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Original Article

Some biological activities and element levels of *Lycoperdon pratense*

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ABSTRACT

Mushrooms are natural products with many biological activities. In this study, antioxidant, antimicrobial, anticholinesterase activities and element levels of *Lycoperdon pratense* Pers. were determined. In this context, the mushroom was extracted with ethanol in a soxhlet device. Antioxidant potential was determined using Rel assay kits. Antimicrobial activity was determined using the agar dilution method. Antialzheimer activity was determined by acetyl- (AChE) and butyrylcholinesterase (BChE) potentials. The levels of elements accumulated in the mushroom were scanned using the wet digestion method. As a result of the analysis, the TAS value of the mushroom was measured as 2.589 ± 0.118 mmol Trolox equiv./L, the TOS value was 10.360 ± 0.197 μmol H_2O_2 equiv./L and the OSI value was 0.401±0.013. The mushroom extract was found to have high activity against bacteria. It was determined that the extract had an effect against microorganisms at concentrations between 50-400 μg/mL. It was determined that the anti-AChE IC50 value of the mushroom extract was 14.48 ± 0.80 μg/mL and the anti-BChE IC50 value was 23.10±1.21 μg/mL. It is also thought that it can be used as an indicator in terms of element levels within the mushroom. As a result, it was determined that the mushroom has antioxidant, antimicrobial and antiallergic potential.

KEYWORDS: Antialzheimer, antimicrobial, antioxidant, *Lycoperdon*, medicinal mushroom.

1. Introduction

People have benefited from many natural products that nature has offered them for different purposes [1]. Since ancient times, many natural products have been used for many purposes such as food, spices, and fight against diseases [2,3]. Mushrooms are unique natural products that stand out with their nutritional properties. Many nutritional elements such as vitamins, minerals and nutrients make mushrooms important natural products [4]. In addition to their nutritional properties, many mushroom species have been reported to have many biological activities such as anticancer, antimicrobial, antiallergic, DNA protective, antioxidant, hepatoprotective, and antiaging [5-12]. In this context, determining the biological activity of mushroom is very important in terms of their potential use. In this study, antioxidant, antimicrobial and antialzheimer activities as well as element levels of *Lycoperdon pratense* Pers. were determined.

Lycoperdon pratense (Lycoperdaceae) is commonly known as the tea mushroom. It is abundant in sand dune systems and grasslands in many parts of the world.

2. Matherials and Methods

L. pratense samples used in our study were collected from Ankara/Turkey. 10 g of the mushroom samples were ground into powder and extracted with 150 mL of ethanol in a soxhlet device for approximately 6 hours at 50 °C.

After the extraction process, the solvents were concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

2.1. Antioxidant tests

Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the mushroom's extract were measured using Rel Assay kits. Antioxidant activity was measured with the TAS kit and expressed as mmol Trolox equiv./L. Oxidant activity was measured with a TOS kit and expressed as μmol H_2O_2 equiv./L [13,14]. When determining the oxidative stress index, the TOS value was equalized to the TAS value. Then, the TOS value was proportioned to the TAS value and the ratio was calculated [15].

2.2. Antimicrobial tests

The effects of the mushroom's extract against bacterial and fungal strains were measured by the agar dilution method. The bacterial strains used in our study are *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606. The fungal strains used in our study are *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, *Candida glabrata* ATCC 90030. Bacterial strains were precultured in Muller Hinton Broth medium. Fungal strains were pre-cultured in RPMI 1640 Broth medium. The lowest concentration that prevented the growth of bacterial and fungal strains used in stock solutions was specified in μg/mL [16-19].

2.3. Antialzheimer tests

Anticholinesterase activity of the mushroom's extract was measured by the Ellman method [20]. Solutions were prepared from the mushroom's extract in concentration ranges of 200-3.125 μg/mL. 130 μL of 0.1 M pH 8 phosphate buffer, 10 μL of stock solution, and 20 μL of enzyme (acetylcholinesterase or butyrylcholinesterase) solution were mixed in the microplate well and incubated for 10 min at 25 °C in the dark. Then, 20 μL of DTNB (5,5"-dithiobis-(2-nitrobenzoic acid)) solution and 20 μL of substrate (acetylcholine iodide or butyrylcholine iodide) were added and reading was performed at 412 nm. The samples were studied in 3 replicates. IC50 values of the results were calculated and expressed as μg/mL.

2.4. Elemental analysis

Mushroom samples were ground into powder using a mechanical grinder. Mushroom samples were studied in triplicate. 1 gram of each sample was weighed and placed in conical flasks. 10 mL HNO_3 was added to it. Then it was left at room temperature for approximately 48 hours. Then, the solution was heated with an adjustable hot plate until it became clear. After the heating process, 20 mL of dilute HCl solution was added to the solution and the samples were filtered using filter paper [21]. Fe, Zn, Pb, Ni, Mn, and Cr concentrations in the mushroom were determined with a Perkin Elmer (AAnalyst 400) atomic absorption spectrometer.

3. Results and Discussion

3.1. Total antioxidant and Oxidant status

Reactive oxygen species are oxidant compounds routinely produced as a result of metabolic activities [22]. Free radicals produced through regular metabolic means do not cause cytotoxicity, as they are destroyed by radical-scavenging antioxidant systems [23]. High levels of these compounds cause serious cellular and tissue damage. The antioxidant defense system functions in suppressing oxidant compounds or reducing their effects [24,25]. When the antioxidant defense system is inadequate, oxidative stress occurs. As a result of oxidative stress, many serious diseases such as diabetes, cancer, asthma, Parkinson's, Alzheimer's, and cardiological disorders can occur in humans [26]. Supplementary antioxidants may be beneficial in preventing these diseases [27]. In our study, the antioxidant status of *L. pratense* was evaluated. The findings obtained are shown in Table 1.

Table 1. TAS, TOS and OSI values of *L. pratense*

Sample	TAS (mmol Trolox equiv./L)	TOS (μmol H_2O_2 equiv./L)	OSI (TOS/(TAS* 10))
L. pratense	2.589±0.118	10.360±0.197	0.401±0.013

*Values are presented as mean±SD; number of mushroom samples n=3

The antioxidant potential of *L. pratense* has not been reported before in the literature. Additionally, TAS, TOS and OSI values were not reported. It was reported for the first time in this study. In a different study, the TAS value of *L. molle* was reported as 1.855 mmol Trolox equiv./L, TOS value as 2.201 μmol H_2O_2 equiv./L and OSI value as 0.119 [28]. Compared to this study, the TAS, TOS and OSI values of *L. pratense* used in our study were determined to be higher than for *L. molle*. TAS value is an indicator of all the antioxidant compounds found in natural products. In our study, the high TAS value of *L. pratense* is an indication that the mushroom has high antioxidant potential. The TOS value shows the totality of oxidant-active compounds produced within natural products. The OSI value shows the fraction of oxidant compounds produced in natural products that are suppressed by antioxidant compounds [29]. The high TOS and OSI values of *L. pratense* used in our study indicate that the mushroom is harmful in terms of oxidative stress. Additionally, in studies conducted on different wild mushrooms in the literature, the TAS values of *Suillus granulatus, Helvella leucopus, Agaricus xanthodermus, Terfezia boudieri, Daedaleopsis nitida* and *Laeticutis cristata* were 3.143, 2.181, 4.229, 2.332, 6.072 and 3.623 mmol Trolox equiv./L, respectively. TOS values were 18.933, 14.389, 29.065, 26.945, 7.165 and 27.476 μmol H_2O_2 equiv./L, respectively. The OSI values were 0.603, 0.661, 0.668, 1.156, 0.118 and 0.765, respectively [30-35]. Compared to these studies, the TAS value of L. pratense was higher than *H. leucopus, and T. boudieri, and lower than S. granulatus, A. xanthodermus, D. nitida and L. cristata*. TOS and OSI values of *L. pratense* were lower than *S. granulatus, H. leucopus, A. xanthodermus, T. boudieri*, and *L. cristata*, and higher than *D. nitida*. As a result, it was determined that *L. pratense* has antioxidant potential.

3.2. Antimicrobial Activity

Nowadays, the number of diseases caused by microorganisms is increasing [36]. The number of resistant microorganisms is increasing due to the unconscious use of drugs used against harmful microorganisms [37,38]. Possible side effects of synthetic drugs and the emergence of resistant microorganisms have necessitated the discovery of new antimicrobial drugs [39]. In this context, it is important to determine the antimicrobial activity of mushrooms. In our study, the effects of *L. pratense* against microorganisms were investigated and the findings were shown in Table 2.

Table 2. Antimicrobial activities of *L. pratense*

Sample	MIC (µg/mL)
S. aureus	50
S. aureus MRSA	50
E. faecalis	100
E. coli	200
P. aeruginosa	200
A. baumannii	50
C. albicans	400
C. glabrata	400
C. krusei	200

* The minimum inhibitory concentration (MIC) values are presented in units of µg/mL

As a result of the analyses, *L. pratense* extracts exhibited the highest activity against *S. aureus, S. aureus* MRSA and *A. baumannii* at a concentration of 50 µg/mL. It was then exhibited against *E. faecalis* at a concentration of 100 µg/mL, against *E. coli, P. aeruginosa* and *C. krusei* at a concentration of 200 µg/mL, and against *C. albicans* and *C. glabrata* at a concentration of 400 µg/mL. No study on the antimicrobial activity of *L. pratense* has been found in the literature. In studies on different *Lycoperdon* species, it was reported that ethanol, methanol and water extracts of *L. pusilum* and *L. giganteum* collected from Nigeria were effective against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphytococcus aureus, Aspergillus niger, Aspergillus flavus, Candida albicans, Microsporum boulardii* and *Trichophyton concentrum* [40]. It has been reported that ethanol, methanol and water extracts of *L. perlatum* are effective against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Candida albicans* and *Candida glabrata* [41]. It has been reported that *Lycoperdon lividium* is effective against *Bacillus subtilis, Candida albicans, Enterobacter aerogenes, Enterococcus mavis, E. faecalis, E. faecium, Escherichia coli, E. coli CFAI, Klebsiella pneumoniae, Listeria monocytogenes, Salmonella enteritidis, S. infantis, S. kentucky, S. typhimurium, Staphylococcus aureus, S. carnosus, S. epidermidis* and *Streptococcus agalactiae* [42]. In our study, it was determined that *L. pratense* was effective against the standard bacterial and fungal strains used at a concentration of 50-400 µg/mL. In this context, it has been determined that *L. pratense* has antimicrobial potential.

3.3. Anticholinesterase activity

Nowadays, the incidence of diseases such as Alzheimer's disease and Parkinson's disease, which are among the most common diseases caused by oxidative stress in people over the age of 65, is increasing [43,44]. In our study, the anti acetyl (AChE) and butyrylcholinesterase (BChE) activities of *L. pratense* were determined and the IC50 values of the findings are shown in Table 3.

Table 3. AChE and BChE activities of *L. pratense*

Sample	AChE µg/mL	BChE µg/mL
L. pratense	14.48±0.80	23.10±1.21
Galantamine	7.67±0.64	17.98±0.24

Anticholinestrase activity of *L. pratense* has not been reported in the literature. It was reported for the first time in our study. It is very important to determine the presence of enzymes that can cause diseases and to suppress these enzymes. It may also be beneficial in the treatment of diseases [45]. In our study, it was determined that *L. pratense* exhibited higher anti-AChE activity than the standard - galantamine. In addition, it was observed that the anti-BChE activity was higher than that of galantamine used as a standard. In this context, it was determined that *L. pratense* used in our study had anticholinesterase potential.

3.4. Element Contents

Mushrooms, which play a decomposing role in the ecosystem, accumulate elements in their bodies depending on the element content of the organic material they decompose [46]. In our study, the element levels in *L. pratense* were determined. The findings obtained are shown in Table 4.

Table 4. Element contents of *L. pratense*

Elements	*L. pratense* (mg/kg)	Literature wild mushroom range (mg/kg)
Fe	641.76±25.60	14.6-835
Zn	28.69±1.81	29.8-158.00
Pb	14.37±1.67	0.68-16.54
Ni	1.28±0.19	0.67-5.14
Mn	38.92±1.93	5.25-103
Cr	11.06±1.43	9.63-73.01

* Values are presented as mean±S.D.; n=3

In the literature, the Fe content of *L. pratense* has been reported as 27, Zn content as 420, Pb content as less than 0.01, Ni content as 4.19, and Mn content as 53.8 mg/kg [47]. Compared to this study, it was determined that the Fe and Pb levels of the samples used in our study were higher and the Ni and Mn levels were lower. In a different study, the Fe content of *L. pratense* was reported as 556, Zn content as 145, Pb content as 1.5, Ni content as 2.3, Mn content as 34 and Cr content as 0.7 mg/kg [48]. Compared to this study, the Fe, Pb, Mn and Cr contents of the samples used in our study were found to be higher, while the Zn and Ni contents were lower. It is thought that this difference is due to the composition

of the organic material that the mushrooms decompose in the areas where they are collected. Element levels reported for wild mushrooms in the literature are shown in Table 4 [49-51]. Compared to these studies, it was observed that the Fe, Pb, Ni, Mn and Cr contents of *L. pratense* used in our study were within the literature ranges, and the Zn contents were lower than the literature ranges. In this context, it was observed that the element levels determined in the mushroom were at normal levels.

4. Conclusions

In this study, antioxidant, anticholinesterase, antimicrobial activities and element levels of *L. pratense* were determined. In our study, the biological activity of *L. pratense* was detected for the first time. In addition, element levels were found to be at normal levels according to literature data. It was also determined that the mushroom has biological activity. In this context, it is thought that *L. pratense* can be used as a natural source in pharmacological designs.

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