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Research Article

Biological Activities of *Leucoagaricus leucothites* Mushroom: Evaluation of Antioxidant, Anticholinesterase and Antiproliferative Effects

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ABSTRACT

Mushrooms have constituted a wide research area as natural resources exhibiting medically important biological activities. This study aimed to investigate the biological activities of *Leucoagaricus leucothites* (Vittad.) Wasser mushroom comprehensively. Ethanol extract of the mushroom was obtained by Soxhlet extraction method. To determine the biological potential of the extract, total antioxidant status (TAS) and total oxidant status (TOS) analyses were performed by Rel Assay kits. In addition, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme activities were measured to evaluate the anticholinesterase activity of the mushroom. A549 lung cancer cell line was used to determine the antiproliferative effect. The obtained data showed that the TAS value of *L. leucothites* was 6.109±0.078 mmol/L and the TOS value was 15.364±0.149 µmol/L. Oxidative stress index (OSI) was calculated as 0.251±0.003. In terms of anticholinesterase activity, AChE inhibition value was found as 106.08±1.76 µg/mL, BChE inhibition value was found as 129.88±1.62 µg/mL. Antiproliferative effect increased in parallel with the increase in concentration, and this situation revealed that *L. leucothites* has a strong potential in terms of biological activities. These findings show that *L. leucothites* can be used in health supportive treatment approaches and can be a valuable source in pharmaceutical applications.

Keywords: *Leucoagaricus*, antioxidant activity, anticholinesterase activity, antiproliferative activity, biological activity.

1. INTRODUCTION

Medicinal mushrooms are organisms rich in biologically active compounds that have a long history and are widely used in traditional medicine. These mushroom species offer numerous health benefits supported by modern scientific research.[1] Studies on medicinal mushrooms are followed with great interest due to their benefits such as strengthening the immune system, fighting infections, helping in cancer treatment, exhibiting antioxidant properties and reducing inflammation.[2,3] Polysaccharides, triterpenoids, phenolic compounds and other biologically active compounds found in medicinal mushrooms explain the effects of these species on health. The properties of these compounds, such as modulating the immune response, neutralizing free radicals and reducing inflammation in the body, have made medicinal mushrooms an important source for modern pharmaceutical research.[4,5] In addition, medicinal mushrooms are used as potential therapeutic agents in the treatment of many diseases thanks to the synergistic interactions of organic compounds.[6] Clinical studies conducted in recent years have further emphasized the health-supporting and therapeutic properties of these mushrooms. Research in this field reveals the effectiveness and safety of the active compounds contained in medicinal mushrooms, contributing to the wider use of these species in the pharmaceutical industry.[7,8]

In this study, *Leucoagaricus leucothites* (Vittad.) Wasser mushroom was used as the material.

L. leucothites is a mushroom species known as an edible macrofungus and attracts attention with its various biological activities. Studies have shown that this mushroom does not cause genetic damage on human lymphocytes and does not negatively affect cell proliferation. In addition, it was determined that it increased total antioxidant capacity and reduced oxidative stress when applied at different concentrations.[9] Its antioxidant potential was reported as showing strong activity by DPPH test. Ethanolic extract of *L. leucothites* exhibited antimicrobial activity especially against foodborne pathogens, supporting the

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potential health benefits of this species. Phenolic component analysis also revealed the presence of important phenolic compounds such as catechin. Its chemical composition was examined by gas chromatography/mass spectrometry and the presence of various phenolic compounds was confirmed.[10,11] Taxonomically, *L. leucothites* is in the family Agaricaceae and has close phylogenetic relationships with the genera *Leucoagaricus* and *Leucocoprinus*.[12] The aim of this study was to determine the antioxidant, antiproliferative and anticholinesterase activities of *L. leucothites* mushroom.

2. EXPERIMENTAL

2.1. Materials

L. leucothites mushrooms used in this study were collected from Istanbul, Türkiye. Mushroom samples were dried under laboratory conditions and then prepared for analysis. For the extraction process, 10 grams of mushroom samples were processed by Soxhlet extraction method. Extraction was carried out using 250 mL of ethanol at 50 °C for approximately 6 hours. The obtained crude extracts were concentrated and freed from solvent with Buchi R100 Rotary Evaporator at 40 °C. The extracts were stored at +4 °C until the experimental analyses were completed.

2.2. Methods

2.2.1. Antioxidant Tests

In this study, total antioxidant capacity (TAS) and total oxidant level (TOS) of ethanolic extract obtained from *L. leucothites* mushroom were analyzed using special kits provided by Rel Assay. Analyses were carried out in accordance with the protocols specified by the kit manufacturer. Trolox was used as a calibrator for the TAS test and hydrogen peroxide was used as a calibrator for the TOS test. The obtained TAS and TOS values were expressed in mmol/L and µmol/L units, respectively.[13,14] Oxidative Stress Index (OSI) was calculated by taking the ratio of TOS to TAS after converting the TOS and TAS values to equal units and the results were presented as a percentage value.[15]

2.2.2. Anticholinesterase Activity Tests

In this study, the anticholinesterase activity of ethanolic extracts obtained from *L. leucothites* mushroom was evaluated according to the Ellman method.[16] Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibitory activities were investigated, and galantamine was used as a reference inhibitor. After the extracts were diluted in the concentration range of 200-3.125 µg/mL, stock solutions were prepared. In the experiments, 130 µL of 0.1 M pH=8 phosphate buffer, 10 µL of extract solution and 20 µL of enzyme (AChE or BChE) were added to the microplate wells, respectively, and then incubated at 25 °C in the dark for 10 minutes. After the incubation was completed, 20 µL of DTNB (5.5''-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added to initiate the reaction, respectively. Enzyme activities were determined by spectrophotometric measurements at 412 nm wavelength. Enzyme inhibition potential, percent inhibition of extracts and IC50 values (µg/mL) were calculated and reported by three repeated experiments.

2.2.3. Antiproliferative Activity Test

In this study, the cytotoxic effects of ethanolic extracts obtained from *L. leucothites* mushroom on A549 lung cancer cell line were evaluated by MTT (Cell Viability Test) method. First, stock solutions were prepared at concentrations of 25, 50, 100 and 200 µg/mL. After the cells reached 70-80% confluence, the cells were detached from the culture surface using 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA). The detached cells were seeded on culture plates and incubated for 24 h. After incubation was completed, plant extracts at different concentrations were applied to the cells and the cells were incubated for another 24 h. At the end of the incubation period, the supernatant was removed and 1 mg/mL MTT solution was added and the cells were incubated at 37 °C. After the purple precipitate formed with the MTT solution was observed, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was added to dissolve the precipitate. The viability of the cells was measured at 570 nm wavelength with an Epoch spectrophotometer (BioTek Instruments, Winooski, VT, USA).[17]

3. RESULTS and DISCUSSION

3.1. Antioxidant And Oxidant Status

Mushrooms are organisms that naturally contain rich biological compounds and contain many antioxidant substances that are important for health. Antioxidants prevent free radicals from accumulating in the body and damaging cells, thus helping to reduce the negative effects of oxidative stress.[18,19] Mushrooms contain powerful antioxidants, especially phenolic compounds, flavonoids, tocopherols, and saponins. These compounds play an important role in the prevention of chronic diseases such as cancer, heart disease, and diabetes. Studies among mushroom species have revealed that each has different levels of antioxidant capacity and some species provide strong protection against oxidative stress.[20] Therefore, mushrooms offer potential health benefits as natural antioxidant sources and constitute an important area for pharmaceutical research.[21] In this study, the antioxidant potential of *L. leucothites* mushroom was evaluated. The obtained data are presented in Table 1.

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Table 1. TAS, TOS ve OSI values of *Leucoagaricus leucothites*.

Sample	TAS mmol/L	TOS µmol/L	OSI
Leucoagaricus leucothites	6.109±0.078	15.364±0.149	0.251±0.003

*****Values are presented as mean±SD.

In our study, the data obtained on the antioxidant and oxidant capacity of *L. leucothites* mushroom provide very important findings in terms of biologically active compounds of the species. The TAS value of 6.109±0.078 mmol/L obtained for the sample collected from Istanbul shows that this species contains strong antioxidant compounds with the capacity to neutralize free radicals. This finding emphasizes that *L. leucothites* contains compounds that may have potential health benefits and that the antioxidant properties of this species offer a resource that can be investigated in the field of natural treatment.[22] Data in the literature on the *L. leucothites* sample collected from Bolu similarly show that the mushroom has strong antioxidant properties. The TAS value of the ethanol extract reported by Sevindik et al.[11] was determined as 8.291 mmol/L, which indicates a slightly higher value compared to the Istanbul sample. This difference may be attributed to the ecological conditions of the place where the samples were taken, environmental factors during the collection period, or differences in the extraction methods used. For example, ethanol extraction may have provided more efficient dissolution of the components, which may have led to a higher TAS value. In terms of TOS value, the value measured as 15.364±0.149 µmol/L in the Istanbul sample is significantly higher than the value of 10.797 µmol/L in the Bolu sample. This situation is parallel to the findings showing that oxidant components may be slightly higher in the Istanbul sample, but these oxidant compounds are present in a controlled manner in terms of biological activity and excessive oxidation can be prevented. The controlled presence of oxidant compounds suggests that the fungus has established a balance that will limit potential damages in the biological system. In terms of OSI value, it was determined as 0.251±0.003 in the Istanbul sample and 0.130 in the Bolu sample. The low OSI value shows that both samples are effective in combating oxidative stress, but the Bolu sample has a lower OSI value, indicating that antioxidant compounds suppress oxidant components more strongly and therefore provide a more effective oxidative balance. In general, although there are some differences between the Istanbul and Bolu samples, it is seen that both samples have high antioxidant capacity and effectively balance oxidative stress. These findings reveal that *L. leucothites* is an important species with the potential to reduce the negative effects of oxidative stress among natural resources. This mushroom species is an important candidate in natural antioxidant resource research.

In addition, TAS, TOS and OSI values of different mushroom species have been reported in the literature. TAS values of *Phellinus hartigii*, *Otidea onotica*, *Hericium erinaceus*, *Cantharellus cibarius and Lactarius deliciosus* were reported as 4.98, 8.866, 5.426, 5.511 and 7.468 mmol/L, respectively. TOS values were reported as 9.27, 14.724, 6.621, 7.289 and 13.161 µmol/L, respectively. OSI values were reported as 0.19, 0.166, 0.122, 0.132 and 0.176, respectively.[23-27] Compared to these studies, the TAS value of *L. leucothites* obtained in our study was determined to be higher than *P. hartigii*, *H. erinaceus* and *C. cibarius*, and lower than *O. onotica* and *L. deliciosus*. This shows that *L. leucothites* contains strong antioxidant compounds and has a high capacity to neutralize free radicals. However, the lower TAS value than *O. onotica* and *L. deliciosus* suggests that these species may have more effective antioxidant compounds or environmental conditions may affect antioxidant compound production more. Both mushroom species may have rich biological activity and may synthesize different antioxidant compounds more effectively under certain conditions. Comparisons made in terms of TOS and OSI values reveal that *L. leucothites* is rich in oxidant compounds and antioxidant compounds effectively balance these oxidant compounds. The TOS value of *L. leucothites* was determined to be higher than other mushroom species. This shows that the species contains oxidant compounds but these compounds are controlled in a way that they do not create excessive oxidation in the biological system. In addition, *L. leucothites* has a higher OSI value than *P.s hartigii*, *O. onotica*, *H. erinaceus*, *C. cibarius* and *L. deliciosus*. This may imply that *L. leucothites* has a strong capacity to maintain oxidative balance, but antioxidant compounds should put more pressure on oxidant components. All these findings reveal that *L. leucothites* has a significant potential in combating oxidative stress, but this capacity shows some differences compared to other mushroom species. It can be said that the antioxidant and oxidant balance of this mushroom species plays an active role in establishing a healthy balance in the biological system, but the interaction of environmental conditions and biological components should be further investigated to optimize this balance.

3.2. Anticholinesterase Activity

Some mushrooms contain bioactive compounds that have anticholinesterase effects. These substances inhibit the acetylcholinesterase enzyme that regulates neurotransmission in the nervous system, leading to acetylcholine accumulation.[28] This mechanism offers a therapeutic potential in the treatment of neurodegenerative diseases. Mushrooms with this property found in nature are the subject of drug

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development studies in pharmacological research.[33] In this study, the anticholinesterase activity of *L. leucothites* was investigated and the results obtained are presented in Table 2.

Table 2. Anti-AChE and anti-BChE values of *Leucoagaricus leucothites*.

Sample	AChE µg/mL	BChE µg/mL
Leucoagaricus leucothites	106.08±1.76	129.88±1.62
Galantamine	**8.16±0.09**	**14.98±0.19**

*The values were studied with 3 replications and std values are given.

The IC50 values obtained in this study reveal the inhibitory effects of the ethanol extract of *L. leucothites* sample against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. The findings obtained in our study show that *L. leucothites* has a lower IC50 value compared to galantamine on both enzymes. This result shows that the inhibitory activity of galantamine on both enzymes is much stronger than the ethanol extract of *L. leucothites*. In addition, it has been reported in the literature that the methanol extract of *L. leucothites* inhibits AChE and BChE activities.[29] This finding, compared to the inhibitory effect of the ethanol extract in the present study, suggests that the differences in the solubilization capacity of ethanol and methanol solvents may contribute to these results. In conclusion, although the ethanol extract of *L. leucothites* has some potential as an inhibitor of AChE and BChE, it does not reach the level of efficacy of galantamine. These findings indicate the usability of the ethanol extract of the mushroom in the treatment of neurological diseases, although it presents a lower efficacy compared to compounds that exhibit the effects of stronger inhibitors. Future studies in this area may require more comprehensive analyses with different solvents and extraction methods to better understand the active components of *L. leucothites*.

3.3. Antiproliferative Activity

Mushrooms are important natural resources that can inhibit the growth and spread of cancer cells by exhibiting antiproliferative activity thanks to their bioactive compounds. The polysaccharides, terpenoids, phenolic compounds and other secondary metabolites they contain can exhibit antitumor effects through mechanisms such as cell cycle arrest, apoptosis stimulation and immune system modulation.[30] Some mushroom species can be effective against cancer through multiple pathways such as preventing DNA damage, suppressing angiogenesis and preventing metastasis.[31,32] Therefore, mushrooms have an important place in pharmacological research due to their complementary or supportive potential in cancer treatment. In this study, the effects of *L. leucothites* on A549 lung cancer cells were examined and the results obtained are presented in Figure 1 as µg/mL.

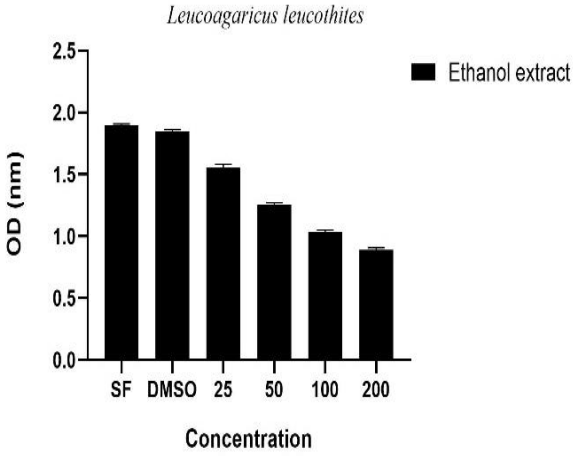

Figure 1. Antiproliferative activity of *Leucoagaricus leucothites.*

In our study, antiproliferative effects of ethanol extract of *L. leucothites* mushroom on cell viability against A549 lung cancer cell line was determined by MTT test. The findings show that the extract inhibited cell proliferation depending on the concentration. Especially, the highest inhibition rate was reached at 200 µg/mL concentration, indicating the anticancer potential of *L. leucothites*. When compared to the control groups (SF and DMSO), a significant decrease in cell viability was observed in the applications at concentrations of 25, 50, 100 and 200 µg/mL. This suggests that the possible bioactive components of the extract create an antiproliferative effect in the A549 cell line. No study was found in the literature evaluating the effect of *L. leucothites* against A549 lung cancer cell line. However, it has been previously reported that different mushroom species have anticancer effects on A549 cells.[34-36] In parallel with the results of these studies, the antiproliferative effect of *L. leucothites* extract on A549 cells suggests that mushroom species may contain important biological components for cancer treatment. In this context, determination of potential bioactive components such as phenolic compounds, polysaccharides or terpenoids contained in *L. leucothites* and elucidation of their mechanisms will provide a better understanding of its anticancer activity. In conclusion, our study reveals the pharmacological potential of *L. leucothites* and paves the way for advanced molecular mechanism studies.

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4.CONCLUSION

In this study, the biological activities of *L. leucothites* mushroom was evaluated comprehensively. Ethanol extract of the mushroom exhibited important biological properties such as increasing antioxidant capacity and reducing oxidative stress. It also shows potential health benefits with its antioxidant, anticholinesterase and antiproliferative activities. In particular, the ability of this mushroom to inhibit acetylcholinesterase and butyrylcholinesterase enzymes has been a promising finding for the treatment of neurological diseases. Antiproliferative tests performed on A549 lung cancer cell line revealed that *L. leucothites* has the potential to inhibit the growth of cancer cells. In the future, it is important to examine the biological activities of this mushroom in more detail and especially to investigate its potential for use in pharmaceutical areas. In addition, comparison of different extraction methods and biological activities can help us better understand the effectiveness of the mushroom. However, evaluating the potential of this mushroom for clinical applications will be an important step in providing safe and effective treatment options.

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Conflict of Interest

There is no conflict of interest with any person, institution, company, etc.

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