high nutritional values. Nutritional values of edible mushroom species have been reported to be quite good compared to meat, eggs and milk. They also contain a good balance of thiamine, riboflavin, ascorbic acid and vitamin D2, and a good protein content and a high amount of trace mineral. They are poor in calories and fat and contain a considerable amount of dietary fibre. However, nutritional properties can be changed by cultivation, watering, fruit-giving and storage conditions affecting the chemical composition (Valentão et al., 2005; Baba et al., 2012; Grimm and Wösten, 2018). Besides, organoleptic values, medicinal properties and economic importance because of their great interest. Also, there is no easy distinction between edible and medical mushroom. Many of the common edible species have therapeutic properties and many of them are edible for medical purposes (Valverde et al., 2015).

Turkey, Bulgaria and Serbia are leading European countries regarding *Cantharellus cibarius* growth. This mushroom is also important in terms of export to the international market (Šumić et al., 2015). Chanterelle word, funnel-like shape similar to "cup", "goblet" or "drinking vessel" comes from the Greek meaning "kantharoi" is derived from the word (Pilz et al., 2003). The name of the European golden *C. cibarius* is derived from the "cibarius" word which means Latin "food". The name of the united species is translated as *C. cibarius*, quite conveniently as cup food. It is very well known for its flavour and easy to collect and consume. (Pilz et al., 2003).

It has been reported by many investigators that the *Cantharellus* species have antioxidant potential as well as their nutritional properties (Table 1).

Table 1 Antioxidant activities of Wild *Cantharellus* species

| Species | Extract | Country | R |
|---|---|---|---|
| *Cantharellus cibarius Fr.* | Methanol, Alcoholic, Aqueous, Ethanol | Portugal, Nigeria, Romania, India, Serbia, Mexico, Turkey, China, Iran | 1 |
| *C. isabellinus* Heinem | Alcoholic, Aqueous | India | 2 |
| *C. rhodophyllus* Heinem | Alcoholic, Aqueous | India | 2 |
| *C. miniatescens* Heinem | Alcoholic, Aqueous | India | 2 |
| *C. appalachiensis* R.H. Petersen | Alcoholic, Aqueous | India | 2 |
| *C. fibrillosus* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. lateritius* (Berk.) Singer | Alcoholic, Aqueous | India | 2 |
| *C. applanatus* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. himalayensis* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. elongatipes* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. indicus* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. pseudoformosus* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. umbonatus* D. Kumari, Ram. Upadhyay & Mod.S. Reddy | Alcoholic, Aqueous | India | 2 |
| *C. minor* Peck | Alcoholic, Aqueous | India | 2 |
| *C. friesii* Quél. | Methanol, Aqueous | India | 3 |
| *C. subcibarius* Corner | Methanol, Aqueous | India | 3 |
| *C. cinereus* (Pers.) Fr. | Methanol, Aqueous | India | 3 |
| *C. tomentosus* Eyssart. & Buyck | Methanol | Tanzania | 4 |
| *C. congolensis* Beeli | Methanol | Tanzania | 4 |
| *C. cyanoxanthus* R. Heim ex Heinem | Methanol | Tanzania | 4 |
| *C. rufopunctatus* (Beeli) Heinem. | Methanol | Tanzania | 4 |
| *C. pseudocibarius* Henn | Methanol | Tanzania | 4 |
| *Afrocantharellus platyphyllus* (Heinem.) Tibuhwa (Syn: *C. platyphyllus* Heinem) | Methanol | Ivory Coast | 5 |

R: References; 1: (Valentao et al., 2005; Queiros et al., 2009; Ebrahimzadeh et al., 2010; Wani et al., 2010; Aina et al., 2012; Akata et al., 2012; Kosanic et al., 2013; Leahu et al., 2015; Kozarski et al., 2015; Kumari et al., 2016; Zavastin et al., 2016; López-Vázquez et al., 2017; Yıldız et al., 2017; Vamanu and Voica, 2017; Zhao et al., 2018); 2: (Kumari et al., 2016); 3: (Kumari et al., 2011); 4: (Tibuhwa, 2014); 5: (Kouassi et al., 2016)

In this study, it was aimed to determine total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and DPPH free radical scavenging activity of wild edible mushroom *C. cibarius* collected from Antalya (Turkey) province.

## Materials and Methods

*C. cibarius* samples used in the study were collected from the oak forest in Antalya (Turkey) province (Figure 1). The mushrooms collected in field studies were extracted in soxhlet extractor for about 6 hours at 50°C with ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) (Gerhardt EV 14). The extracts obtained were concentrated by rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Figure 1 *Cantharellus cibarius*

*Antioxidant Activity*

TAS, TOS and OSI values of *C. cibarius* samples were determined by using Rel Assay kits. Trolox was used as calibrator for the determination of TAS values. Results are shown in mmoL Trolox equiv./L (Erel 2004). $H_2O_2$ (Hydrogen peroxide) was used as calibrator in determining TOS values and the results are shown as μmoL $H_2O_2$ equiv./L (Erel, 2005). OSI (Arbitrary Unit = AU) value was determined according to the following formula (Erel, 2005).

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

The free radical scavenging activity of EtOH, MeOH and DCM extracts from *C. cibarius* samples was determined using 1-diphenyl-2-picrylhydrazase (DPPH). Stock solutions were prepared from the mushroom extracts at 1 mg/mL concentration using DMSO. 50μL of the prepared solution was added to 160μL %0.039 DPPH. It was then incubated for 30 minutes at room temperature in dark place. The absorbance at 517 nm wavelength was scanned. (Shimada et al. 1992). Separate processes were repeated for all extracts. Ascorbic acid was used as reference antioxidant.

DPPH free radical scavenging percentages;

The scavenging activity was calculated according to the formula

(%) = [(ADPPH-ASample)/(ADPPH)]×100

## Results and Discussion

*Antioxidant Activity*

Antioxidants compounds are compounds that combine many different chemical forms. Antioxidant compounds have the ability to counteract the effects of highly reactive, harmful free radicals, which are normally caused by the basic oxidation reactions in food. Natural antioxidant compounds are found in many foods (Anbudhasan et al., 2014). Mushrooms are important natural antioxidant sources. In this study, DPPH free radical activity was investigated by using EtOH, MeOH and DCM extracts of *C. cibarius* mushroom. The results are shown in Table 2.

In previous studies, it was reported that the methanol extract of *C. cibarius* collected from Portugal had high DPPH activity (Queiros et al., 2009). The extracts of ethyl acetate and methanol from *C. cibarius* collected from Iran have been reported to have high DPPH activity (Ebrahimzadeh et al., 2010). In another study, acetone and methanol extracts of *C. cibarius* collected from Serbia were reported to have high DPPH activity (Kosanic et al., 2013). In a different study, it has been reported that the ethanolic and hydromethanolic extracts of *C. cibarius* collected from Romania have DPPH activities (Zavastin et al., 2016). In our study, DPPH inhibition percentages of 1 mg / mL concentrations of EtOH, MeOH and DCM extracts of *C. cibarius* were determined as 70.52 ± 0.50, 64.34 ± 1.54 and 61.72 ± 0.59, respectively. EtOH extracts showed the highest activity. It was then observed in MeOH and DCM extracts, respectively. All concentrations showed low activity compared to the standard antioxidant ascorbic acid used. In this context, it was determined that *C. cibarius* may be a natural antioxidant source.

In addition, in our study, TAS, TOS and OSI values of *C. cibarius* mushroom were determined for the first time. The values obtained are shown in Table 3.

In studies conducted on previously edible natural mushroom species, TAS value of *Gyrodon lividus* was reported 2.077 mmol/L, TOS value was 13.465 μmol/L and OSI value was 0.651(Bal 2018). TAS value of *Lepista nuda* was reported 3.102 mmol/L, TOS value was 36.920 μmol/L and OSI value was 1.190 (Bal et al., 2019). TAS value of *Cyclocybe cylindracea* was reported 4.325 mmol/L, TOS value was 21.109 μmol/L and an OSI value was 0.488 (Sevindik et al., 2018). The TAS value of *Macrolepiota procera* mushroom was reported 2.805 mmol/L, TOS value was 6.596 μmol/L and OSI value was 0.235 (Akgül et al., 2016). In our study, TAS of *C. cibarius* was determined as 5.268±0.059 mmol/L, TOS value was 6.380±0.256 μmol/L and OSI value was 0.121±0.005. Compared to these studies, the TAS value of *C. cibarius* mushroom was higher than that of *G. lividus, L. nuda, C. cylindracea* and *M. procera*. The reason for the difference between TAS values of mushroom species is thought to be caused by the differences in the capacity of mushrooms to produce antioxidant compounds.

Also TOS and OSI values of *C. cibarius* mushroom was lower than *G. lividus, L. nuda, C. cylindracea* and *M. procera*.

The main reason for this difference in TOS values among mushroom is, as a result of differences in metabolic processes due to areas, where mushroom are collected, it is thought that mushroom are caused by their capacity to

produce and accumulate oxidant compounds in their bodies. With the increase in oxidative stress level, the formation of chronic and degenerative diseases such as cancer, aging, autoimmune diseases, cardiovascular and neurodegenerative diseases are increasing. The human body has various mechanisms for eliminating oxidative stress by means of endogenous antioxidants and exogenous antioxidants supplied through dietary foods and/or supplements. Endogenous and exogenous antioxidants act as "free radical scavengers le by preventing and repairing the damage caused by ROS and RNS, thereby increasing immune defences and reducing the risk of cancer and degenerative disease (Pham-Huy et al., 2008). In this study, it was determined that *C. cibarius*, which has high antioxidant potential, may be from natural antioxidant sources that can be taken by diet. However, it is recommended that the consumption of the fungus or any natural product with a high TOS value is controlled more controlled. In our study, because of the more potent and effective total antioxidant system of *C. cibarius*, OSI values were found to be low. Oxidative stress induced by oxidant molecules has been prevented by TAS, which covers the whole of enzymatic and nonenzymatic systems and as a result OSI values were obtained at low levels. According to these results, consumption of *C. cibarius* is thought to be beneficial in the scope of antioxidant supplementation.

Table 2 DPPH Activity of *C. cibarius*

| MC | EtOH | MeOH | DCM |
|---|---|---|---|
| C. *cibarius* | 70.52±0.50 | 64.34±1.54 | 61.72±0.59 |
| Ascorbic acid | 93.86±0.87 | 93.86±0.87 | 93.86±0.87 |

MC: Mushroom and Control

Table 3 TAS, TOS and OSI values of *C. cibarius*

| Sample | TAS | TOS | OSI |
|---|---|---|---|
| *C. cibarius* | 5.268±0.059 | 6.380±0.256 | 0.121±0.005 |

## Conclusion

In this study, the total antioxidant capacity of *C. cibarius*, total oxidant capacity, oxidative stress index and DPPH free radical scavenging activity were determined. TAS, TOS and OSI values of *C. cibarius* were determined for the first time. As a result of the studies, it was determined that *C. cibarius* may be a natural supplementary antioxidant source in reducing the effects of oxidative stress.

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