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Original Article

Bioactivity of EtOH and MeOH Extracts of Basidiomycetes Mushroom (*Stereum hirsutum)* on Atherosclerosis

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Abstract

Mushrooms are cosmopolitan organisms living on different substrates and have different pharmacological properties, such as antioxidant, antimicrobial, and anti-inflammatory effects thanks to many bioactive compounds. Edible and medicinal higher fungi have been used by humankind for millennia. They are collected and used directly not only for their nutritional values as a main source of food or as a part of a regular diet but also for their medicinal purpose as a source of powerful new bioactive compounds. Antioxidative and anti-inflammatory functions and therefore lipid-lowering effects correlate with antiatherogenic effects. This study determined the total antioxidant capacity (TAS), total oxidant capacity (TOS), oxidative stress index (OSI), 1-diphenyl-2-picrylhydrazyl (DPPH) activity, and antimicrobial activity of ethanolic and methanolic extracts of *Stereum hirsutum* (Willd.) Pers. Moreover, the effects on atherosclerosis are discussed according to the antioxidant activity of the mushroom. The TAS, TOS, and OSI values of *S. hirsutum* were determined using Rel Assay kits. According to the results, the TAS, TOS, and OSI values were determined at 5.289±0.113 mmol/L, 20.540±0.416 µmol/L, and 0.389±0.012. Furthermore, free radical scavenging activity was determined by the DPPH method. The ethanol (EtOH) extracts of *S. hirsutum* showed higher DPPH activity than methanol extracts. The EtOH extracts at a concentration of 2 mg/mL showed a DPPH inhibition of 45.84±0.81%. Antimicrobial activities were tested on 9 standard bacterial and fungal strains, including *Staphylococcus aureus*, *S. aureus MRSA*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *C. krusei*, and *C. glabrata* using a modified agar dilution method. Extracts showed high activity against *S. aureus*, *S. aureus MRSA*, and *A.baumannii*. In conclusion, it was suggested that *S. hirsutum* can be used as a natural source related to the effects on atherosclerosis due to its antioxidant and antimicrobial activities.

Keywords: Antioxidant, Antimicrobial, Atherosclerosis, Mushroom, *Stereum hirsutum*

Bioactivité des Extraits EtOH et MeOH de Champignon Basidiomycète (*Stereum hirsutum*) sur L'athérosclérose

Résumé: Les champignons sont des organismes cosmopolites vivant sur différents substrats et ont des propriétés pharmacologiques différentes, telles que des effets antioxydants, antimicrobiens et anti-inflammatoires grâce à de nombreux composés bioactifs. Les champignons supérieurs comestibles et médicinaux sont utilisés par l'humanité depuis des millénaires. Ils sont collectés et utilisés directement non seulement pour leurs valeurs nutritionnelles en tant que principale source de nourriture ou dans le cadre d'un régime alimentaire normal, mais aussi à des fins médicinales en tant que source de puissants nouveaux composés bioactifs. Les fonctions antioxydantes et anti-inflammatoires et donc les effets hypolipidémiants sont en corrélation avec les effets

antiathérogènes. Cette étude a déterminé la capacité antioxydante totale (TAS), la capacité oxydante totale (TOS), l'indice de stress oxydatif (OSI), l'activité 1-diphényl-2-picrylhydrazyl (DPPH) et l'activité antimicrobienne des extraits éthanoliques et méthanoliques de *Stereum hirsutum* (Willd.) Pers (Le Stérée hirsute). De plus, les effets sur l'athérosclérose sont discutés en fonction de l'activité antioxydante du champignon. Les valeurs TAS, TOS et OSI de *S. hirsutum* ont été déterminées à l'aide de kits Rel Assay. Selon les résultats, les valeurs TAS, TOS et OSI ont été déterminées à 5.289 ± 0.113 mmol / L, 20.540 ± 0.416 µmol / L et 0.389 ± 0.012. En outre, l'activité de piégeage des radicaux libres a été déterminée par la méthode DPPH. Les extraits à l'éthanol (EtOH) de *S. hirsutum* ont montré une activité DPPH plus élevée que les extraits au méthanol. Les extraits d'EtOH à une concentration de 2 mg/ml ont montré une inhibition de la DPPH de 45.84 ± 0.81%. Les activités antimicrobiennes ont été testées sur 9 souches bactériennes et fongiques standard, dont *Staphylococcus aureus*, *S. aureus MRSA*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida albicans*, *C. krusei* et *C. glabrata* en utilisant une méthode de dilution en gélose modifiée. Les extraits ont montré une activité élevée contre *S. aureus*, *S. aureus MRSA* et *A.baumannii*. En conclusion, il a été suggéré que *S. hirsutum* puisse être utilisé comme source naturelle liée aux effets sur l'athérosclérose en raison de ses activités antioxydantes et antimicrobiennes.
Mots-clés: Antioxidant, Antimicrobien, Athérosclérose, Champignon, *Stereum hirsutum*

Introduction

Cardiovascular diseases are among the most common causes of morbidity and mortality in the world. There are lots of risk factors in cardiovascular diseases, such as hyperlipidemia, hypertension, cigarette smoking, obesity, diabetes mellitus, and a positive family history of cardiovascular disease. Some risk factors can be altered or eliminated followed by changes in lifestyle and diet. Since several food ingredients reduce hyperlipidemia and hypertension, the presence of antioxidant and anti-inflammatory compounds in mushrooms might be clinically relevant in the management of heart and circulation health complications. Foods constitute antioxidative and anti-inflammatory properties, which have anti-inflammatory functions followed by lipid-lowering and antiatherogenic effects. Moreover, the consumption of dietary antioxidants could be important in the prevention of cardiovascular diseases (Balsano and Alisi, 2009), and there is evidence that the oxidative modification of low-density lipoproteins (LDL) (lipids or protein components) plays a crucial role in atherogenesis (Witztum and Steinberg, 2001).

Edible and medicinal higher fungi have been used by humankind for millennia (Sevindik, 2019). They are collected and used directly not only for their nutritional values as a main source of food or as a part of a regular diet but also for their medicinal purpose as a source of powerful new bioactive compounds (Sevindik et al., 2018). Several studies have reported several health benefits of these species, such as antioxidant, anti-aging, antimicrobial, hypoglycemic, antiulcer, and anti-inflammatory effects (Kalač, 2009; Gürgen et al., 2017). Polysaccharides are essential fatty acids and secondary bioactive compounds that are best known as potent mushroom-derived substances with high values. These properties, along with low cytotoxicity, raise the possibility that it could be effective in the cancer patients receiving conventional chemotherapy and/or radiation treatment to build up immune resistance and decrease toxicity (Bishop et al., 2015; Bal et al., 2017).

The *Stereum* fungus belongs to the *Stereaceae* family (basidiomycetes) and is widely distributed throughout the world, which can produce diversiform secondary metabolites. The mushroom *S. hirsutum* is very widely distributed, lives on dead wood of limbs and trunks of both hardwoods and conifers, and plays an important role in the wood deterioration process. However, it is parasitized by the fungus *Tremella aurantia* Schwein to form a heterogeneous complex basidiocarp Jin'er (golden jelly fungus) (Liu and Zheng, 1994) which has been traditionally used as food and crude medicine in Chinese society. Mushrooms have been used as food and medicine since ancient times, and they are widely consumed in foods with medical features. Moreover, they produce secondary metabolites that have many different biological effects (Calvo et al., 2002).

Basidiomycetes mushrooms contain biologically active compounds in fruit bodies, as well as cultured mycelium and broth. They had long been used as a folk remedy to promote health and longevity in many oriental countries for their important chemical and nutritional characteristics (Kalač, 2009; Selamoglu et al., 2020).

Actually, basidiomycetes are clinically confirmed and constitute a strong base for intensive research and development of basidiomycetes biologically active metabolites, such as organic acids, alkaloids, terpenoids, steroids, and phenolics (Prasad et al., 2015). Basidiomycetes are also valuable health food, which is high in vitamins, minerals, and fibers; moreover, they contain nutritive compounds, such as protein, essential amino acids, and polyunsaturated fatty acids. The fermented mycelia of *S. hirsutum* have been reported to produce abundantly active secondary metabolites, including phytotoxic active acetylenic compounds, multiple active sesquiterpenoids, antibacterial epidioxysterols, and benzoate derivatives (Aqueveque et al., 2017).

This study aimed to determine the total antioxidant capacity, total oxidant capacity, and antimicrobial activity of *S. hirsutum*; moreover, it was attempted to investigate the related anti-atherosclerosis effects of this fungus.

Material and Methods

Samples Collection. The *S. hirsutum* samples used in this study were collected from Gaziantep province, Turkey. Morphological (shape, color, size) and ecological characteristics of the samples were recorded in the field conditions. Moreover, the microscopic characteristics of the specimens transported to the laboratory under appropriate conditions were determined using light microscopy with a 3% KOH solution (Leica DM750).

Laboratory Studies. After the identification of the collected mushroom samples, they were dried at 40˚C. Subsequently, they were pulverized in a mechanical grinder, and pulverized mushroom samples were extracted with ethanol (EtOH) and methanol (MeOH) in a Soxhlet apparatus at 50˚C that lasted 6 h (Gerhardt EV 14). The mushroom extracts were then concentrated under pressure at 40˚C in a rotary evaporator (Heidolph Laboratory 4000 Rotary Evaporator) and stored at +4˚C.

Antimicrobial Activity. Antimicrobial activity tests were conducted on the mushroom EtOH and MeOH extracts using the agar dilution method as recommended by the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing. Minimal inhibitor concentrations (MIC) for each extract were determined against standard bacterial and fungal strains. The microorganisms used for this purpose included *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, and *Enterococcus faecalis* ATCC 29212.

Moreover, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606 were used as gram-negative bacteria. Furthermore, *C. albicans* ATCC 10231, *C. krusei* ATCC 34135, and *Candida glabrata* ATCC 90030 were employed as fungi and were obtained from the American culture collection. Bacteria strains were pre-cultured in Muller Hinton Broth medium, and fungal strains were pre-cultured in RPMI 1640 Broth medium. To obtain standard inoculum, the turbidity of the bacteria and fungi was set based on the McFarland 0.5 scale. All extracts were tested at 800-12.5 μg/mL concentrations, and distilled water was used in all dilutions. The solvents used in extracts were also individually tested for antimicrobial activity. In addition, Fluconazole and Amphotericin B were employed as reference drugs for the fungi.

Amikacin, Ampicillin, and Ciprofloxacin were used as reference drugs for the bacteria. The lowest concentration that prevented the proliferation of bacteria and fungi was determined as the MIC (Bauer et al., 1966; Hindler et al., 1992; CLSI, 2012; EUCAST, 2014; Matuschek et al., 2014; EUCAST, 2015).

Determination of Total Antioxidant Status, Total Oxidant Status, and Oxidative Stress Index. Total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of mushroom extracts were determined with Rel Assay Kits (Rel Assay Kit Diagnostics, Turkey). The TAS value was expressed as mmol Trolox equiv./L, and Trolox was used as the calibrator (Erel, 2004). The TOS value was expressed as μmol H_2O_2 equiv./L, and hydrogen peroxide was utilized as the calibrator (Erel, 2005). The OSI (Arbitrary Unit) was calculated using the following formula.

$$\text{OSI (AU)} = \frac{\text{TOS, μmol } H_2O_2 \text{ equiv./L}}{\text{TAS, mmol Trolox equiv./L X 10}}$$

DPPH Free Radical Scavenging Activity Test. The free radical scavenging activity of the fungus was determined using 1-diphenyl-2-picrylhydrazyl (DPPH). Stock solutions containing 0.25, 0.5, 1, and 2 mg/mL extract were prepared by dimethyl sulfoxide. In addition, 50μL of the solution was added to 160μL of 0.039% DPPH and incubated for 30 min in the dark at room temperature. Following that, it was read at 527 nm absorbance, and separate processes were repeated for all concentrations and samples (Shimada et al., 1992). It is worth mentioning that rosmarinic acid was used as a reference antioxidant.

DPPH Free Radical Scavenging Percentages. The sweeping activity was calculated according to the formula (%)=[(ADPPH-AExample)/(ADPPH)]×100.

Results

This study analyzed the anti-oxidative, antimicrobial, and DPPH activity of ethanolic and methanolic extracts of *S. hirsutum*.

Total Antioxidant Status, Total Oxidant Status, and Oxidative Stress Index Results. In total, six different mushroom samples were taken and run in 5 replicates to obtain *S. hirsutum* extract. According to the results, the TAS, TOS, and OSI values were determined at 5.289±0.113 mmol/L, 20.540±0.416 μmol/L, and 0.389±0.012. Table 1 tabulates the obtained findings.

Table 1. Total antioxidant status, total oxidant status, and oxidative stress index values of *S. hirsutum*

	TAS	TOS	OSI
S. hirsutum	5.289±0.113	20.540±0.416	0.389±0.012

Values are presented as mean±S.D.; n=6 (Experiments were made as 5 parallel)

DPPH Activity Results. This study determined the DPPH free radical scavenging activities of EtOH and MeOH extracts of *S. hirsutum* (Table 2).

Table 2. DPPH activity of *S. hirsutum*

	0.25 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL
Rosmarinic Acid	37.68±1.36	52.23±2.43	66.63±1.21	78.14±0.46
EtOH	24.95±1.62	26.98±1.12	34.18±3.25	45.84±0.81
MeOH	11.61±2.08	21.39±0.91	31.54±1.62	41.23±0.45

Values are presented as mean±S.D.; n=6 (Experiments were made as 5 parallel)

According to the results, it is clear that the EtOH extract of *S. hirsutum* shows higher activity, compared to MeOH. However, EtOH and MeOH extracts of S. hirsutum showed lower activities, compared to the standard antioxidant rosmarinic acid.

Antimicrobial Activity Results. Table 3 tabulates the results of utilizing EtOH and MeOH extracts of S. hirsutum and investigating antimicrobial activities against 9 microorganisms.

Table 3. Antimicrobial activity of *S. hirsutum*

	S. aureus	*S. aureus MRSA*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A.baumannii*	*C. albicans*	*C. glabrata*	*C. krusei*
EtOH	25	25	100	200	100	25	100	100	100
MeOH	25	25	200	200	100	50	100	200	200
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

The MIC values are presented in units of µg/mL

The EtOH extracts of the mushroom showed higher activity than that of the MeOH extracts. In addition, the extracts showed the highest activity against *S. aureus*, *S. aureus* MRSA, and *A. baumannii*.

Discussion

Antioxidant Activity. Antioxidants play a very important role in the defense system against reactive oxygen species (ROS). Moreover, they are inhibitors of the oxidation process, even at relatively small concentrations. In cases where antioxidants are inadequate, they may reduce the effects of ROS (Young and Woodside, 2001; Yadav et al., 2016; Krupodorova and Sevindik, 2020). This study determined the antioxidant potential of *S. hirsutum*. There are studies conducted on the data relevant to different types of mushrooms in the literature. The TAS, TOS, and OSI values of *Ganoderma lucidum* were estimated at 5.509, 10.177, and 0.185, respectively (Bal, 2019). In other studies, the TAS, TOS, and OSI values of *Gyrodon lividus* were obtained at 2.077, 13.465, and 0.651 (Bal, 2018), respectively. Moreover, the TAS, TOS, and OSI values of *Fomitopsis pinicola* were 1.44, 14.21, and 0.99, respectively (Sevindik et al., 2017).

Compared to these studies, the TAS value of *S. hirsutum* used in the current study is lower than that of the *G. lucidum*. However, the TAS value of *S. hirsutum* is higher than those of *G. lividus* and *F. pinicola*. Regarding the TOS values, *S. hirsutum* was found to be higher than those of *G. lucidum*, *G. lividus*, and *F. pinicola*. Furthermore, the OSI values of *S. hirsutum* were higher than those of *G. lucidum*. However, the OSI values of *S. hirsutum* were lower than those of *G. lividus* and *F. pinicola*.

The reason for these differences in TAS, TOS, and OSI among mushrooms is due to the differences in the capacity of the product oxidant compounds, capacity of endogenous antioxidant compounds, and their ability to tolerate oxidant compounds with endogenous antioxidants with environmental and metabolic factors (Sevindik, 2020). As a result, it is thought that *S. hirsutum* has high antioxidant potential, and it can be used as a natural antioxidant agent in pharmacological design by detecting the compounds that cause this effect.

In addition, the protective effect on the atherosclerosis of *S. hirsutum* can be utilized due to its antioxidant effects. It is well-known that atherosclerosis risk factors are associated with excess ROS generation and oxidation of LDL. The oxidized-LDL acting on cell types promotes atherogenesis. Many pharmacologic agents that are currently in use modulate oxidative stress and improve atherogenesis (Li et al., 2013). In some studies, traditional antioxidant supplements failed to improve cardiovascular event rates; however, other studies reported no failure in this regard. The factors that may have effects on this discrepancy include the late initiation of the anti-oxidant therapy or lack of exogenous antioxidant impacts on this pathway.

Our findings represent that free radical scavenging (DPPH) potential increases with an increase in the concentration of mushroom extracts. Previously conducted studies used water, ethanol, and acetonic extracts of *S. hirsutum* and reported DPPH free radical activities (Asatiani et al., 2007; Nowacka et al., 2015; Torres et al., 2016). The DPPH free radical scavenging activities of EtOH and MeOH extracts of *S. hirsutum* were also determined in this study. In this context, it is thought that *S. hirsutum* can be used as a natural antioxidant protective agent related to its bioactivities on atherosclerosis with pharmacological designs.

Antimicrobial Activity. Infections with drug-resistant organisms remain a major problem that is difficult to resolve in clinical practice (Bal et al., 2019). According to clinical epidemiology analysis reports, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* became the most common hospital infections and drug-resistant strains with infection rates of up to 50% (Su et al., 2015).

Therefore, the detection of new antimicrobial agents has become inevitable. In previous studies, acetonic extracts of *S. hirsutum* have been used and reported to be effective at different concentrations against *Escherichia coli, Salmonella typhi*, and *S. typhimurium* (Torres et al., 2016).

In another study, ethanolic extracts of *S. hirsutum* were used and reported to be effective at different concentrations against *Staphylococcus epidermidis, S. aureus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Proteus mirabilis* (Nowacka et al., 2015). In our study, EtOH and MeOH extracts of *S. hirsutum* were used and found to be effective at concentrations of 25-200 μg/mL against *S. aureus, S. aureus* MRSA, *E. faecalis, E. coli, P. aeruginosa, A. baumannii, C. albicans, C. Krusei*, and *C. glabrata*. In this context, it has been determined that *S. hirsutum* may be a natural antimicrobial agent against test microorganisms.

Conclusion

This study determined the antioxidant, oxidant, and antimicrobial potentials of *S. hirsutum*. Moreover, the antioxidant and anti-inflammatory compounds occurring in mushrooms also may contribute to reducing atherosclerosis risk. As a result, it is recommended to detect antioxidant and antimicrobial effects of the bioactive compounds in the mushrooms. In addition, it has been observed that mushrooms can be used as a natural pharmacological source in drug production due to the determined biological activities.

Authors' Contribution

Study concept and design: M. S. and Z. S.
Acquisition of data: M. S., Z. S. and C. B.
Analysis and interpretation of data: M. S., Z. S. and C. B.
Drafting of the manuscript: M. S., Z. S. and B. O.
Critical revision of the manuscript for important intellectual content: M. S. and B. O.
Statistical analysis: M. S. and B. O.
Administrative, technical, and material support: M. S. and C. B.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare no conflict of interest.

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References

Aqueveque, P., Cespedes, C.L., Becerra, J., Aranda, M., Sterner, O., 2017. Antifungal activities of secondary metabolites isolated from liquid fermentations of *Stereum hirsutum* (Sh134-11) against Botrytis cinerea (grey mould agent). Food Chem Toxicol 109, 1048-1054.

Asatiani, M.D., Elisashvili, V.I., Wasser, S.P., Reznick, A.Z., Nevo, E., 2007. Free-radical scavenging activity of submerged mycelium extracts from higher basidiomycetes mushrooms. Biosci Biotechnol Biochem 71, 3090-3092.

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by astandardized single disk method. Am J Clin Pathol, 45, 493-96.

Bal, C., 2018. A Study on Antioxidant Properties of *Gyrodon lividus*. Eurasian J For Sci 6, 40-43.

Bal, C., 2019. Antioxidant and antimicrobial capacities of *Ganoderma lucidum*. J Bacteriol Mycol Open Access 7, 5-7.

Bal, C., Akgul, H., Sevindik, M., Akata, I., Yumrutas, O., 2017. Determination of the anti-oxidative activities of six mushrooms. Fresenius Envir Bull, 26(10), 6246-6252.

Bal, C., Sevindik, M., Akgul, H., Selamoglu, Z., 2019. Oxidative stress index and antioxidant capacity of *Lepista nuda* collected from Gaziantep/Turkey. Sigma, 37(1), 1-5.

Balsano, C., Alisi, A., 2009. Antioxidant effects of natural bioactive compounds. Current pharmaceutical design. Curr Pharm Des 15, 3063-3073.

Bishop, K.S., Kao, C.H., Xu, Y., Glucina, M.P., Paterson, R.R., Ferguson, L.R., 2015. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. Phytochemistry 114, 56-65.

Calvo, A.M., Wilson, R.A., Bok, J.W., Keller, N.P., 2002. Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66, 447-459.

CLSI (The Clinical and Laboratory Standards Institute). 2012. Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard-Eighth Edition (M11-A8).

Erel, O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin biochem 37, 277-285.

Erel, O., 2005. A new automated colorimetric method for measuring total oxidant status. Clin biochem 38, 1103-1111.

EUCAST (European Committee on Antimicrobial Susceptibility Testing). 2014. Breakpoint tables Fungal isolate for interpretation of MICs. 2014; Version 7.0

EUCAST (European Committee on Antimicrobial Susceptibility Testing). 2015. Breakpoint tables for Bacteria interpretation of MICs and zone diameters 2015; Version 5.0

Gürgen, A., Yildiz, S., Kılıç, C., Can, Z., 2017. Protein contents and antioxidant properties of *Pleurotus ostreatus* cultivated on tea and espresso wastes. Int J Sec Metabol 4, 177-186.

Hindler, J., Hochstein, L., Howell, A., 1992. Preparation of routine media and reagents used in antimicrobial susceptibility testing. Part 1. McFarland standards, p. 5.19.1-5.19.6. In H. D. Isenberg (ed) Clinical microbiology procedures handbook, 1. American Society for Microbiology, Washington, D.C

Kalač, P., 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem 113, 9-16.

Krupodorova, T., Sevindik, M., 2020. Antioxidant potential and some mineral contents of wild edible mushroom *Ramaria stricta*. AgroLife Sci. J, 9, 186-191.

Li, H., Horke, S., Forstermann, U., 2013. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci 34, 313-319.

Liu, Z.N., Zheng, S.F., 1994. Structural correction of the fruiting body for Golden Ears. Edible Fungi 16, 12-13.

Matuschek, E., Brown, D.F., Kahlmeter, G., 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiolog laboratories. Clin Microbiol Infect, 20, 255-266

Nowacka, N., Nowak, R., Drozd, M., Olech, M., Los, R., Malm, A., 2015. Antibacterial, Antiradical Potential and Phenolic Compounds of Thirty-One Polish Mushrooms. PloS one 10, e0140355.

Prasad, S., Rathore, H., Sharma, S., As, Y., 2015. Medicinal Mushrooms as a Source of Novel Functional Food. Int J Food Sci Nutr Diet 4, 221-225.

Selamoglu, Z., Sevindik, M., Bal, C., Ozaltun, B., Sen, İ., Pasdaran, A., 2020. Antioxidant, antimicrobial and DNA protection activities of phenolic content of *Tricholoma* *virgatum* (Fr.) P.Kumm. Biointerface Res Appl Chem, 10(3), 5500-5506.

Sevindik, M., Akgul, H., Akata, I., Alli, H., Selamoglu, Z., 2017. *Fomitopsis pinicola* in healthful dietary approach and their therapeutic potentials. Acta Aliment 46, 46, 464.

Sevindik, M., Akgul, H., Bal, C., Selamoglu, Z., (2018). Phenolic contents, oxidant/antioxidant potential and heavy metal levels in *Cyclocybe cylindracea*. Int J Pharm Edu Res, 52(3), 437-441.

Sevindik, M., 2019. The novel biological tests on various extracts of *Cerioporus varius*. Fresen Environ Bull, 28(5), 3713-3717.

Sevindik, M., 2020. Antioxidant and antimicrobial capacity of *Lactifluus rugatus* and its antiproliferative activity on A549 cells. Indian J Tradit Knowl, 19(2), 423-427.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agr Food Chem 40, 945-948.

Su, P.W., Yang, C.H., Yang, J.F., Su, P.Y., Chuang, L.Y., 2015. Antibacterial Activities and Antibacterial Mechanism of *Polygonum cuspidatum* Extracts against Nosocomial Drug-Resistant Pathogens. Molecules 20, 11119-11130.

Torres, S., Cabrera-Pardo, J.R., Alonso, F., Bustos, E., Perez, C., Palfner, G., *et al.*, 2016. Changes in secondary metabolites profiles and biological activity of the fresh fruiting bodies of *Stereum hirsutum* exposed to high-dose UV-B radiation. J Chil Chem Soc 61, 3224-3227.

Witztum, J.L., Steinberg, D., 2001. The Oxidative Modification Hypothesis of Atherosclerosis: Does It Hold for Humans? Trends Cardiovasc Med 11, 93-102.

Yadav, A., Kumari, R., Yadav, A., Mishra, J.P., Srivastava, D.S., Prabha, S., 2016. Antioxidants and its functions in human body - A Review. Res Env Life Sci 9, 1328-1331.

Young, I.S., Woodside, J.V., 2001. Antioxidants in health and disease. J Clin Path 54, 176-186.