



Total Phenolic, Total Flavonoid Contents and Antioxidant Potential of The Wild Edible Mushroom *Clitocybe odora*

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ABSTRACT

The nutritional value of the edible fungus *Clitocybe odora* (Bull.) P. Kumm was evaluated by measuring its total phenolic, total antioxidant, total oxidant, and total flavonoid contents. In this case, a soxhlet was used to extract the methanol from the mushroom. The investigation involved the utilisation of Rel Assay kits to ascertain the total antioxidant status and total oxidant status. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) test was used to measure the ability to quench free radicals. Folin-Ciocalteu reagent was used to measure total phenolic content. Aluminum chloride analysis was used to determine the total flavonoid content. As a result of the study, the total antioxidant status of *C. odora* was determined to be 6.801 ± 0.243 mmol L⁻¹, the total oxidant status was 5.748 ± 0.137 µmol L⁻¹, and the oxidative stress index was 0.085 ± 0.003 . The extract has a scavenging activity of 73.38 ± 1.60 percent against DPPH free radicals at a concentration of 2 mg mL⁻¹. Total phenolic content was determined as 82.646 ± 1.623 mg g⁻¹ and total flavanoid content as 117.753 ± 3.491 mg g⁻¹. This led to the conclusion that the mushroom had significant antioxidant potential.

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Yenilebilir Doğal Mantar *Clitocybe odora*'nın Toplam Fenolik, Toplam Flavonoid İçeriği ve Antioksidan Potansiyeli

ÖZET

Yenilebilir mantar *Clitocybe odora* (Bull.) P. Kumm'nın toplam fenolik, toplam antioksidan, toplam oksidan ve toplam flavonoid içerikleri ölçülerek değerlendirildi. Bu kapsamda, mantardan metanol özütü elde etmek için soxhlet cihazı kullanıldı. Toplam antioksidan durumunu (TAS) ve toplam oksidan durumunu (TOS) belirlemek için Rel Assay kitleri kullanıldı. DPPH (2,2-Diphenyl-1-picrylhydrazyl) testi, serbest radikal süpürme yeteneğini ölçmek için kullanıldı. Toplam fenolik içeriği ölçmek için Folin-Ciocalteu reaktifi kullanıldı. Toplam flavonoid içeriğini belirlemek için alüminyum klorür analizi kullanıldı. Çalışma sonucunda *C. odora*'nın total antioksidan durumu 6.801 ± 0.243 mmol L⁻¹, total oksidan durumu 5.748 ± 0.137 µmol L⁻¹ ve oksidatif stres indeksi 0.085 ± 0.003 olarak belirlendi. Mantar özütünün 2 mg mL⁻¹'lik konsantrasyonda DPPH serbest radikallerine karşı 73.38 ± 1.60 'lik bir süpürme aktivitesine sahip olduğu belirlendi. Toplam fenolik madde içeriği 82.646 ± 1.623 mg g⁻¹ ve toplam flavaoid içeriği 117.753 ± 3.491 mg g⁻¹ olarak belirlendi. Bu sonuçlar mantarın önemli bir antioksidan potansiyelinin olduğunu gösterdi.

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INTRODUCTION

Mushrooms have been used for many purposes in different communities around the world (Eralan et al.,

2021). They are distributed in different ecosystems (Akata et al., 2018). In religious rituals, natural products that are of significant importance are utilized

as a source of sustenance or for medicinal purposes (Sevindik and Bal, 2021). They are invariable elements of diets in many countries (Gürgen et al., 2021). They are natural products with high nutritional properties such as protein, carbohydrates, vitamins, essential amino acids and nutritional elements (Torres-Gómez et al., 2022; Bal et al., 2023). In addition to nutritional properties, they are very useful sources from a medical point of view (Baba et al., 2020). Numerous studies have reported the diverse activities of fungi, such as antimicrobial, anticancer, antiproliferative, hepatoprotective, antioxidant, and DNA-protective properties (Canli et al., 2017; Oloke et al., 2017; Bal et

al., 2019; Atila et al., 2021; Majeed et al., 2021; Bal et al., 2022; Peng et al., 2022; Sevindik et al., 2023).

Clitocybe odora (Bull.) P. Kumm (Agaricales) are mushrooms that are abundant in conifer-dominated forests and broad-leaved forests. It can spread intensively from late summer to late spring. It is an edible type of mushroom. But when consumed heavily, it causes gastrointestinal syndrome (Walther et al., 2005; Sahin et al., 2021). Antioxidant activity studies on members of the genus *Clitocybe* in the literature are shown in Table 1.

Çizelge 1. *Clitocybe* türlerinin Antioksidan aktiviteleri
 Table 1. Antioxidant activities of *Clitocybe* species

Species	Extract	Country	References
<i>Clitocybe alexandri</i>	Methanol, ethanol	Portugal	Vaz et al., 2010
<i>Clitocybe maxima</i>	Hot water, Methanol, ethanol, aqueous	China, Taiwan	Tsai et al., 2009; Liu et al., 2012; Hu et al., 2017
<i>Clitocybe geotropa</i> (Current name: <i>Infundibulicybe</i> <i>geotropa</i>)	Ethanol, Metanol, acetone	Turkey, Serbia	Kosanić et al., 2020; Sevindik et al., 2020
<i>Clitocybe odora</i>	Ethanol, water	Portugal, Nigeria, Serbia	Egwim et al., 2011; Vaz et al., 2011; Dimitrijevic et al., 2015
<i>Clitocybe squamulosa</i>	aqueous	China	Yuan et al., 2022
<i>Clitocybe</i> <i>brunneocaperata</i>	methanol	India	Debnath et al., 2020
<i>Clitocybe nebularis</i>	Acetone, ethanol, methanol, distilled water	Serbia	Dimitrijevic et al., 2019; Kosanić et al., 2020
<i>Clitocybe nuda</i>	Water	Slovakia	Strapáč et al., 2019
<i>Clitocybe gibba</i>	Methanol	Korea	Kim et al., 2012

Clitocybe species have been shown to exhibit antioxidant activity in a variety of published research (Table 1). Research shows that antioxidant properties may be found in *Clitocybe* species from all over the world. We analyzed *C. odora* for its total phenolic and flavonoid content, as well as its antioxidant and oxidant potential and DPPH activity. In this study, total antioxidant and total oxidant status of *C. odora* was determined for the first time using Rel Assay kits. In addition, the suppression ratio (OSI) with antioxidant compounds included in the oxidant compounds was determined for the first time.

MATERIAL and METHOD

C. odora samples (MS-352) used in the study were collected from a fir forest in Kocaeli province. After collecting the fruiting bodies of mushroom samples, they were extracted with methanol (MeOH) for about six hours at 50 °C using a soxhlet extractor. Solvents of the resulting extracts were made using a rotary evaporator (Heidolph Laborota 4000 Rotary

Evaporator).

Total Phenolic and Flavonoid Tests

Dilution with distilled water brought the volume of the MeOH extracts to 0.1 mL. After that, we added 1 mL of Folin-Ciocalteu reagent (1:9, v/v) and gave it a good whirl. 0.75 mL of a 1% Na₂CO₃ solution was added to the mixture. Following a 2-hour incubation period at room temperature, the absorbance was measured at 760 nm. From the gallic acid standard solution calibration curve, we were able to determine the total phenolic content (TPC) in mg GAE (Gallic Acid Equivalent) g⁻¹ (Yurmutas et al., 2009).

Aluminium chloride analysis was used to determine the total flavonoid content (TFC) of the mushroom (Chang et al., 2002). Combined the Quercetin (0.5 mL), mushroom sample (0.5 mL), MeOH (4.3 mL), 10% Al (NO₃)₃, and NH₄CH₃COO (1 M) to make the final solution. Finally, a 40-minute incubation period was performed. The absorbance was checked at 415 nm. Flavonoids were expressed as mg QE (Quercetin

Equivalent) g⁻¹. In order to ensure accuracy, we triple-checked all of the results. Standard deviations were computed using the averages of the data sets used in the investigation.

Antioxidant activity tests

The TAS, TOS, and OSI values of *C. odora* were determined using Rel Assay kits (Mega Tıp/Türkiye). Trolox was utilized as a calibrator in TAS tests. The results were presented as mmol Trolox equiv./L (Erel, 2004). In order to determine the TOS values, hydrogen peroxide (H₂O₂) was utilized as a calibrator and the resulting outcomes were presented as μmol H₂O₂ equivalent L⁻¹, as reported by Erel in 2005. OSI values were determined by dividing the TOS values from the TAS values and taking the percentage (Sevindik et al., 2017).

Samples of *C. odora* were tested for their MeOH extract's ability to scavenge free radicals using 1-diphenyl-2-picrylhydrazyl (DPPH). The mushroom extracts were dissolved in 10% DMSO to make stock solutions with concentrations of 0.25, 0.50, 1, and 2 mg mL⁻¹. To 160 mL of %0.039 DPPH, 50 mL of the produced solution was added. After that, we let it sit in the dark and at room temperature for 30 minutes. The 517 nm absorbance was then measured (Shimada et al., 1992). Each extract had its unique series of procedures. The antioxidant ascorbic acid served as a standard.

DPPH free radical scavenging percentages (1);

The scavenging activity was calculated according to the formula (%) = [(ADPPH-ASample)/(ADPPH)]x100 (1)

Çizelge 2. *Clitocybe odora*'nın DPPH Aktivitesi

Table 2 DPPH Activity of *Clitocybe odora*

Mushroom and Control (%)	0.25 mg mL ⁻¹	0.50 mg mL ⁻¹	1 mg mL ⁻¹	2 mg mL ⁻¹
<i>Clitocybe odora</i>	37.49±1.30 ^a	52.94±1.46 ^b	66.29±1.23 ^c	73.38±1.60 ^d
Ascorbic acid	76.16±2.12 ^a	90.56±0.30 ^b	93.29±0.31 ^c	96.49±0.54 ^d

*Means followed by different letter(s) differ significantly at p < 0.05 (Duncan's multiple range test)

The study found that higher concentrations of mushroom extract resulted in greater DPPH activity. Activity was measured to be 73.38±1.60 at a 2 mg mL⁻¹ concentration. The ascorbic acid control showed 96.49±0.54 activity at 2 mg mL⁻¹. Also, the DPPH activity of different concentrations of the sample was found statistically different (p < 0.05). The mushroom extract was found to be less active than the reference standard. Multiple research conducted in many countries have indicated that *C. odora* possesses antioxidant activity (Egwim et al., 2011; Vaz et al., 2011; Dimitrijevic et al., 2015). This study shows that the MeOH extract of *C. odora* has potent free radical

Statistical Analysis

The analysis of all assays was performed in triplicate. The data were recorded as means ± standard deviations and analyzed in a completely randomized by using Statistical Package for Social Sciences (SPSS version 22.0). Statistically significant differences (p<0.05) among means of experimental results were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

RESULTS and DISCUSSION

Antioxidant activity

Organisms are constantly under stress due to environmental factors. These organisms produce oxidising free radicals as a result of their metabolic activities in response to environmental influences (Mohammed et al., 2019). As the levels of these compounds increase, the antioxidant defence system in living organisms is activated. The antioxidant defence system suppresses oxidant compounds. However, in cases where the antioxidant defence system is insufficient, oxidative stress occurs (Korkmaz et al., 2018). The occurrence of significant health conditions such as cancer, cardiovascular diseases, Alzheimer's, and Parkinson's can be attributed to oxidative stress in humans (Saridoğan et al., 2021). Supplementation with exogenous antioxidants can be utilized to reinforce the inadequate antioxidant defence system, which falls short in reducing the effects of oxidative stress. In this context, it is highly important to evaluate the potential use of mushrooms as a supplementary antioxidant (Unal et al., 2022). In this study, the MeOH extract of *C. odora* was evaluated for its DPPH free radical scavenging activity at concentrations of 0.25, 0.50, 1, and 2 mg mL⁻¹. The results obtained have been presented in Table 2.

scavenging action against DPPH radicals.

In this study, TAS, TOS and OSI values of *C. odora* were determined for the first time. The results obtained are shown in Table 3.

TAS value represents the antioxidant-effective compounds detected in the mushroom (Sevindik, 2020). TAS value of *C. odora* was calculated to be 6.801±0.243 in this investigation. Previously, different wild mushrooms *Gyrodon lividus* (Bull.) Sacc. (TAS:2.077, TOS:13.465, OSI:0.651), *Hohenbuehelia myxotricha* (Lév.) Singer (TAS:4.549, TOS:2.623, OSI:0.058), *Ramaria stricta* (Pers.) Quél. (TAS:4.223, TOS:8.201, OSI: 0.194), *Laetiporus sulphureus* (Bull.)

Çizelge 3. *Clitocybe odora*'nın TAS, TOS, OSI, TPC ve TFC değerleri

Table 3 TAS, TOS, OSI, TPC and TFC values of *Clitocybe odora*

	TAS mmol L ⁻¹	TOS µmol L ⁻¹	OSI	TPC mg g ⁻¹	TFC mg g ⁻¹
<i>Clitocybe odora</i>	6.801±0.243 ^c	5.748±0.137 ^b	0.085±0.003 ^a	82.646±1.623 ^d	117.753±3.491 ^e

*Means followed by different letter(s) differ significantly at p < 0,05 (Duncan's multiple range test)

Murrill (TAS:2.195, TOS:1.303, OSI:0.059), *Tricholoma virgatum* (Fr.) P. Kumm (TAS:3.754, TOS:8.362, OSI:0.223), *Suillus granulatus* (TAS:3.143, TOS:18.933, OSI:0.603), *Helvella leucopus* Pers (TAS:2.181, TOS:14.389, OSI:0.661) and *Cerioporus varius* (Pers.) Zmitr. & Kovalenko (TAS:2.312, TOS:14.358, OSI:0.627) have been reported (Bal, 2018; Sevindik et al., 2018; Sevindik, 2019; Krupodorova and Sevindik, 2020; Mushtaq et al., 2020; Selamoğlu et al., 2020; Sevindik and Akata, 2020; Krupodorova et al., 2022). TAS values for *G. lividus*, *H. myxotricha*, *R. stricta*, *L. sulphureus*, *T. virgatum*, *S. granulatus*, *H. leucopus*, and *C. varius* were found to be lower than those for *C. odora* in this research. To combat free radical damage, mushrooms make a plethora of chemicals that act as antioxidants (Sevindik, 2020). We can observe that *C. odora* has a strong antioxidant capacity in this setting.

The total oxidant potential (TOS) is a measure of all chemicals present in fungus that have oxidizing effects (Sevindik, 2020). *C. odora* had a greater TOS value than *H. myxotricha* and *L. sulphureus*, but a lower TOS value than *G. lividus*, *R. stricta*, *T. virgatum*, *S. granulatus*, *H. leucopus*, and *C. varius*. We found that the mushrooms utilized in this investigation had decreased oxidant levels. The level of suppression of oxidant chemicals generated in mushrooms by antioxidant compounds is represented by the OSI value (Sevindik, 2020). This study showed that the OSI for the *C. odora* we utilized was lower than that of *G. lividus*, *R. stricta*, *T. virgatum*, *S. granulatus*, *H. leucopus*, and *C. varius*, and higher than that of *H. myxotricha* and *L. sulphureus*. From these findings, it is clear that the *C. odora* we utilized in this study significantly mitigates the harmful effects of oxidant chemicals.

Total Phenolic and Flavonoid Values

Antioxidant actions are linked to total phenolic content, as is well known (Alispahić et al., 2015). Different types of wild mushrooms have been shown to have varying amounts of total phenolic contents, according to numerous research (Wong et al., 2013; Salachna et al., 2021; Bristy et al., 2022). The total phenolic content of the ethanol extract of *C. odora*, previously collected from Serbia, was reported as 38.112 mg g⁻¹ (Dimitrijevic et al., 2015). *C. odora* used in this study was determined as 82.646±1.623 mg g⁻¹. It is speculated that the solvent and the site where the fungus is gathered are the primary contributors to this variation. In contrast, the *C. odora* we employed in this

research has the potential to be a significant source due to its high phenolic content. It is generally agreed that flavonoids play a crucial role in protecting human health and vigor through their powerful antioxidant impact (Gašević et al., 2016; Shi et al., 2019). The flavonoid content of *C. brunneocaperata* has been reported in the past to be 13 g g⁻¹ (Debnath et al., 2020). Using a different species, *C. odora*, we were able to determine its total phenolic content to be 117.753±3.491 mg g⁻¹. Mushrooms, in this regard, are considered a potential resource for the extraction of flavonoids. In addition, the TAS, TOS, OSI, TPC and TFC values of sample were found statistically different (p < 0,05).

CONCLUSION

C. odora, a wild edible mushroom, has its antioxidant, oxidant, phenolic, flavonoid, and oxidative stress index levels analyzed in this article. The results indicated that the mushroom had significant anti-oxidant potential. Moreover, both the phenolic and flavonoid content levels were discovered to be rather high. Mushrooms, it is believed, can serve as a natural supply of antioxidants in this setting.

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Author's Contributions

The contribution of the authors is equal.

CONFLICT of INTEREST

The authors report no declarations of interest.

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