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Original Article

ANTIOXIDANT POTENTIAL AND FATTY ACID COMPOSITION OF *LACTARIUS SANGUIFLUUS*

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ABSTRACT

Edible mushrooms have been valued as a source of food and traditional medicines since ancient times. Mushrooms have drawn the attention of numerous researchers in recent decades since they contain a variety of bioactive compounds with antibacterial, antiallergic, antidiabetic, antitumoral, and antioxidant activities. As a member of the Russulaceae family *Lactarius sanguifluus* (Paulet) Fr. (1838) (also known as bloody milk cap) is an ectomycorrhizal edible mushroom distributed in Asia, the Mediterranean shores and mid-Europe. In this study it was aimed to identify the mushroom sample both by physical traits according to the current literature and by molecular techniques. The antioxidant capability of *L. sanguifluus* methanol extract was evaluated by stable radical scavenging activity and metal ion reducing capability methods. The content of total phenolic compounds and the fatty acid composition of the mushroom sample were also investigated. The mushroom sample was identified both by comparing the physical traits with the current literature and by comparing the sequence data of the ITS region of rDNA with GenBank. The methanol extract of the dried mushroom sample was used to perform the analysis to determine the antioxidant activity and content of total phenolic compounds. It was found that *L. sanguifluus* methanol extract contained 3.677±0.207 mg GAE/g DW phenolic compounds and exhibited radical scavenging activities as 211.652±0.537 mg TE/g DW against DPPH and 73.389±1.003 mg TE/g DW against ABTS. *L. sanguifluus* methanol extract also had metal ion reducing capabilities with ferric reducing and cupric reducing antioxidant capacities of 191.903±0.659 mg AAE/g DW and 379.598±7.527 mg TE/g DW, respectively. The GC-MS analysis of the hexane extract of the mushroom sample revealed that palmitic acid, linoleic acid, oleic acid and stearic acid were the predominant fatty acids found in *L. sanguifluus*.

KEYWORDS: *Lactarius sanguifluus*, Molecular identification, Antioxidant activity, Total phenolics, Fatty acids.

1. Introduction

A fungus is a type of eukaryotic heterotroph organism made up of cells called hyphae that resemble tubes. Fungi obtain their nourishment from dissolved substances in their surroundings through the secretion of extracellular digesting enzymes or from the host plants through the symbiotic relationship called mycorhhiza. Generally found above ground, where the fungal mycelia are located, mushrooms are spore-bearing fruiting bodies of fungus with fleshy structures [1].

People have valued edible mushrooms as a source of food and traditional medicines since ancient times. Mushrooms have drawn the attention of numerous researchers in recent decades since they contain a variety of bioactive compounds with antibacterial, antiallergic, antidiabetic, antitumoral, and antioxidant activities [2]. As a member of the Russulaceae family *Lactarius sanguifluus* (Paulet) Fr. (1838) (also known as bloody milk cap) is an ectomycorrhizal edible mushroom distributed in Asia, the Mediterranean shores and mid-Europe.

The focus of studies with wild *Lactarius* species such as *L. deliciosus*, *L. volemus*, *L. sanguifluus*, *L. semisanguifluus* and *L. piperatus* is on the investigation of their biological properties (antioxidant, antimicrobial, cytotoxicity and neuroprotective activity) and the determination of total phenols [3].

Mushrooms have the potential to be used as sources of antioxidants because they contain bioactive substances such as polysaccharides, vitamins, minerals, polyphenols,

and carotenoids [4-6]. As reactive oxygen species build up from normal metabolic processes, they can lead to oxidative stress, which may accelerate cell aging through lipid peroxidation and cause serious diseases, including Parkinson's disease, cancer, and Alzheimer's disease. Hence, considerable research has been conducted to identify new antioxidant substances that can be used as supplements to prevent oxidative stress while possessing the fewest possible negative effects [7].

Fatty acids have important metabolic functions and are molecules composed of carbon chains with methyl and carboxyl groups at either end. They perform a variety of functions, including energy storage and transit, insulation against mechanical, thermal, and electrical forces, signal transmission, and gene regulation, and are used as components of cell membranes [8].

An abundance of mushroom species can be found in Türkiye because of its rich biodiverse environment [9]. However, little research has been conducted on the biological activities of indigenous mushrooms [10]. In this study it was aimed to determine the content of total phenolic compounds, free radical scavenging activity, metal ion reducing capacity, and fatty acid composition following molecular identification of *Lactarius sanguifluus* collected from Niğde.

2. Materials and Methods

2.1. Material

Macrofungi sample was obtained in May 2019 from a local bazaar in the Ulukışla district of Niğde, Türkiye. The current literature [11-13] was used to identify the sample based on its morphological features. After being identified, the sample was freeze-dried and stored in laboratory conditions until use.

2.2. Molecular identification

Additional molecular identification was carried out to support morphological identification using the Internal Transcribed Spacer (ITS) region of the rDNA, which is often utilized to discriminate fungal species [14].

With a few minor adjustments to the supplier's instructions, DNA was extracted from the samples using the Macherey-Nagel Nucleospin Plant II DNA extraction kit. Polymerase Chain Reaction (PCR) was used to amplify the ITS regions using the primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') [15]. The PCR product was delivered to BM Labosis (Ankara, Türkiye) for sequence analysis after agarose gel electrophoresis. The macrofungus sample was identified by using the Basic Local Alignment Search Tool (BLAST) to compare data from sequence analysis with the NCBI GenBank database.

2.3. Extract preparation

Dried fungal samples were mashed into a powder using a mortar and pestle. Then, 45 mL of methanol and 2 g of powdered material were combined and the mixture was homogenized for 5 min. at 8000 rpm using a Daihan HG-15D homogenizer. The mixture was then incubated overnight at ambient temperature and for 30 min at 30°C in an ultrasonic bath (Sonorex, Bandelin). Following incubation, the mixture was filtered with Macherey Nagel filter paper and dried using a rotary evaporator (Hei-Vap Value, Heidolph). To create an extract with a concentration of 100 mg/mL, the residue was resuspended in methanol. The antioxidative characteristics of the fungal sample and the total phenolic compounds were analyzed using this extract.

2.4. Total phenolic compounds

The total phenolic content in the methanol extract was determined using Folin-Ciocalteu's phenol reagent method [16]. The measurements were conducted in triplicate and the results were expressed as mg GAE/g DW.

2.5. DPPH assay

A commonly employed technique to examine the fungal samples' antioxidant activity is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity. The highest absorbance of DPPH, a dark-colored powder containing stable free radicals, was observed at 517 nm [17]. DPPH radical scavenging assay was performed as reported by Blois [18]. A standard curve was established using the DPPH scavenging values of Trolox solutions and the obtained data were expressed in mg TE/g DW.

2.6. ABTS assay

Another commonly used stable radical for assessing the ability of samples with antioxidant qualities to inhibit radicals is 2-azino-bis-(3-ethylbenzothiozoline-6-sulphonic acid) (ABTS). The ABTS radical scavenging activity of the macrofungus extract was evaluated according to the method modified by Cam et al. [19]. The inhibitory percentage values of Trolox solutions at different concentrations were used to create a standard curve, and the findings were represented as mg TE/g DW.

2.7. PFRAP assay

An increase in the absorbance of ferric ferrocyanide which is a blue-colored complex with a maximum absorbance at 700 nm, can be related to the antioxidative activity of the sample which forms potassium ferrocyanide ($K_4[Fe(CN)_6]$) by reacting potassium ferricyanide ($K_3[Fe(CN)_6]$) [20]. The PFRAP assay was performed according to current litetature [21,22] with slight modifications. The ascorbic acid standard curve was used for data evaluation, and the results were represented as mg AAE/g DW.

2.8. CUPRAC assay

The color change of neocuproine and $Cu(II)Cl_2$ mixture from bright yellow to orange in the presence of compounds acting as electron donors indicates the antioxidant capacity of a sample by reducing cupric (Cu^{+2}) ions to cuprous (Cu^{+}) ions. The CUPRAC assay was performed according to the method originally reported by Apak et al. [23], with slight modifications. The absorbance of all samples was measured at 450 nm against a blank solution. The Trolox standard curve was established to evaluate the antioxidant capacity of mushroom samples with different concentrations and the obtained data was expressed as mg TE/g DW.

2.9. Determination of fatty acid composition

The fatty acid composition of fungus sample was determined by gas chromatography–mass spectrometry (GC-MS). One g of powdered mushroom sample was mixed

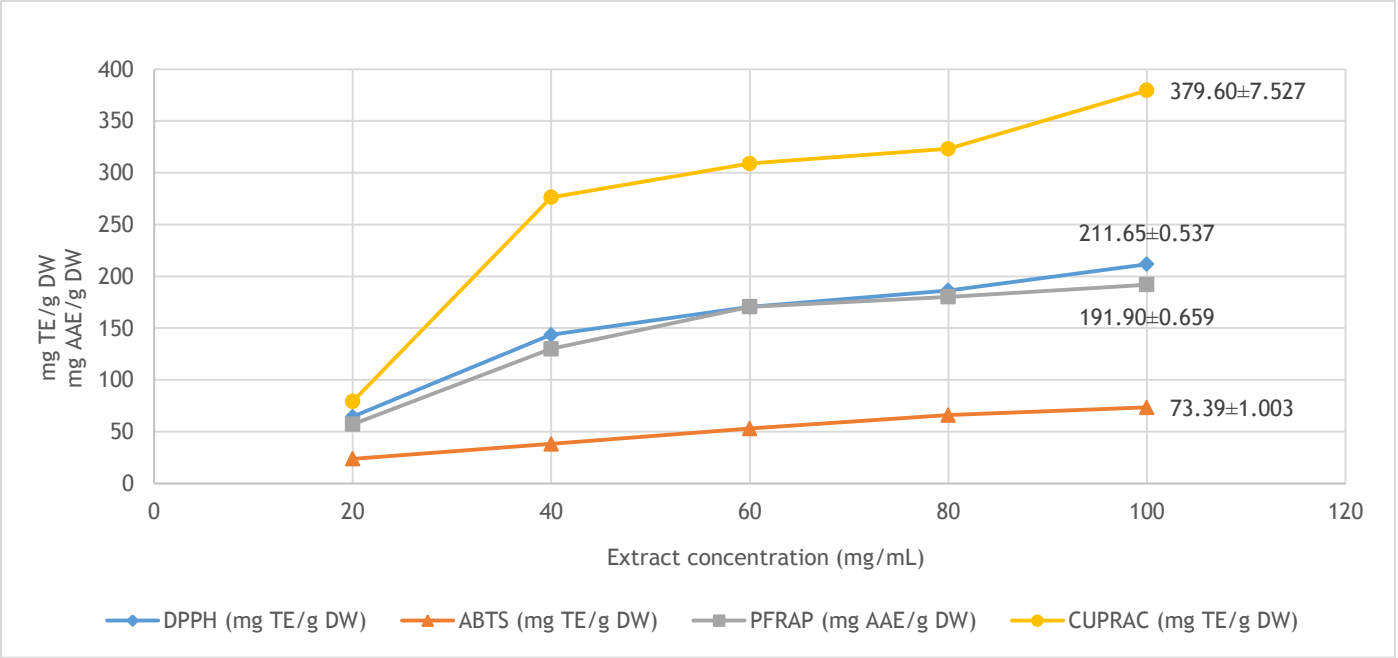

Fig. 1. Free radical scavenging activity and metal ion reducing capacity of *L. sanguifluus* methanol extract. (DPPH: DPPH scavenging activity, mg TE/g DW; ABTS: ABTS scavenging activity, mg TE/g DW; PFRAP: Potassium ferricyanide reducing antioxidant power, mg AAE/g DW; CUPRAC: Cupric ion reducing antioxidant capacity, mg TE/g DW; TE: Trolox equivalent; AAE: Ascorbic acid equivalent; DW: Dry weight).

with 20 mL hexane and homogenized for 5 min. at 8000 rpm. The mixture was then incubated in an ultrasonic bath for 30 min at 30°C. After filtering the homogenate the solvent was evaporated and the residue was resuspended with 5 mL of hexane. Methyl esters of the fatty acids were derived from the hexane extract with 2 M KOH in methanol and 1 N HCl solution. The clear upper layer after the phase separation was dried with anhydrous Na_2SO_4 and passed through a 0.45 µm syringe filter. An aliquote of 1 µL sample was analyzed by a Shimadzu QP2010 Ultra GC-MS system equipped with Restek Rxi-5MS column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The fatty acids were characterized by comparing the obtained spectra with those from the Wiley (W9N11) mass spectra library and Flavor and Fragrance Natural and Synthetic Compounds (FFNSC 1.2) library [24].

3. Results and Discussion

3.1. Identification of macrofungus

The macrofungus sample investigated in this study was identified both in terms of morphological characteristics according to the current literature [11-13] and by comparison of sequence data with those deposited in the GenBank database via BLAST. Most of the data in GenBank comprise sequence data of ITS fragments belonging to fungal samples. According to results the macrofungus sample was identified as *Lactarius sanguifluus* (Paulet) Fr. (1838) with a similarity rate of 99.85%.

3.2. Antioxidant activity

The antioxidant capability of the *L. sanguifluus* methanol extract was investigated using four different methods, along with the content of total phenolic compounds. The results are shown in Fig. 1 and Table 1.

According to Fig. 1, *L. sanguifluus* methanol extract exhibited high DPPH radical scavenging activity with 211.65±0.537 mg TE/g DW and 73.39±1.003 mg TE/g DW ABTS radical scavenging activity and high metal ion reducing activities with 191.90±0.659 mg AAE/g DW potassium ferricyanide reducing activity and 379.60±7.527 mg TE/g DW cupric reducing activity. These findings indicate that the free radical scavenging activity and metal ion reducing capacity of *L. sanguifluus* collected from Türkiye are similar to those reported in the literature [25-27].

Table 1. Biochemical properties of *L. sanguifluus* methanol extract.

TPC	DPPH	ABTS	PFRAP	CUPRAC
3.677 ±0.207	211.65 ±0.537	73.39±1.003	191.90 ±0.659	379.60 ±7.527

(TPC: Total phenolic compounds, mg GAE/g DW; DPPH: DPPH scavenging activity mg TE/g DW; ABTS: ABTS scavenging activity, mg TE/g DW; PFRAP: Potassium ferricyanide reducing antioxidant power, mg AAE/g DW; CUPRAC: Cupric ion reducing antioxidant capacity, mg TE/g DW; GAE: Gallic acid equivalent TE: Trolox equivalent; AAE: Ascorbic acid equivalent; DW: Dry weight)

The phenolic compounds are generally correlated with the antioxidant capability since they can act as hydrogen donors, reducing agents, and singlet oxygen scavengers [28]. The content of total phenolic compounds was determined as 3.667±0.207 mg GAE/g DW for *L. sanguifluus*. Yıldız and Gürgen [29] stated that the content of total phenolic compounds of three *Lactarius* species (*L. deciousus*, *L. insulsus* and

Table 2. Fatty acid composition of *L. sanguifluus* determined by GC-MS.

No.	Compound	RT	MW+CF	RC	SI
1	Myristic acid, methyl ester	32.761	242 ($C_{15}H_{30}O_2$)	0.54	87
2	Palmitic acid methyl ester	37.912	270 ($C_{17}H_{34}O_2$)	12.31	94
3	3,5-Bis(1,1-dimethylethyl)-4-hydroxy-benzenepropanoic acid methyl ester	38.396	292 ($C_{18}H_{28}O_3$)	1.28	86
4	Linoleic acid, methyl ester	41.614	294 ($C_{19}H_{34}O_2$)	6.69	97
5	Oleic acid, methyl ester	41.736	296 ($C_{19}H_{36}O_2$)	7.86	95
6	Stearic acid, methyl ester	42.244	298 ($C_{19}H_{38}O_2$)	25.03	97
7	2-Propenoic acid, tetradecyl ester	45.471	268 ($C_{17}H_{32}O_2$)	2.06	88
8	Arachidic acid methyl ester	46.044	326 ($C_{21}H_{42}O_2$)	0.48	85

*RT: Retention time, min; MW: Molecular weight; CF: Chemical formula; RC: Relative concentration, %; SI: Similarity index, %

L. vellereus) varied between 1.124±0.032 and 2.247±0.086 mg GAE/g. *L. vellereus*, which had the highest phenolic compounds with a value of 2.247±0.086 mg GAE/g, also demonstrated the highest antioxidant activity with a FRAP value of 5.668±0.042 µmol $FeSO_4$/g. These findings supported the correlation between the phenolic compounds and the antioxidant activity.

Erdoğan et al. determined the DPPH scavenging activity and the amount of phenolic compounds as 1283.51 µmol TE/100 g DW and 1133.12 mg GAE/100 g DW, respectively. Researchers have stated that the possible mechanism of antioxidant activity of mushroom extracts occurs through the hydrogen bonding capacity and oxidation of peroxyl radicals [30]. Stankovic et al. examined four species of mushrooms in a 2022 study: *L. deliciosus, L. volemus, L. sanguifluus, L. semisanguifluus* and *L. piperatus* [31]. The mushroom species *L. sanguifluus* exhibited the highest levels of total phenolic compounds and the greatest antioxidant activity as measured by ABTS and CUPRAC assays. It has been stated that the *Lactarius* genus is not only a food source but also contains components with high antioxidant potential.

The factors affecting the difference in the content of total phenolic compounds and antioxidant capabilities between this study and the literature can be considered to be the geographical position and altitude of the sampling area, environmental and meteorological conditions, growth stage of the macrofungus, extraction method and solvent.

3.3. Determination of fatty acid composition

The fatty acid composition of *L. sanguifluus* was also investigated by GC-MS and the results are shown in Table 2.

Stearic acid (25.03%, RT: 42.244 min), palmitic acid (12.31%, RT: 37.912 min), oleic acid (7.86%, RT: 41.736 min), and linoleic acid (6.69%, RT: 41.614 min) are found to be the predominant fatty acids in *L. sanguifluus*. Although the saturated fatty acid concentrations were found higher than the unsaturated fatty acids, which are considered healthy components for human diet, it was reported that stearic acid did not affect the increase in total cholesterol and low density lipoproteins in human blood [32]. Myristic acid (0.54%, RT: 32.761 min), which was associated with increased cholesterol levels, along with palmitic and lauric acids [33], was also determined. 2-Propenoic acid (2.06%, RT: 45.471 min), 3,5-Bis(1,1-dimethylethyl)-4-hydroxy-benzenepropanoic acid (1.28%, RT: 38.396 min) and arachidic acid (0.48%, RT: 46.044 min) were also detected by GC-MS analysis of fatty acids in *L. sanguifluus* collected from Niğde. In this study, stearic acid was the major fatty acid detected in *L. sanguifluus*, and it is one of the most important essential fatty acids. Mushrooms are rich in fat and fatty acids, which are necessary for human metabolism. Myristic, palmitic, stearic, oleic, and linoleic fatty acids are commonly found in *Lactarius* species [4,34-36]. Supporting our findings, it was reported that palmitic, stearic, oleic, and linoleic acids were determined as predominant fatty acids in the study conducted with three *Lactarius* species, namely *L. piperatus, L. quietus* and *L. vellereus* [37]. Erbiai et al. [25] reported that *Lactarius sanguifluus* is rich in organic compounds and contains high amounts of phenolic acids, ascorbic acid, flavonoids, tannins, sugars, fatty acids. The researchers stated that the high amounts of these chemical compounds in the extracts of *L. sanguifluus* may be linked to the significant biological activities observed [25].

4. Conclusions

In conclusion, the results of this study provide valuable insights into the total phenolic compound content, antioxidant activity and fatty acid composition of *L. sanguifluus* collected from Niğde, highlighting its potential as a promising source of bioactive compounds for various applications. Future studies should focus on investigating the bioactive compounds present in *L. sanguifluus* and their potential health benefits as well as exploring the antioxidant activity and fatty acid composition of other mushroom species.

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References

1. Webster, J.; Weber, R. Introduction to Fungi, 3rd ed.; Cambridge University Press: UK, **2007**; pp. 1-39.
2. Ildız, E.; Canpolat, Ş.; İşlek, C.; Canpolat, E.Y.; İşlek, Y.; Akata, I. *Bjerkandera adusta* collected from Niğde: analysis of total phenolic compound, antioxidant, and antimicrobial properties. *Turkish JAF Sci. Tech*. **2022**, *10*, 2996-3000. DOI: 10.24925/turjaf.v10isp2.2996-3000.5750
3. Kosanic, M.; Petrović, N.; Milosevic-Djordjevic, O.; Grujičić, D.; Tubic, J.; Marković, A.; Stanojkovic, T.P. The health promoting effects of the fruiting bodies extract of the peppery milk cap mushroom *Lactarius piperatus* (Agaricomycetes) from Serbia. *Int. J. Med. Mushrooms* **2020**, *22*(4), 347–357. DOI: 10.1615/IntJMedMushrooms.2020034167
4. Barros, L.; Venturini, B.A.; Baptista, P.; Estevinho, L.M.; Ferreira, I.C.F.R. Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. *J. Agric. Food Chem.* **2008**, *56*(10), 3856-3862. DOI: 10.1021/jf8003114
5. Kumar, K.; Mehra, R.; Guiné, R.P.; Lima, M.J.; Kumar, N.; Kaushik, R.; Ahmed, N.; Yadav, A.N.; Kumar, H. Edible mushrooms: A comprehensive review on bioactive compounds with health benefits and processing aspects. *Foods* **2021**, *10*(12), 2996-3006. DOI: 10.3390/foods10122996
6. Yim, H.S.; Chye, F.Y.; Tan, C.T.; Ng, Y.C.; Ho, C.W. Antioxidant activities and total phenolic content of aqueous extract of *Pleurotus ostreatus* (cultivated oyster mushroom). *Malays. J. Nutr.* **2010**, *16*(2), 281-291.
7. Fu, H.Y.; Shieh, D.E.; Ho, C.T. Antioxidant and free radical scavenging activities of edible mushrooms. *J. Food Lipids* **2002**, *9*(1), 35-43. DOI: 10.1111/j.1745-4522.2002.tb00206.x
8. Rustan, A.C. and Drevon, C.A. Fatty acids: structures and properties. *Proc. Natl. Acad. Sci.* **2012**, *109*(16), 6241–6246. DOI: 10.1038/npg.els.0003894
9. Sevindik, M.; Akgül, H.; Bal, C. Determination of oxidative stress status of *Ompholatus olearius* gathered from Adana and Antalya provinces in Turkey. *SAUJS* **2017**, *21*(3), 324-327. DOI: 10.16984/saufenbilder.298974
10. Bal, C.; Akgul, H.; Sevindik, M.; Akata, I.; Yumrutas, O. Determination of the anti-oxidative activities of six mushrooms. *Fresenius Environ. Bull.* **2017**, *26*(10), 6246-6252.
11. Breitenbach, J.; Kränzlin, F. Fungi of Switzerland,2nd ed.; Verlag Mykologia: Lucerna, Switzerland, **1986**.
12. Cannon, P.F. and Kirk, P.M. Fungal Families of the World, 1st ed.; CABI: Netherland, **2007**.
13. Hansen, L. and Knudsen, H. Nordic Macromycetes: Polyporales, Boletales, Agaricales, Russulales, Nordsvamp: Copenhagen, **1992**.
14. Schoch, C.L.; Seifert, K.A.; Huhndorf, S.; Robert, V.; Spouge, J.L.; Levesque, C.A.; Chen, W.; Consortium, F.B.; List, F.B.C.A.; Bolchacova, E. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci.* **2012**, *109*(16), 6241-6246. DOI: 10.1073/pnas.1117018109
15. White, T.J.; Bruns, T.; Lee, S.J.W.T.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications, Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., Eds., Academic Press: New York, **1990**, *18*(1), 315-322.
16. Singleton, V.L.; R, O.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152-178. DOI: 10.1016/S0076-6879(99)99017-1
17. Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **1992**, *40*(6), 945-948. DOI: doi.org/10.1021/jf00018a005
18. Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*(4617), 1199-1200. DOI: 10.1038/1811199a0
19. Cam, M.; Basyigit, B.; Alasalvar, H.; Yilmaztekin, M.; Ahhmed, A.; Sagdic, O.; Konca, Y.; Telci, I. Bioactive properties of powdered peppermint and spearmint extracts: Inhibition of key enzymes linked to hypertension and type 2 diabetes. *Food Biosci.* **2020**, *35*, Art. No: 100577. DOI: 10.1016/j.fbio.2020.100577
20. Xiao, F.; Xu, T.; Lu, B.; Liu, R. Guidelines for antioxidant assays for food components. *Food Front.* **2020**, *1*(1), 60-69. DOI: 10.1002/fft2.10
21. Mokrani, A.; Krisa, S.; Cluzet, S.; Da Costa, G.; Temsamani, H.; Renouf, E.; Mérillon, J.-M.; Madani, K.; Mesnil, M.; Monvoisin, A. Phenolic contents and bioactive potential of peach fruit extracts. *Food Chem.* **2016**, *202*, 212-220. DOI: 10.1016/j.foodchem.2015.12.026
22. Düşgün, C.; Kankılıç, T.; İşlek, C.; Balı, D.F.; Kankılıç, Ö. Antioxidant and cytotoxic potential of local endemic plant *Pastinaca zozimoides* Fenzl. *Turkish JAF Sci. Tech.* **2021**, *9*(4), 646-649. DOI: 10.24925/turjaf.v9i4.646-649.3715
23. Apak, R.; Güçlü, K.; Özyürek, M.; Esin Karademir, S.; Erçağ, E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int. J. Food Sci. Nutr.* **2006**, *57*(5/6), 292-304 DOI: 10.1080/09637480600798132.
24. Canpolat, Ş. and Canpolat, E.Y. Antioxidant and Antimicrobial Activity of a Medicinal Mushroom, *Ganoderma lucidum*. *J. Adv. Biol. Biotechnol.* **2023**, *26*(11), 60-67. DOI: 10.9734/jabb/2023/v26i11667
25. Erbiai, E.H.; Bouchra, B.; da Silva, L.P.; Lamrani, Z.; Pinto, E.; da Silva, J.C.E.; Maouni, A. Chemical composition and antioxidant and antimicrobial activities of *Lactarius sanguifluus*, a wild edible mushroom from northern Morocco. *Euro-Mediterr. J. Environ. Integr.* **2021**, *6*, 1-12. DOI:10.1007/s41207-021-00247-6
26. Kalogeropoulos, N.; Yanni, A.E.; Koutrotsios, G.; Aloupi, M. Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. *Food Chem. Toxicol.* **2013**, *55*, 378-385.DOI: 10.1016/j.fct.2013.01.010

27. Sharma, S.; Atri, N.S.; Kaur, M.; Verma, B. Nutritional and neutraceutical potential of some wild edible Russulaceous mushrooms from North West Himalayas, India. *Kavaka* **2017**, *48*, 41-46.

28. Emsen, B.; Guven, B.; Uzun, Y.; Kaya, A. Antioxidant and genotoxic effects of aqueous and methanol extracts from two edible mushrooms from Turkey in human peripheral lymphocytes. *Int. J. Med. Mushrooms* **2020**, *22*(2), 1-6. DOI: 10.1615/IntJMedMushrooms. 2020033845

29. Yıldız, S. and Gürgen, A. Bazı yenilebilir yabani *Lactarius* mantarlarının biyoaktif ve radyoaktif özellikleri. *Orman. Araşt. Derg.* **2022**, *9*(Özel Sayı), 254-263. DOI: 10.17568/ogmoad.1111983

30. Erdoğan, S.; Soylu, M.K.; Başer, K.H.C. Bazı yabani mantarların antioksidan özellikleri. *Nevşehir Bil. Teknol. Derg.* **2017**, *6*, 254-260. DOI: 10.17100/ nevbiltek.334595

31. Stanković, M.; Mitić, V.; Stankov Jovanović, V.; Dimitrijević, M.; Nikolić, J.; & Stojanović, G. (2022). Selected fungi of the genus Lactarius-screening of antioxidant capacity, antimicrobial activity, and genotoxicity. *J. Toxicol. Env. Heal. A*, 85(17), 699-714. DOI: 10.1080/15287394.2022.2075502

32. Bonanome, A. and Grundy, S.M. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **1988**, *318*(19), 1244-1248. DOI: 10.1056/NEJM198805123181905

33. Williams, C.M. Dietary fatty acids and human health. *Ann. Zootech.* **2000**, *49*(3), 165-180. DOI: 10.1051/ animres:2000116

34. Kalač, P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chem.* **2009**, *113*(1), 9-16. DOI: 10.1016/ j.foodchem.2008.07.077

35. Üstün, O. Makrofungusların besin değeri ve biyolojik etkileri. *Türk Hij. Deney.Biyol. Derg.* **2011**, *68*(4), 223-240. DOI: : 10.5505/TurkHijyen.2011.00922

36. Yılmaz, H.Ç. and Bengü, A.Ş. The ınvestigation of fatty acids and mineral profiles of some edible *Lactarius* species (*L. deliciosus, L. deterrimus, L. salmonicolor, L. sanguifluus, L. semisanguifluus*) in the Uşak/Turkey province of Aegean Region. *Biol. Divers. Conserv.* **2018**, *11*(1), 95-104.

37. Kostić, M.; Ivanov, M.; Fernandes, Â.; Calhelha, R.C.; Glamočlija, J.; Barros, L.; Soković, M.; Ćirić, A. A comparative study of *Lactarius* mushrooms: Chemical characterization, antibacterial, antibiofilm, antioxidant and cytotoxic activity. *J. Fungi* **2023**, *9*(1), 70-79. DOI: 10.3390/jof9010070