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Research Article

Anticholinesterase, Antimicrobial and Antioxidant Activities of Wild Mushroom *Suillus bellinii* (Inzenga) Kuntze

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ABSTRACT

Mushrooms are invariable components of natural ecosystems. Today, they are used by humans for different purposes. In this study, some biological activities of wild mushroom *Suillus bellinii* (Inzenga) Kuntze were determined. In this regard, ethanol and methanol extracts of the mushroom were obtained using Soxhlet apparatus. Anticholinesterase activity was determined by determining acetyl and butyrylcholinesterase activities. Antimicrobial activity was determined by agar dilution method using standard bacterial and fungal strains. Total antioxidant level (TAS), total oxidant level (TOS) and oxidative stress index (OSI) of the mushroom were determined using Rel Assay kits According to the results obtained, it was observed that methanol extract of mushroom had higher acetyl and butyrylcholinesterase activity than ethanol extract. The mushroom extracts were found to be effective against microorganisms at concentrations between 100-400 µg/Ml. In addition, It was determined that the methanol extract of the mushroom had higher TAS and TOS values than the ethanol extract. OSI value was found to be higher in ethanol extract. In conclusion, it was determined that *Suillus bellinii* has antioxidant, antimicrobial and anticholinesterase activities and can be used as natural material in this concept.

Introduction

Wild edible fungi are natural resources with various potentials in the medicinal field. In particular, they may contain bioactive components and are used in traditional medicine and modern drug development processes [1-3].

The medicinal potential of mushrooms includes anticholinesterase, antioxidant, anti-inflammatory, antiviral, antibacterial, antitumoral and immunomodulatory effects [2, 4-9].

Mushrooms may contain natural components with anticholinesterase activity [10]. Anticholinesterases are enzymes that inhibit the breakdown of acetylcholine [11]. The anticholinesterase inhibitors contained in mushrooms block anticholinesterase enzymes, slowing the breakdown of acetylcholine and improving nerve conduction. Therefore, mushrooms could potentially be a natural source for the treatment of neurodegenerative diseases such as Alzheimer's disease [12-14].

Mushrooms naturally contain bioactive components with antioxidant activity [15]. Antioxidants are substances that prevent or reduce oxidative damage by neutralizing harmful

free radicals caused by oxidative stress in cells. The antioxidant activity of mushrooms can help support the healthy aging process, reduce the risk of chronic diseases and provide positive effects on overall health [16-18]. Therefore, regular consumption of antioxidant-rich mushrooms can be recommended as part of a healthy lifestyle and a balanced diet [19].

Mushrooms contain natural components with antimicrobial activity and these properties have the potential to prevent or treat infections by inhibiting the growth or killing microorganisms [20]. Antimicrobial activity is related to the effects of the bioactive components contained in mushrooms on microorganisms [21]. The antimicrobial activity of mushrooms may be a promising natural resource for the control and treatment of infections [22].

Research on the medicinal use of mushrooms is continuing and more scientific research is needed on their full effects and safe use [23]. *Suillus bellinii* mushroom is an edible mushroom species that is particularly widespread in Europe [24]. However, there are limited studies on the medicinal activities of this mushroom species. The aim of the study is investigation of anticholinesterase, antimicrobial and antioxidant activity of *Suillus bellini* mushroom.

Material and Method

Mushroom extraction

*Suillus bellini*i samples were collected from Oğuzeli/Gaziantep in 2022. 30 g of dry mushroom samples were extracted with ethanol at 50 ℃ for about 6 hours in a Soxhlet apparatus. The process was repeated for methanol extraction.

Anticholinesterase activity

The anticholinesterase activity (acetylcholinesterase and butyrylcholinesterase) of ethanol and methanol extracts of the mushroom was measured by the Ellman method [25]. Stock solutions were prepared from mushroom extracts in the range of 3.125-200 µg/mL. 130 µL 0.1 M pH=8 phosphate buffer, 10 µL stock solution, 20 µL enzyme (AChE or BChE enzyme solution) were added to the microplane. Then, incubation was performed at 25 °C in the dark for 10 min. To the resulting solution, 20 µL DTNB (5,5''-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL substrate (acetylcholine iodide or butyrylcholine iodide) were added. Finally, readings were taken at 412 nm and the IC50 values of the results were calculated and expressed as µg/mL.

Antimicrobial activity

Antimicrobial activities of the mushroom extract were determined by agar dilution method. The lowest concentration of the extract that inhibited the growth of bacterial and fungal strains used in the study was determined. Stock solutions were prepared from mushroom extract at concentrations of 12.5, 25, 50, 100, 200, 400 and 800 µg/mL.
Bacterial strains (*Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606) were cultured in Muller Hinton Broth medium. Fungi strains (*Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 ve *C. glabrata* ATCC 90030) RPMI 1640 were cultured in RPMI 1640 Broth medium [26-28]

Antioxidant activity

Total antioxidant and total oxidant tests of mushroom extract were performed using Rel assay TAS and TOS kits. Trolox for TAS kit and hydrogen peroxide for TOS kit were used as calibrators. TAS value was expressed in mmoL Trolox equiv./L. TOS value was expressed as µmoL H2O2 equiv./L [29, 30]. OSI (Arbitrary Unit: AU) was determined by the ratio of TOS values to TAS values [31]. In the study, mushroom samples were studied in 3 replicates. Values were expressed as mean±SD.

Results and Discussion

Anticholinesterase activity

Anticholinesterase inhibitors may enhance nerve conduction and contribute to the improvement of neurological functions by inhibiting the breakdown of acetylcholine [32]. Therefore, the discovery and evaluation of anticholinesterase inhibitors derived from natural sources such as mushrooms may lead to the development of new

and effective therapeutic agents for the treatment of neurological diseases [33].

Anticholinesterase activity of ethanol and methanol extracts of *Suillus bellini* mushroom were given in Figure 1.

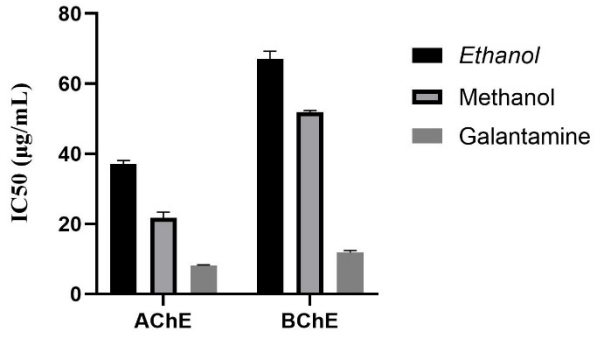

Figure 1. Anticholinesterase activity of *S. bellinii*

There is no study on the anticholinesterase activity of Suillus bellini in the literature. In the present study, acetyl and butyrylcholinesterase activities of ethanol and methanol extracts of the mushroom were determined. In the study, it was determined that the methanol extract of the mushroom had higher activity than the ethanol extract. It was also found to have lower activity than galantamine used as a standard. Investigation of the presence of enzymes that play a role in the occurrence of diseases and the suppression of these enzymes is very important in the fight against diseases [34]. Cholinesterase inhibitors are generally used in the treatment of Alzeihmer's disease. In the study, the anticholinesterase activity of *Suillus bellini* was determined. According to the findings obtained, it was observed that the mushroom has anticholinesterase activity.

Antimicrobial activity

Mushrooms and other natural materials may contain a variety of antimicrobial compounds, and the discovery of these compounds may play an important role in combating antibiotic resistance and in the development of new treatment methods [35, 36]. Antimicrobial compounds from these natural sources are considered to be a potential source for developing new drugs and therapeutic agents [37, 38]. Therefore, it is very important to determine the antimicrobial potential of mushrooms.

Antimicrobial activity of ethanol and methanol extracts of *Suillus bellini* mushroom were given in Table 1.

Table 1. Antimicrobial activity of *S. bellinii*

Microorganism	Extract solvent µg/mL	
	Ethanol	Methanol
S. aureus	200	200
***S. aureus* MRSA**	100	200
E. faecalis	200	100
E. coli	400	400
P. aeruginosa	400	200
A. baumannii	400	400
C. glabrata	200	200
C. albicans	100	200
C. krusei	200	200

There is no study on the antimicrobial activity of *Suillus bellinii* in the literature. In our study, the effects of ethanol and methanol extracts of the mushroom were investigated against standard bacterial and fungal strains. The ethanol extract of the mushroom showed the highest activity against *S. aureus* MRSA and *C. albicans* at a concentration of 100 µg/mL. It was also effective against *S. aureus*, *E. faecalis*, *C. glabrata* and *C. krusei* at a concentration of 200 µg/mL. The mushroom extract was effective against *E. coli*, *P. aeruginosa* and *A. baumannii* at 400 µg/mL. The methanol extract of the mushroom showed the highest effect against *E. faecalis* at a concentration of 100 µg/mL. It was also found to be effective against *S. aureus*, *P. aeruginosa*, *S. aureus* MRSA, *C. glabrata*, *C. albicans* and *C. krusei* at a concentration of 200 µg/mL. In addition, extracts at a concentration of 400 µg/mL were effective against *E. coli* and *A. baumannii*. In the previous studies, *Suillus granulatus*, a different *Suillus* species, was reported to be effective against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Listeria monocytogenes* and *Salmonella typhimurium* [39]. In this context, it was determined that *Suillus bellinii* used in our

study has antimicrobial potential against test microorganisms.

Antioxidant activity

Free radicals are oxidant compounds produced as a result of metabolic activities. These compounds, which can be tolerated at low levels, can cause serious damage as their levels increase [40]. The antioxidant defence system acts to reduce the effects of oxidant compounds [41]. However, in some cases, the antioxidant defence system may be insufficient. In such cases, oxidative stress occurs [42]. As a result of oxidative stress, cancer, cardiological disorders, neurodegenerative diseases can be observed in humans [43, 44]. Supplementary antioxidants can be used to reduce the effects of oxidative stress [45]. Mushrooms are important sources of natural antioxidants. In this context, the antioxidant potential of *Suillus bellinii* was determined in the present study.

TAS, TOS and OSI values of ethanol and methanol extracts of *Suillus bellini* mushroom were given in Figure 2.

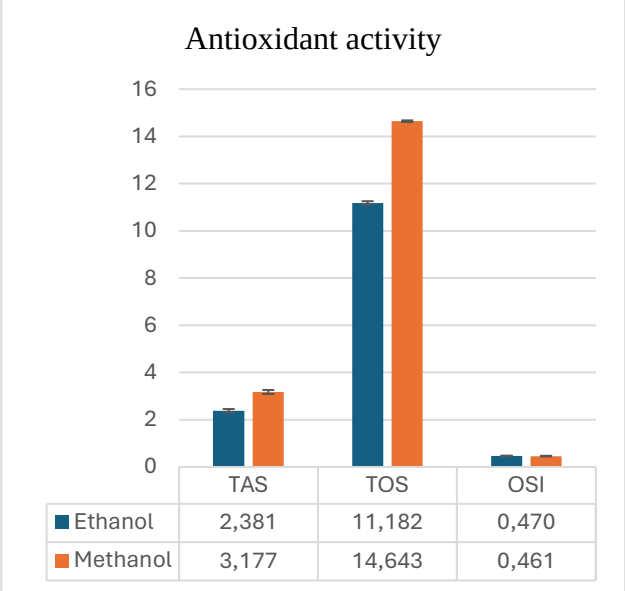

Figure 2. Antioxidant activity of *S. bellinii*

In the literature, antioxidant potential of *Suillus bellinii* has been reported by different methods [46].

TAS, TOS and OSI values of Suillus bellini have not been reported before. In this study, these values were determined for the first time. TAS values of ethanol and methanol extracts of *S. bellinii* were determined as 2.381±0.073 and 3.177±0.086 mmol/L, respectively. TOS values of ethanol and methanol extracts of *S. bellinii* were determined as 11.182±0.074 and 14.642±0.035 µmol/L, respectively. OSI values of ethanol and methanol extracts of *S. bellinii* were determined as 0.470±0.011 and 0.461±0.013, respectively. In previous mushroom studies, TAS values of *Suillus granulatus*, *Chanterelle cibarius*, *Clavariadelphus truncatus*, *Helvella leucopus*, *Terfezia boudieri*, *Clitocybe odora* and *Entoloma sinuatum* were reported as 3.143, 5.268, 2.415, 2.181, 2.332, 6.801 and 2.64 mmol/L, respectively. TOS values were reported as 18.933, 6.380, 3.367, 14.389, 26.945, 5.748 and 6.58 µmol/L, respectively. In addition, OSI values were reported as 0.603, 0.121, 0.140, 0.661, 1.156, 0.085 and 0.25, respectively [40, 47-52].

TAS value is an indicator of the totality of compounds with antioxidant potential in natural products [53]. High TAS values indicate that the natural product has high antioxidant potential. The TAS value of the ethanol extract of *Suillus bellinii* used in this study was lower *than Suillus granulatus*, *Chanterelle cibarius*, *Clavariadelphus truncates*, *Clitocybe odora* and *Entoloma sinuatum*, and higher than *Terfezia boudieri* and *Clitocybe odora*. In addition, the TAS value of the methanol extract of *Suillus bellinii* was lower than that of *Chanterelle cibarius* and *Clitocybe odora*, and higher than that of *Suillus granulatus*, *Clavariadelphus truncatus*, *Helvella leucopus*, *Terfezia boudieri*, *Entoloma sinuatum*. In this context, it was observed that *Suillus bellinii* has antioxidant potential.

TOS value is an indicator of the totality of oxidant compounds found in natural products [54].

The TOS value of the ethanol extract of *Suillus bellinii* used in this study was lower than *Suillus granulatus*, *Helvella leucopus*, *Terfezia boudieri* and higher than *Chanterellus cibarius*, *Clavariadelphus truncatus*, *Clitocybe odora* and *Entoloma sinuatum*. In addition, TOS value of methanol extract of *Suillus bellinii* was lower than *Suillus granulatus*, *Terfezia boudieri* and higher than *Cantharellus cibarius*, *Clavariadelphus*

truncatus, *Helvella leucopus*, *Clitocybe odora* and *Entoloma sinuatum*.

OSI value shows how much the antioxidant compounds detected in natural products suppress oxidant compounds in percentage. As a result of the increase in the OSI value, the consumption of natural products is not recommended because it will be harmful [50]. The OSI values of both ethanol and methanol extracts of *Suillus bellinii* used in the present study were lower than *Suillus granulatus*, *Helvella leucopus*, *Terfezia boudieri* and higher than *Chanterellus cibarius*, *Clavariadelphus truncatus*, *Clitocybe odora* and *Entoloma sinuatum*. In this context, it was determined that *Suillus bellinii* used in our study has antioxidant potential. In conclusion, it was concluded that *S. bellinii* can be used in pharmacological preparations due to its potential anticholinesterase, antimicrobial and antioxidant activities.

Ethics committee approval and conflict of interest statement

There is no need to obtain permission from the ethics committee for the article prepared.

There is no conflict of interest with any person / institution in the article prepared.

Authors' Contributions

-Study conception and design: Sevindik, M., Bal, C.

-Acquisition of data: Sevindik, E., Bal, C, Krupodorova, T.

-Analysis and interpretation of data: Sevindik E., Bal, C., Krupodorova, T.

-Drafting of manuscript: Gürgen, A., Sevindik M.

-Critical revision: Sevindik M., Gürgen, A.

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