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Research Article

Wild mushroom *Tylopilus felleus* antioxidant, anticholinesterase and oxidant status and heavy metal contents

Celal Bal[1*], **Aras Fahrettin Korkmaz**[2], **Hayri Baba**[3], **Ilgaz Akata**[4], **Mustafa Sevindik**[5]

[1]Gaziantep University, Oguzeli Vocational School, Gaziantep, Türkiye
[2]İstanbul Kültür University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Istanbul, Türkiye
[3]Hatay Mustafa Kemal University, Faculty of Science and Literature, Department of Biology, Hatay, Türkiye
[4]Ankara University, Faculty of Science, Department of Biology, Ankara, Türkiye
[5]Osmaniye Korkut Ata University, Faculty of Engineering and Natural Sciences, Department of Biology, Osmaniye, Türkiye

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Abstract: Mushrooms play essential roles in natural ecosystems due to their ecological functions and bioactive potential, and many species are also valued for nutritional and medicinal purposes. In the present study, the antioxidant and oxidant status, anticholinesterase activity, and heavy metal contents of the fruiting bodies of the wild mushroom *Tylopilus felleus* (Bull.) P. Karst. were investigated. Total antioxidant status (TAS) and total oxidant status (TOS) were determined using Rel Assay commercial kits, and the oxidative stress index (OSI) was calculated accordingly. Anticholinesterase activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were evaluated based on the Ellman method, with galantamine used as a reference inhibitor. Heavy metal contents, including Fe, Zn, Cu, Pb, and Ni, were quantified using an atomic absorption spectrophotometer (Agilent 240FS AA). The TAS, TOS, and OSI values of *T. felleus* were found to be 3.427±0.147, 20.512±0.266, and 0.601±0.031, respectively. Elemental analysis revealed Fe, Zn, Cu, Pb, and Ni contents of 119.57±12.78, 21.07±1.69, 44.10±2.07, 2.89±0.47, and 0.82±0.011 mg/kg, respectively, all of which were within ranges reported in the literature. The mushroom extract exhibited moderate inhibitory activity against AChE (IC_{50}: 192.16 ± 3.15 µg/mL) and BChE (IC_{50}: 236.99±1.22 µg/mL) compared to galantamine. Overall, the findings suggest that *T. felleus* represents a potential natural source of antioxidant compounds with supplementary anticholinesterase activity.

INTRODUCTION

As integral components of terrestrial ecosystems, mushrooms contribute substantially to both environmental sustainability and human well-being. Their fruiting bodies typically emerge in abundance following rainfall, reflecting their close ecological dependence on environmental conditions. Throughout history, mushrooms have been widely utilized in indigenous cultures

*Corresponding Author: Mustafa SEVİNDİK sevindik27@gmail.com Osmaniye Korkut Ata University, Faculty of Engineering and Natural Sciences, Department of Biology, Osmaniye, Türkiye

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for medicinal, ceremonial, and nutritional purposes (Wu *et al.*, 2010). Owing to their constant interaction with soil, plants, and microorganisms, mushrooms are capable of synthesizing and accumulating a diverse array of bioactive compounds, which underpin their broad therapeutic potential (Nitha *et al*., 2007). Numerous studies have demonstrated that mushrooms exhibit a wide spectrum of biological activities, including antioxidant, antibacterial, anticancer, antiproliferative, anti-inflammatory, and DNA-protective effects (Lee and Jang, 2004; Ren *et al.*, 2012; Ma *et al.*, 2013; Sevindik *et al.*, 2023; Sevindik *et al.*, 2024).

Free radicals are continuously generated in living organisms as by-products of normal metabolic processes and environmental exposures. When produced in excess, these reactive species may induce oxidative damage to cellular components, ultimately leading to oxidative stress (Lee *et al.*, 2017). The antioxidant defense system plays a crucial role in neutralizing free radicals; however, when endogenous defenses are insufficient, exogenous antioxidants may help alleviate oxidative stress-related damage (Wang *et al.*, 2019). Due to their rich antioxidant profiles, mushrooms are considered valuable natural sources of exogenous antioxidants, and their antioxidant capacity warrants thorough investigation (Sevindik *et al.*, 2025).

In addition to oxidative stress, the dysregulation of the cholinergic system (particularly through the excessive activity of acetylcholinesterase and butyrylcholinesterase enzymes) has been associated with neurodegenerative disorders such as Alzheimer's disease. Natural anticholinesterase agents have therefore gained increasing attention as potential neuroprotective compounds. Recent research has highlighted mushrooms as promising sources of cholinesterase inhibitors, attributed to their diverse secondary metabolites and phenolic constituents (Mišković *et al.*, 2021; Murray *et al.*, 2024; Ünal *et al.*, 2025).

Moreover, mushrooms are globally recognized as nutritious foods due to their high contents of proteins, vitamins, minerals, essential amino acids, chitin, and their low fat and caloric values (Manzi *et al.*, 2001; Pedneault *et al.*, 2008; Valverde *et al.*, 2015). They also have the ability to accumulate various elements from their environment, with elemental composition varying according to ecological conditions (Wu *et al.*, 2020). Determining elemental content is therefore essential for evaluating both their nutritional value and potential health risks. In this context, the present study aimed to investigate the antioxidant and oxidant status, anticholinesterase activity, and elemental accumulation of the fruiting bodies of *Tylopilus felleus* (Bull.) P. Karst.

METHOD

Fruiting body samples of *T. felleus* (10 g) were dried in a laboratory oven at 40 °C until constant weight and subsequently ground into a fine powder. The powdered material (10 g) was extracted with 200 mL of ethanol using a heated magnetic stirrer for 24 h. Following extraction, the mixture was filtered through Whatman filter paper, and the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator.

Antioxidant and Oxidant Tests

The antioxidant and oxidant capacities of the mushroom extracts were determined using commercial Rel Assay total antioxidant status (TAS) and total oxidant status (TOS) kits, respectively, following the protocols described by Erel (2004, 2005). In the TAS assay, Trolox was employed as the calibration standard, whereas hydrogen peroxide served as the calibrator for the TOS measurements. The oxidative stress index (OSI) was calculated by normalizing TOS values to TAS values to ensure unit consistency, followed by expressing the resulting ratio as a percentage (Sevindik, 2025).

Anticholinesterase Activity Test

The anticholinesterase potentials of mushroom samples were evaluated based on the Ellman method (Ellman *et al.*, 1961). Galantamine was used as a positive control in the experimental design. Extract dilutions were prepared to obtain final assay concentrations ranging from 400 to 3.125 µg/mL in the reaction mixture. In the experimental application, 130 µL of 0.1 M phosphate buffer adjusted to pH 8.0 was first added to the microplate wells; then 10 µL of

extract solution and 20 µL of AChE or BChE enzyme solution were added, respectively. The mixtures were incubated in the dark at 25 °C for 10 minutes. After incubation, 20 µL of DTNB solution [5,5'-dithiobis-(2-nitrobenzoic acid)], along with 20 µL of suitable substrate (acetylthiocholine iodide or butyrylthiocholine iodide), was added to each well. Absorbance values of the reactions were measured at a wavelength of 412 nm; based on the obtained data, enzyme inhibition percentages and IC_{50} values were calculated and expressed as µg/mL.

Determination of Element Content

The concentrations of heavy metals (Fe, Zn, Cu, Pb, and Ni) in the mushroom samples were determined using an atomic absorption spectrophotometer (Agilent 240FS AA). Before analysis, the samples were dried at 80 °C to constant weight. For mineralization, 0.5 g of the dried material was digested with a mixture of 9 mL HNO_3 and 1 mL H_2O_2 using a microwave digestion system (Milestone Ethos Easy). Following digestion, elemental concentrations were quantified according to the method described by Sevindik and Akata (2019).

Statistical Analysis

Experimental results are presented as mean ± standard deviation (SD) based on triplicate measurements. Statistical analyses were performed using Microsoft Excel (Microsoft Office 2021). Differences were considered statistically significant at $p < 0.05$.

RESULTS and DISCUSSION

Antioxidant and Oxidant Potentials

Mushrooms may exhibit considerable variation in the levels of antioxidant enzymes and reduced coenzymes. In addition, they represent redox-active natural systems that incorporate vitamins such as A, C, and E into their metabolic processes (Baba *et al.*, 2025). The evaluation of total antioxidant status (TAS) is therefore a key parameter for assessing the overall antioxidant potential of natural materials, as TAS reflects the combined contribution of both enzymatic and non-enzymatic antioxidant components synthesized and accumulated by fungi (Gürgen & Sevindik, 2025). Despite their biological relevance, comprehensive data on TAS, total oxidant status (TOS), and oxidative stress index (OSI) values in mushrooms remain limited in the literature. In this context, the present study calculated the TAS, TOS, and OSI values of *T. felleus*, and the obtained results are presented in Table 1.

Table 1. *TAS, TOS, OSI, AChE and BChE values of Tylopilus felleus.*

Parameters	Tylopilus felleus	Galantamine
TAS	3.427±0.147	-
TOS	20.512±0.266	-
OSI	0.601±0.031	-
AChE	192.16±3.15	7.61±0.37
BChE	236.99±1.22	15.44±0.34

No findings regarding the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) values of *T. felleus* were found in the literature. Previous studies have demonstrated that ethanol or methanol extracts of *T. felleus* possess antioxidant activity (Macáková *et al.*, 2009; Ahmed *et al.*, 2015); however, comprehensive redox profiling of this species has remained unexplored. Several studies have previously reported TAS, TOS, and OSI values for different wild mushroom species. For example, *Ramaria stricta* exhibited TAS, TOS, and OSI values of 4.223 mmol/L, 8.201 µmol/L, and 0.194, respectively (Krupodorova and Sevindik, 2020), while *Candolleomyces candolleanus* showed values of 5.547 mmol/L, 8.572 µmol/L, and 0.155 (Karalti *et al.*, 2022). Similarly, TAS, TOS, and OSI values were reported as 3.683 mmol/L, 6.303 µmol/L, and 0.171 for *Leucopaxillus gentianeus* (Sevindik, 2021). In contrast, *Infundibulicybe geotropa* displayed a relatively low TAS (1.854 mmol/L) and markedly high TOS (30.385 µmol/L), resulting in an elevated OSI value of 1.639 (Sevindik *et*

al., 2020). Comparable assessments have also been reported for *Lactifluus rugatus*, *Lepista nuda*, *Lycoperdon molle*, and *Gyrodon lividus* (Bal *et al.*, 2019; Sevindik, 2020; Emsen *et al.*, 2019; Bal, 2018). In the present study, the TAS value of *T. felleus* was lower than those reported for *R. stricta*, *C. candolleanus*, *L. gentianeus*, and *L. molle*, but higher than those of *I. geotropa*, *L. rugatus*, *L. nuda*, and *G. lividus*. Since TAS reflects the cumulative contribution of antioxidant-active compounds synthesized by mushrooms (Gürgen and Sevindik, 2022), these differences may be attributed to species-specific metabolic capacities as well as environmental and ecological factors influencing antioxidant biosynthesis. Comparative evaluation of TOS values revealed that *T. felleus* exhibited lower oxidant levels than *I. geotropa* and *L. nuda*, but higher levels than *C. candolleanus*, *L. gentianeus*, *L. rugatus*, *R. stricta*, and *G. lividus*. The wide variation in TOS values reported across studies highlights the strong influence of collection site, environmental pollution, and habitat characteristics on oxidant accumulation in mushrooms. Given that elevated TOS levels may pose potential risks, careful regulation of the collection and consumption of mushrooms with high oxidant loads is advisable. The oxidative stress index (OSI), which reflects the balance between oxidant burden and antioxidant defense capacity (Gürgen and Sevindik, 2022), further supported these observations. *T. felleus* exhibited higher OSI values than *C. candolleanus* and *L. gentianeus*, but lower values than *I. geotropa*, *L. nuda*, and G. lividus. The relatively moderate OSI value of *T. felleus* suggests that its antioxidant defense mechanisms are partially effective in counteracting oxidative stress. Overall, mushrooms collected from environments associated with moderate oxidative stress may still serve as nutritional resources; however, their consumption should be evaluated cautiously with respect to redox balance and environmental conditions.

Anticholinesterase Activity

Mushrooms may exhibit substantial variation in their capacity to produce and accumulate bioactive compounds with neuroprotective potential, including natural cholinesterase inhibitors (Andrade *et al.*, 2022). These organisms synthesize a diverse range of secondary metabolites capable of interacting with key enzymes of the cholinergic system, particularly acetylcholinesterase and butyrylcholinesterase. Assessment of anticholinesterase activity is therefore a critical parameter for evaluating the neuropharmacological potential of natural materials, as it reflects the ability of bioactive constituents to inhibit enzymatic acetylcholine degradation (Wei *et al.*, 2022; Sutthibutpong *et al.*, 2024). Despite this relevance, information regarding the anticholinesterase properties of wild mushrooms remains limited. In the present study, the inhibitory effects of *T. felleus* against acetylcholinesterase and butyrylcholinesterase were determined using the Ellman method, and the obtained results are presented in Table 1.

The anticholinesterase activity of the *T. felleus* extract was evaluated against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using the Ellman method, and the results are presented in Table 1. The extract exhibited inhibitory effects on both enzymes, with IC_{50} values of 192.16±3.15 µg/mL for AChE and 236.99±1.22 µg/mL for BChE. These findings indicate that *T. felleus* possesses moderate anticholinesterase activity, showing slightly stronger inhibition toward AChE than BChE. As expected, galantamine, used as a reference inhibitor, demonstrated substantially higher inhibitory potency against both enzymes. To the best of our knowledge, no previous study has reported the anticholinesterase activity of *T. felleus*. Although direct comparisons with the same species are not possible, several studies conducted on different wild mushroom species have documented their ability to inhibit cholinesterase enzymes. These reports collectively support the view that wild mushrooms constitute a promising source of natural anticholinesterase agents (Tel *et al.*, 2015; Dundar *et al.*, 2016; Nguyen *et al.*, 2016; Korkmaz *et al.*, 2025). The moderate inhibitory activity observed for *T. felleus* may be attributed to its complex secondary metabolite profile. Mushroom-derived phenolic compounds and other low-molecular-weight metabolites are known to interact with cholinesterase enzymes through reversible binding mechanisms, resulting in partial enzyme inhibition (Hamed *et al.*, 2023). The slightly higher inhibition of AChE compared to BChE

observed in this study suggests a potential preference toward neuronal acetylcholinesterase, which plays a central role in the regulation of synaptic acetylcholine levels. Although the inhibitory potency of the *T. felleus* extract was lower than that of galantamine, this outcome is not unexpected when comparing crude natural extracts with purified pharmaceutical agents. Importantly, moderate anticholinesterase activity in natural products may still be biologically relevant, particularly when considered alongside potential synergistic effects and lower toxicity profiles. These characteristics are often regarded as advantageous in the early stages of natural product-based neuroprotective research. Overall, the present results expand the current knowledge on the neurobiological potential of wild mushrooms by providing the first anticholinesterase data for *T. felleus*. Further studies focusing on bioactive compound isolation, structure–activity relationships, and in vivo assessments are warranted to better elucidate the therapeutic relevance of these findings.

Antioxidant and Oxidant Potentials

Mushrooms constitute a fundamental component of ecological systems due to their role in the decomposition of dead organic matter. Through this process, they are able to absorb and accumulate various elements from their surroundings, with the resulting elemental composition largely influenced by the characteristics of the substrate on which they grow (Mleczek *et al.*, 2021). Depending on the type and concentration of accumulated elements, mushrooms may exert either beneficial or toxic effects. Therefore, determination of the elemental composition of edible or medicinal mushrooms is of critical importance for evaluating both their nutritional value and potential health risks (Siwulski *et al.*, 2019). In the present study, the concentrations of Fe, Zn, Cu, Pb, and Ni in *T. felleus* were determined, and the corresponding results are presented in Table 2.

Table 2. *Heavy metal contents of the fruiting body of Tylopilus felleus*

Elements	*Tylopilus felleus*
Fe	119.57±12.78
Zn	21.07±1.69
Cu	44.10±2.07
Pb	2.89±0.47
Ni	0.82±0.011

The elemental composition of *T. felleus* was determined as 119.57 ± 12.78 mg kg^{-1} for Fe, 21.07±1.69 mg/kg for Zn, 44.10±2.07 mg/kg for Cu, 2.89±0.47 mg/kg for Pb, and 0.82±0.011 mg/kg for Ni. A previous study conducted on *T. felleus* fruiting bodies collected from the Yunnan region of China reported Fe, Zn, Cu, and Pb contents of 75.57, 293.06, 12.72, and 0.73 mg/kg, respectively (Ahmed *et al.*, 2015). Compared to these findings, the present samples exhibited higher concentrations of Fe, Cu, and Pb, whereas the Zn content was notably lower. Such variations in elemental composition are not unexpected and may be attributed to differences in environmental conditions, soil characteristics, substrate composition, and geographical location. Indeed, the literature reports wide concentration ranges for wild mushrooms, including 14.60-1714 ppm for Fe, 7.63-40 ppm for Zn, 1.90-180 ppm for Cu, 0.18-16.54 ppm for Pb, and 0.67-6.72 ppm for Ni (Falandysz et al., 2007, 2008; Krupodorova & Sevindik, 2020; Nikkarinena & Mertanen, 2004; Sarikurkcu et al., 2011; Vetter, 1990). When evaluated within this broader context, the Fe, Zn, Cu, Pb, and Ni concentrations detected in *T. felleus* in the present study fall well within the ranges previously reported for wild mushrooms. Overall, these findings suggest that the elemental profile of *T. felleus* is consistent with existing literature data and reflects the strong influence of environmental and ecological factors on metal accumulation in wild mushroom species.

CONCLUSION

In the present study, *T. felleus* was evaluated in terms of its antioxidant and oxidant status, anticholinesterase activity, and elemental composition, including Fe, Zn, Cu, Pb, and Ni. The results demonstrated that *T. felleus* possesses a notable antioxidant capacity, while also exhibiting measurable oxidant levels. Additionally, the mushroom extract showed moderate inhibitory activity against acetylcholinesterase and butyrylcholinesterase enzymes, indicating its potential neurobiological relevance. Analysis of heavy metal contents revealed that the concentrations of Fe, Zn, Cu, Pb, and Ni were within the ranges reported in the literature for wild mushrooms. Overall, these findings suggest that *T. felleus* may serve as a promising natural source of antioxidant compounds with supplementary anticholinesterase activity.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent via email upon request.

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C.B.: Investigation, Resources, Methodology, Visualization, Software, Formal Analysis, and Writing-original draft. **A.F.K.**: Investigation, Resources, Methodology, Visualization, Software, Formal Analysis, and Writing-original draft. **H.B.**: Investigation, Resources, Methodology, Visualization, Software, Formal Analysis, and Writing-original draft. **I.A.**: Investigation, Resources, Methodology, Visualization, Software, Formal Analysis, and Writing-original draft. **M.S.**: Investigation, Resources, Methodology, Visualization, Software, Formal Analysis, Writing-original draft, Supervision, and Validation. Authors may edit this part based on their case.

Orcid

Celal Bal https://orcid.org/0000-0001-6856-3254
Aras Fahrettin Korkmaz https://orcid.org/0000-0002-3827-3018
Hayri Baba https://orcid.org/0000-0002-1837-4321
Ilgaz Akata https://orcid.org/0000-0002-1731-1302
Mustafa Sevindik https://orcid.org/0000-0001-7223-2220

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