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Chemical Composition, Antimicrobial and Antioxidant Activities of *Lactifluus vellereus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors contributed equally to all sections of the study. All authors read and approved the final manuscript.

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ABSTRACT

The consumption of cultivated or wild mushrooms has increased significantly in recent years This is because of their beneficial features, particularly their nutritional value and antioxidant capacity. Vitamins, fiber, protein, amino acids, and phenolic compounds are all abundant in mushrooms. Belonging to the family Russulaceae (Russulales), *Lactifluus* (Pers.) Roussel is a genus of milkcaps, which is predominantly represented in subtropical and tropical regions in the World. In this study it was aimed to determine the chemical composition (ash, total protein, total fat, and fatty acid composition), total amount of phenolic compounds, antioxidant capability and antimicrobial activity of *Lactifluus vellereus* (Fr.) Kuntze (1891) provided from Kastamonu, Türkiye. According to the existing literature, mushroom samples were identified based on their morphological characteristics. Dried mushroom samples were extracted with two different solvents, methanol and acetone, to determine antimicrobial activity against test microorganisms by disk diffusion method. The highest inhibition was recorded against *Staphylococcus aureus* ATCC 25923 strain with 16±0.81 mm zone

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diameter for acetone extract while it was 15.33±0.47 mm against *Salmonella typhimurium* SL1344 strain for methanol extract. The amount of total phenolic compounds and antioxidant capability of *L. vellereus* were also determined using the methanol extract via Folin-Ciocalteu phenol reagent method and DPPH radical scavenging activity and potassium ferricyanide reducing antioxidant power (PFRAP) methods, respectively. The amount of total phenolic compounds was found as 3.58±0.12 mg GAE/g DW while DPPH scavenging activity and PFRAP values were 334.88±0.29 mg TE/g DW and 381.17±0.99 mg AAE/g DW, respectively. The ash, total protein and total fat content of *L. vellereus* were 8.47±0.17%, 20.48±0.07% and 2.28±0.04%, respectively. The fatty acid composition of the mushroom sample was also analyzed by GC-MS and oleic acid was found to be the predominant fatty acid with a concentration of 18.95% followed by elaidic acid (9.30%) and linoleic acid (5.81%). These findings indicated that *L. vellereus* should be considered a valuable source for its nutritional and therapeutic uses.

Keywords: Lactifluus vellereus; antioxidant activity; antimicrobial activity; chemical composition; GC-MS.

1. INTRODUCTION

In recent times, there has been a notable surge in the consumption of both wild and cultivated mushrooms. This is because of their advantageous qualities, particularly their nutritional value and antioxidant content. Mushrooms are a rich source of protein, amino acids, fiber, phenolic compounds, and vitamins (Lalotra et al. 2016). Since they include various levels of minerals and trace elements required for specific biochemical reactions and cellular functions, they are regarded as one of the key foods that promote health (Proskura et al. 2017).

A large number of edible and medicinal mushrooms represent an under-utilised source of substances that can be used in the treatment of a variety of diseases. Mushrooms are mainly used to improve the immune response to various diseases due to their high content of proteins and secondary metabolites. Although items made from mushrooms cannot totally replace prescription medications, using them can help the patient's overall health (Anusiya et al. 2021, Bhambri et al. 2022). The cultivation and commercialisation of naturally growing mushrooms that can serve as nutraceutical, food and/or pharmaceutical ingredients is a promising area of research for scientists. This is because factors such as nutrition, healthcare and socio-economic changes can be supplemented with existing foods and medicines by considering these mushrooms. It is clear that native mushroom species represent an important and largely unexplored source of biologically active compounds with a wide range of potential applications in medicine, pharmacy and the food industry (Anusiya et al. 2021, Kostic et al. 2023). Mushrooms are natural foods that can combat bacterial resistance thanks to their natural compounds with different mechanisms of action (Pancu et al. 2021). Mushroom extracts inhibit pathogenic microorganisms. It is advantageous over the use of synthetic antimicrobial compounds, especially because it has fewer unwanted side effects and can fight microbial resistance (Suleiman et al. 2022).

Lactifluus (Pers) is a genus of milkcaps that is mainly found in tropical and subtropical areas and is a member of the Russulaceae (Russulales) family (Buyck et al. 2008). It is one of the wild edible mushroom genus collected from rural areas. It is of economic importance for many local markets. More than 600 species of the genus *Lactifluus* Pers. (Russulales) have been described worldwide (Lee et al. 2018). A considerable number of novel *Lactifluus* species have been identified in the last decade (De Crop et al. 2021, Boonmee et al. 2021). They are generally known for their medicinal and nutritional properties and are considered promising fungi in the medical industry (Vieira et al. 2014). Since *Lactifluus* species are ectomycorrhizal species, they are associated with a variety of plants and hence have an ecological role in terrestrial ecosystems (Lee et al. 2018). For example, *Lactarius deliciosus* (L.) Grey is a species widely consumed by rural populations and commercialized in some countries. It is also widely consumed in some parts of Türkiye (Martins et al. 2002). Since it is more consumed by people it attracts more attention from the researchers compared to other *Lactarius* and *Lactifluus* species. Several research have been conducted on the mineral and chemical composition of *Lactifluus vellereus* (Fr.) Fr. and *Lactarius piperatus* (L.) Pers., including analyses of their nutritional value, phenolic acid, fatty acids, protein and tocopherol contents (Barros et al. 2007, Heleno et al. 2012,

Niedzielski et al. 2023). Furthermore, some studies have investigated the antioxidant activity and antimicrobial activity of these species against different ATCC strains (Dogan and Aydin 2013, Özen et al. 2016, Fogarasi et al. 2020). The purpose of this study was to determine the chemical composition, antioxidant and antimicrobial effects of *L. vellereus* mushroom.

2. MATERIALS AND METHODS

2.1 Materials

The mushroom samples used in this study were obtained from a local bazaar in Kastamonu Province, Türkiye in 2019. Identification of the samples was performed based on morphological traits, such as size, shape and color of the cap, stipe and spores, according to current literature (Breitenbach and Kranzlin 2000, Cannon and Kirk 2007). The mushroom samples were dried using a conventional vegetable drier at 40°C until the samples reached a constant weight. After that, the dried samples were pulverized and kept in laboratory conditions away from direct sunlight.

2.2 Preliminary Analyses

The chemical composition of *L. vellereus* was ascertained in terms of ash, total protein and total fat content for the evaluation of nutritional value. The ash content was determined using a Protherm PC442T furnace at 550 °C for 5 h. The total protein content was measured by Kjeldahl method via full automated Opsis KjelROC KD310 vehicle following the digestion of the sample with H_2SO_4 and titanium tablets at 400 °C. Using an Ankom XT15 device the total fat content was determined. The results were presented as % for all three parameters.

2.3 Extract Preparation

2 g of powdered sample was mixed with 30 mL of acetone or methanol at analytical grade, homogenized by Daihan HG-15D homogenizer at 9000 rpm for 3 min and incubated for 24 h at 30 ℃ 150 rpm by a shaking incubator. Following that, extracts were filtered through Whatman No:1 filter papers. A rotary evaporator was used to remove solvents until the material was dry. The residues were recovered with the same solvents to obtain solutions with a concentration of 200 mg/mL. The methanol extract was used to assess the total phenolic compound and antioxidant capability analyses. Both extracts were used to perform the antimicrobial activity assay. The extracts were kept at +4 ℃ until use.

2.4 Total Phenolic Compounds and Antioxidant Activity

Determination of the amount of total phenolics was performed by the Folin-Ciocalteu's phenol reagent method (Karadeniz et al. 2024). An aliquot of 100 µL methanolic extract was added to 1 mL Folin-Ciocalteu's phenol reagent and incubated at ambient temperature for 5 min. Then 1 mL 7.5% Na_2CO_3 solution was added to the mixture and incubated for an additional 90 min at ambient temperature in the dark. Following the incubation the absorbance of the sample tubes was measured by a spectrophotometer at 760 nm against blank. The amount of total phenolic compounds was calculated as mg GAE/g DW.

A commonly employed technique to examine the fungal samples' antioxidant activity is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The DPPH radical scavenging activity assay was conducted as reported by Kebaili et al. (2021). The absorbance of test tubes including the DPPH solution and the extract was measured by a spectrophotometer at 517 nm against a blank. A standard curve was established using the DPPH scavenging values of Trolox solutions with various concentrations and the obtained data were presented in mg TE/g DW.

The antioxidative activity of the sample can be related to an increase in the absorbance of ferric ferrocyanide, a blue-colored complex with a maximum absorbance at 700 nm, which is formed by potassium ferricyanide reacting to potassium ferrocyanide. The antioxidant capability of *L. vellereus* methanol extract was also evaluated using the PFRAP method according to current literature. The ascorbic acid standard curve was used for data evaluation and the results were expressed as mg AAE/g DW (Mokrani et al. 2016).

2.5 Antimicrobial Activity

Acetone and methanol extracts of *L. vellereus* were tested against pathogen microorganisms to evaluate the antimicrobial activity of the mushroom sample. *Escherichia coli* ATCC25922*, Salmonella typhimurium* SL1344, *Salmonella kentucky*, *Salmonella infantis*, *Pseudomonas aeruginosa* DSMZ50071, *Staphylococcus aureus*

ATCC25923, *S. epidermidis* DSMZ20044, *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC29212 and *Candida albicans* ATCC10231 strains were used as test microorganisms. The strains were regularly cultured at 36.5 °C in Luria-Bertani (LB) agar. Each microorganism-bearing mixture was used to inoculate a single colony, which was then incubated at 36.5 °C for 18-24 h. The test microorganisms' turbidity complied with the 0.5 McFarland standard.

The antimicrobial activity of *L. vellereus* extracts was assessed using the disc diffusion method. By a sterile Drigalski spatula, 100 µL of each microbial culture in broth media was plated on agar media. Sterile discs of 6 mm in diameter were covered with 25 µL aliquots of extracts and placed on agar media. Subsequently, the plates were incubated for 24 hours at 36.5 °C. Discs containing gentamycin (10 µg) and vancomycin (30 µg) served as the positive control, while the negative control consisted of empty discs, acetone, and methanol. After the incubation period, the sizes of the inhibitory zones were measured. Every experiment was performed as three replicates, and the mean and standard deviations in mm were reported as results.

2.6 Fatty Acid Composition

The fatty acid composition of *L. vellereus* was determined using gas chromatography-mass spectrometry (GC-MS). In this context, 1 g of powdered sample was mixed with 20 mL n-hexane and homogenized. The mixture was then incubated in an ultrasonic bath (Sonorex, Bandelin) for 30 min at 40 °C. After filtering the homogenate through a Whatman No:1 filter paper. A rotary evaporator was used to remove the solvent.The residue was resuspended with 5 mL of n-hexane. Methyl esters of the fatty acids in hexane extract were derived by 2M KOH in methanol and 1N HCl. After the phase separation, the clear upper layer was dried with anhydrous Na_2SO_4 and passed through a 0.45 µm syringe filter. Using a Restek Rxi-5MS column, a Shimadzu QP2010 Ultra GC-MS system examined an aliquot of 1 µL of sample. The carrier gas, helium, was employed at a 1 mL/min flow rate. 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 (Canpolat and Canpolat 2023).

3. RESULTS AND DISCUSSION

3.1 Identification of the Mushroom

Edible mushrooms are a great resource for both culinary and therapeutic uses. As a result, research into creating viable cultivation methods and reproducing wild species keeps growing. The samples of mushrooms used in the present study were purchased from a local bazaar in Kastamonu province, Türkiye. The current literature was followed in the identification process, which was based on the morphological characteristics of the mushroom samples. The mushroom samples were identified as *Lactifluus vellereus* (Fr) Kuntze (1891) (*syn. Agaricus vellereus* Fr. (1821), *Lactarius vellereus* (Fr.) Fr. (1838), *Galorrheus vellereus* (Fr.) P. Kumm. (1871)).

3.2 Preliminary Analysis

The mushroom samples were analyzed in terms of nutritional value including the ash, total protein and total fat content. The results are shown in Table 1.

According to the results *L. vellereus* contains 8.4717±0.173% ash, while the total protein and total fat contents were determined as 20.4837±0.074 and 2.28±0.041%, respectively. Consuming 200 g of dried mushrooms per day in place of meat has the effect of substituting the meat and maintaining a balanced protein intake. One kilogram of dried mushrooms is equivalent to two times the amount of albumin found in beef and eleven times the amount found in milk. The studies on the nutritional value of Lactarius species revealed that the content of ash, total protein and total fat might vary between 5.1-8.3%, 13.06-31.81 and 2-2.69%, respectively (Barros et al. 2007, Vieira et al. 2014, Nagy et al. 2017). The findings of our study are in accordance with the current literature in terms of ash, total protein and total fat content of the *L. vellereus*.

3.3 Total Phenolic Compounds and Antioxidant Capability

L. vellereus was found to exhibit strong antioxidant activity in numerous investigations examining the antioxidant capacity of mushroom extracts using a variety of methodologies. Studies looking at the overall amount of the antioxidant and phenolic compounds potential of

Table 1. Ash, total protein and total fat content of *L. vellereus*

Ash (%)	Total protein (%)	Total fat (%)
8.4717±0.173	20.4837±0.074	2.28±0.041

Table 2. Amount of total phenolic compounds and antioxidant capability of *L. vellereus* methanol extract

TPC (mg GAE/g DW)	DPPH (mg TE/g DW)	PFRAP (mg AAE/g DW)
3.5804±0.117	334.8812±0.286	381.1705±0.989

mushroom extracts all agree that there is a positive relationship between phenolic compounds and antioxidative activity (Heleno et al. 2012, Dogan and Aydin 2013, Gurgen et al. 2018, Kostić et al. 2023).

The amount of total phenolic compounds (TPC) and the antioxidant capability via DPPH radical scavenging activity and PFRAP assays of *L. vellereus* methanol extract were demonstrated in Table 2.

According to the results of individual experiments conducted as triplicates, the methanol extract of L. vellereus exhibited 334.8812±0.286 mg TE/g DW DPPH radical scavenging activity and 381.1705±0.989 mg AAE/g DW PFRAP capacity with a TPC value of 3.5804±0.117 mg GAE/g DW.

Determination of the phenolic content of natural extracts provides a first insight into the potential of the extracts. In a study, total phenolic contents of 3 different *Lactarius* species were found between 6.55-9.64 mg GAE/g. It has been observed in the literature that the total phenolic contents of *Lactarius* species have different values (Kosanic et al. 2016, Bozdogan et al. 2018, Ayvaz et al. 2019, Su et al. 2019, Ozen et al. 2019, Alkan et al. 2020, Rosa et al. 2020, Volcao et al. 2021). In a study using *L. deliciosus* and *L. salmonicolor* mushroom extracts, total phenolic compound amounts were calculated as 6.281±0.0006 and 8.615±0.0008 mg GAE/100 g dry material, respectively (Dizeci and Yıldırım 2023). These results can be explained by differences in the place and time of collection or the methods used in extraction procedures (Zeng et al. 2012, Petrović et al. 2014, Boonsong et al., 2016).

Synthetic antioxidants should be replaced with safer antioxidants derived from natural sources. In the present study, the antioxidant properties of *Lactarius vellereus* were determined using different test systems and in this way, it was aimed to reach a more precise conclusion about the antioxidant properties of the extracts studied. DPPH is the most commonly used radical in antioxidant capacity studies and provides the evaluation of radical scavenging properties of extracts. Plant antioxidants neutralize radicals by donating electrons or hydrogen. The reducing power of *Lactarius* extract was also determined by FRAP test. There is a positive correlation between total phenolic content and radical scavenging and reducing power. Confirming these correlation results, many studies have reported the existence of a positive relationship between total phenolic content and radical scavenging and reducing power (Mwamatope et al. 2020, Sarikurkcu et al. 2020). There is a consensus that phenolic compounds, especially phenolic acids, rank first among the phytochemicals responsible for the antioxidant activity of fungi. This is because these structures contain one or more aromatic rings and one or more hydroxyl (-OH) groups. Thanks to these properties, they have the potential to scavenge free radicals (Wright et al. 2001, Sarikurkcu et al. 2010). Antioxidant activity of *Lactarius* species indicates that these species can be used as a source of natural antioxidants due to concerns about the use of synthetic ones.

3.4 Antimicrobial Activity

In this study, *in vitro* antimicrobial activities of 30 µL aliquots of *L. vellereus* acetone and methanol extracts with a concentration of 200 mg/mL were investigated against *E. coli* ATCC25922, *S. typhimurium* SL1344, *S. kentucky*, *S. infantis*, *P. aeruginosa* DSMZ50071, *S. aureus* ATCC25923, *S. epidermidis* DSMZ20044, *L. monocytogenes* ATCC 7644, *E. faecalis* ATCC29212 and *C. albicans* ATCC10231 strains as test microorganisms. The results are shown in Table 3.

According to the results, the highest inhibition was observed against *S. aureus* with a zone diameter of 16.33±0.94 mm by *L. vellereus*

acetone extract. Interestingly, the acetone extract did not show any effect against other pathogen strains. On the other hand, the methanol extract exhibited low to moderate inhibition against all pathogen microorganisms. The highest inhibition zone diameter was measured as 15.66±0.94 mm against *S. typhimurium* while the lowest was 9.33±0.47 mm against *E. coli* for the methanol extract.

According to the literature, there are many studies on the antimicrobial activity of *Lactarius sp.* (*L. deterrimus, L. deliciosus, L. sanguifluus, L. piperatus, L. semisangiufluus, L. vellereus*, *L. salmonicolor, L. rufus*) (Lindequist et al. 2005, Oyetayo 2009, Özen et al. 2011, Todorović et al. 2023, Karakas et al. 2023).

Lactarius deliciosus showed inhibitory effect against the growth of *Klebsiella pneumoniae* and *Escherichia coli* under *in vitro* conditions. It was reported that *Lactarius deliciosus* showed antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, and *Mycobacterium smegmatis* (Dülger et al. 2002), *Bacillus cereus*, *Bacillus subtilis* (Barros et al. 2007), *Candida albicans* (Santoyo et al. 2009). It was stated that antimicrobial properties may be affected by factors such as solvent type, duration and concentration of extracts (Barros et al. 2007).

The antibacterial properties of *L. controversus* methanol and ethanol extracts against a variety of microorganisms (*Pseudomonas aeruginosa* DMS50071, *Bacillus megaterium* DSM32, *Klebsiella pneumoniae* ATCC700603, *Escherichia coli* ATCC25922, *Staphylococcus aureus* COWAN1, *Candida glabrata* ATCC 66032, *Candida albicans* FMC17, and *Trichophyton* sp.) were examined in a study. The methanol and ethanol extracts of *L. controversus* were reported to have an antibacterial (8.3-25.3 mm) effect against the microorganisms utilized, based on the disk diffusion method (İnci and Kırbağ 2024).

The methanol extract obtained from the maceration method of *L. vellereus*, which has the highest antimicrobial activity among all the extracts we used in the study, showed high activity especially against *S. pyogenes, K. pneumonia, P. aeruginosa* and *S. enteriditis* at low concentrations (0.0048; 0.0024; 0.0097 mg/mL, respectively) (Aydın 2009).

In another study, it was reported that this species had no antimicrobial effect against *E. coli, C. albicans*, *P. aeruginosa, S. enterica* but formed an 8 mm inhibition zone against *S. flexneri* (Altuner and Akata 2010). It was reported that the methanol extracts of *Lactarius controversus* and *L. musteus* exhibited a range of inhibition zones between 12.00-17.50 mm against test microorganisms including *S. aureus, P. aeruginosa*, *E. coli*, and *C. albicans.* It was also stated that the difference in antimicrobial activities between the wild and cultivated species might be due to difference in the chemical compositions of the mushrooms (Özen et al. 2016).

Dulger et al. (2002) reported that *L. controversus* has antimicrobial effect against Gram (+) and Gram (-) bacteria but not against yeasts. MIC values of some *Lactarius* species against *P. aeruginosa, E. coli, S. aureus* were found to be in the range of 9.38-37.50 mg/mL (Stanković et al. 2022). Methanol and ethanol extracts of *L. piperatus*, *L. quietus,* and *L. vellereus* species were reported to have MIC values in the range of 12.5-25 mg/mL against *S. aureus* (Kostić et al. 2023).

The difference in antimicrobial activity results may vary depending on the species of the fungus, the place of collection, the components it contains, the solvent used in extraction, and the type of pathogenic microorganism. Therefore, it is important to analyze the chemical contents and antimicrobial substances of mushroom species growing naturally in different geographical regions and to compare these analyses (Puttaraju et al. 2006, Özen et al. 2016, Avcı and Avcı 2019, Erbiai et al. 2021, Stanković et al. 2022, Kostić et al. 2023).

Using the data obtained from the studies, the active ingredients in the extracts with known antimicrobial activity can be determined in future studies. The obtained compounds can be isolated from fungi and used in diseases caused by pathogenic microorganisms after their effects on living tissues are tested.

3.5 Chemical Composition

In this study, the chemical composition of *L. vellereus* n-hexane extract was also investigated by GC-MS technique and the results are shown in Table 4.

According to the results oleic acid (18.95%, RT: 42.956), elaidic acid (9.3%, RT: 41.621), linoleic acid (5.81%, RT: 44.507),

Table 3. Antimicrobial activity of *L. vellereus* methanol and acetone extracts against test microorganisms

			Inhibition Zone Diameters (mm)	
Test Microorganisms	**VA**	**CN**	**Acetone**	**Methanol**
Gram-negative bacteria				
S. typhimurium SL1344	17	22	nd	15.66±0.94
S. kentucky	7	14	nd	12±0.82
S. infantis	8	21	nd	10.33±0.47
E. coli ATCC 25922	12	20	nd	9.33±0.47
P. aeuruginosa DSMZ 50071	8	18	nd	12.33±1.25
Gram-positive bacteria				
S. aureus ATCC 25923	7	10	16.33±0.94	10.33±0.47
S. epidermidis DSMZ 20044	nd	20	nd	11.66±0.47
E. faecalis ATCC 29212	nd	nd	nd	13.66±0.47
L. monocytogenes ATCC 7644	9	19	nd	13.5±1.47
Yeast				
C. albicans ATCC 10231	nd	nd	nd	14.66±0.47

**VA: Vancomycin (30 µg), *CN: Gentamycin (10 µg)*

Table 4. Chemical constituents derived from L. vellereus n-hexane extract determined by GC-MS

No.	Compound	RT	MW+CF	RC	SI
1	Palmitic acid methyl ester	37.795	270 ($C_{17}H_{34}O_2$)	1.72	91
2	Elaidic acid methyl ester	41.621	270 ($C_{19}H_{36}O_2$)	9.3	94
3	Stearic acid methyl ester	42.132	298 ($C_{19}H_{38}O_2$)	4.61	93
4	Oleic acid ethyl ester	42.956	310 ($C_{20}H_{38}O_2$)	18.95	95
5	Linoleic acid methyl ester	44.507	298 ($C_{19}H_{34}O_2$)	5.81	97
6	Hexacontane	46.474	842 ($C_{60}H_{122}$)	7.34	82
7	Hexatriacontane	46.649	506 ($C_{36}H_{74}$)	16.1	92
8	Hexadecanal	53.342	240 ($C_{16}H_{32}O$)	11.57	84

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

stearic acid (4.61%, RT: 42.132) and palmitic acid (1.72%, RT: 37.795) were found as predominant fatty acids existing in *L. vellereus* n-hexane extract. These findings support that edible mushrooms can be considered a rich source of dietary unsaturated fatty acids, which are valuable secondary metabolites with key roles in human metabolism. Hexacontane (7.34%, RT: 46.474), hexatriacontane (16.1%, RT: 46.649) and hexadecanal (11.57%, RT: 53.342) were also determined in *L. vellereus*.

Dietary fatty acids, which are essential for human metabolism, are abundant in mushrooms. *Lactarius* species are known to have high concentrations of Myristic, Stearic, Palmitic, Linoleic and Oleic fatty acids. It was reported that Lactarius species are rich in organic components and include significant concentrations of sugars, fatty acids, tannins, flavonoids, ascorbic acid, and phenolic acids. According to the common statement of the researchers, the considerable biological activities reported may be related to the high concentrations of these chemical compounds in mushroom extracts (Kalac 2009, Yılmaz and Bengü 2018, Erbiai et al. 2021).

4. CONCLUSION

Mushrooms consumed as food are known to be an ideal food because of their rich phytochemical content, low sugar and fat content and especially because they are good dietary products. In addition, edible mushrooms are recognized as a good food source for cardiovascular disease due to their protein and mineral content. Furthermore, these mushrooms are important for their antibacterial, antifungal, antiparasitic, detoxification and antidiabetic properties. The results of the study show that *L. vellereus* has important nutritional components, bioactive chemicals, antioxidant, and antibacterial properties. The extracts of *L. vellereus* may be used as a natural agent both as nutraceutical and therapeutic purposes. It is recommended that more investigation be done on the therapeutic and medical properties of *L. vellereus*. The results obtained from this study should be confirmed by *In vivo* tests and the mechanisms of action of specific bioactive compounds found in this species should be studied.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Alkan, S., Uysal, A., Kasik, G., Vlaisavljevic, S., Berezni, S., & Zengin, G. (2020). Chemical characterization, antioxidant, enzyme inhibition and antimutagenic properties of eight mushroom species: A Comparative Study. *Journal of Fungi,* 6(3), 1-19. https://doi.org/10.3390/jof6030166

Altuner, E.M., & Akata, I. (2010). Antimicrobial activity of some macrofungi extracts. *Sakarya University Journal of Science*, 14(1), 45-49.

Anusiya, G., Gowthama Prabu, U., Yamini, N.V., Sivarajasekar, N., Rambabu, K., Bharath, G., & Banat, F. (2021). A Review of the Therapeutic and Biological Effects of Edible and Wild Mushrooms. *Bioengineered*, 12(2), 11239-11268. https://doi.org/10.1080/21655979.2021.2001183

Avcı, E., & Avci, G.A. (2019). Antimicrobial and antioxidant activities of medicinally important *Lactarius deliciosus. International Journal of Medical Science and Dental Research*, 2(6), 49-55.

Aydın, S. (2009). The identification of antioxidant, antimicrobial effects and fatty acids composition of *Terfezia boudieri* chatin and *Lactarius vellereus* (Fr.) Fr*.* Selçuk University Graduate School of Natural and Applied Sciences Department of Biology, MS Thesis, p. 107.

Ayvaz, M.Ç., Aksu, F., & Kır, F. (2019). Phenolic profile of three wild edible mushroom extracts from Ordu, Turkey and their antioxidant properties, enzyme inhibitory activities. *British Food Journal*, 121, 1248-1260. DOI 10.1108/BFJ-06-2018-0399

Barros, L., Baptista, P., Estevinho, L.M., & Ferreira, I.C. (2007). Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *Lactarius* sp. mushrooms. *Journal of Agricultural and Food Chemistry,* 55(21), 8766-8771. https://doi.org/10.1021/jf071435+

Bhambri, A., Srivastava, M., Mahale, V.G., Mahale, S., & Karn, S.K. (2022).

Mushrooms as potential sources of active metabolites and medicines. *Frontiers in Microbiology,* 13: 837266. https://doi.org/10.3389/fmicb.2022.837266

Boonmee, S., Wanasinghe, D.N., Calabon, M.S., Huanraluek, N., Chandrasiri, S.K., Jones, G.E., ... & Hyde, K.D. (2021). Fungal diversity notes 1387–1511: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. *Fungal Diversity,* 111, 1-335. https://doi.org/10.1007/s13225-021-00489-3

Boonsong, S., Klaypradit, W., & Wilaipun, P. (2016). Antioxidant activities of extracts from five edible mushrooms using different extractants. *Agriculture and Natural Resources,* 50(2), 89-97. https://doi.org/10.1016/j.anres.2015.07.002

Bozdogan, A., Ulukanli, Z., Bozok, F., Eker, T., Dogan, H.H., & Buyukalaca, S. (2018). Antioxidant Potential of *Lactarius deliciosus* and *Pleurotus ostreatus* from Amanos Mountains. *Advancements in Life Sciences,* 5(3), 113-120.

Breitenbach, J., & Kränzlin, F. (2000). Fungi of Switzerland. Vol.5, Verlag Mykologia Lucerne, Switzerland.

Buyck, B., Hofstetter, V., Eberhardt, U., Verbeken, A., & Kauff, F. (2008). Walking the thin line between *Russula* and *Lactarius*: The dilemma of Russula subsect. Ochricompactae. *Fungal Divers*, 28, 15–40.

Cannon, P.F., & Kirk, P.M. (Eds.). (2007). Fungal families of the world. Cabi.

Canpolat, Ş., & Canpolat, E.Y. (2023). Antioxidant and Antimicrobial Activity of a Medicinal Mushroom, *Ganoderma lucidum*. *Journal of Advances in Biology & Biotechnology*, 26(11), 60-67. https://doi.org/10.9734/jabb/2023/v26i11667

De Crop, E., Delgat, L., Nuytinck, J., Halling, R. E., & Verbeken, A. (2021). A short story of nearly everything in *Lactifluus* (Russulaceae). *Fungal Systematics and Evolution,* 7(1), 133-164. https://doi.org/10.3114/fuse.2021.07.07

Dizeci, N., & Yıldırım, Ö. (2023). Evaluation Of Phenolic Compounds And Antıoxidant Effects of *Lactarius delicious* and *Lactarius salmonicolor* Mushrooms. *Journal of Faculty of Pharmacy of Ankara University,* 47(2), 567-575. https://doi.org/10.33483/jfpau.1225979

Dogan, H.H., & Aydin, S. (2013). Some biological activities of *Lactarius vellereus* (Fr.) Fr. in Turkey. *Pakistan Journal of Biological Sciences,* 16(21), 1279-1286. DOI: 10.3923/pjbs.2013.1279.1286 Doi 10.5943/cream/6/3/2

Dülger, B., Yılmaz, F., & Gucin, F. (2002). Antimicrobial activity of some *Lactarious* species. *Pharmaceutical Biology*, 40(4), 304-306. https://doi.org/10.1076/phbi.40.4.304.8468

Erbiai, E.H., Bouchra, B., da Silva, L.P., Lamrani, Z., Pinto, E., da Silva, J.C.E., & Maouni, A. (2021). Chemical composition and antioxidant and antimicrobial activities of *Lactarius sanguifluus*, a wild edible mushroom from northern Morocco. *Euro-Mediterranean Journal for Environmental Integration,* 6(43), 1-12. https://doi.org/10.1007/s41207-021-00247-6

Fogarasi, M., Diaconeasa, Z.M., Pop, C.R., Fogarasi, S., Semeniuc, C.A., Fărcaş, A.C., ... & Socaci, S.A. (2020). Elemental composition, antioxidant and antibacterial properties of some wild edible mushrooms from Romania. *Agronomy*, 10(12), 1972. https://doi.org/10.3390/agronomy10121972

Gurgen, A., Yildiz, S., Can, Z., Tabbouche, S., & Kilic, A.O. (2018). Antioxidant, antimicrobial and antiquorum sensing activities of somie vvild and cultivated mushroom species collected from Trabzon. *Fresenius Environ Bull.,* 27(6), 4120-4131.

Heleno, S.A., Barros, L., Martins, A., Queiroz, M.J.R., Santos-Buelga, C., & Ferreira, I.C. (2012). Phenolic, polysaccharidic, and lipidic fractions of mushrooms from Northeastern Portugal: chemical compounds with antioxidant properties. *Journal of Agricultural and Food Chemistry.* 60(18), 4634-4640. https://doi.org/10.1021/jf300739m

İnci, Ş., & Kırbağ, S. (2024). Determination of antimicrobial effect of *Lactarius controversus* (Pers.) Pers. *Artvin Coruh University Journal of Forestry Faculty*, 25(1), 1-5. https://doi.org/10.17474/artvinofd.1335292

Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, 113(1), 9-16. https://doi.org/10.1016/j.foodchem.2008.07.077

Karadeniz, M., Bakır, T.K., & Ünal, S. (2024). Investigation of the Phenolic Contents and Antioxidant Activities of Some Natural Edible Mushroom Species. *Journal of*

Bartin Faculty of Forestry, 26(1), 7-12. https://doi.org/10.24011/barofd.1386438

Karakas, F.P., Turker, A.U., & Bozat, B.G. (2023). Phenolic content, antibacterial and antioxidant potential of several edible Agaricomycetes mushrooms sold in public bazaar in Bolu, Turkey. *International Journal of Medicinal Mushrooms,* 25(1), 45-56. DOI: 10.1615/IntJMedMushrooms.2022046526

Kebaili, F.F., Tahar, N., Toumi, M.E., Redouane, R., Chawki, B., Pablo, A., & Perduca, M. (2021). Antioxidant activity and phenolic content of extracts of wild Algerian Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Agaricomycetes). *International Journal of Medicinal Mushrooms,* 23(6), 79-88.DOI: 10.1615/IntJMedMushrooms.2021038424

Kosanic, M., Rankovic, B., Rancic, A., & Stanojkovic, T. (2016). Evaluation of metal concentration and antioxidant, antimicrobial, and anticancer potentials of two edible mushrooms *Lactarius deliciosus* and *Macrolepiota procera*. *Journal of Food and Drug Analysis,* 24(3), 477-484. https://doi.org/10.1016/j.jfda.2016.01.008

Kostić, M., Ivanov, M., Fernandes, Â., Calhelha, R.C., Glamočlija, J., Barros, L., ... & Ćirić, A. (2023). A Comparative study of *Lactarius* mushrooms: chemical characterization, antibacterial, antibiofilm, antioxidant and cytotoxic activity. *Journal of Fungi,* 9(1), 70. https://doi.org/10.3390/jof9010070

Lalotra, P., Gupta, D., Yangdol, R., Sharma. Y.P., & Gupta, S.K. (2016). Bioaccumulation of heavy metals in the sporocarps of some wild mushrooms. *Curr. Res. Environ. Appl. Mycol.,* 6 (3), 159–165.

Lee, H., Park, J.Y., Wisitrassameewong, K., Kim, M.J., Park, M.S., Kim, N.K., ... & Lim, Y.W. (2018). First report of eight milkcap species belonging to *Lactarius* and *Lactifluus* in Korea. *Mycobiology,* 46(1), 1-12.https://doi.org/10.1080/12298093.2018.1454012

Lindequist, U., Niedermeyer, T.H., & Jülich, W.D. (2005). The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine,* 2(3), 285-299. https://doi.org/10.1093/ecam/neh107

Martins, A., Baptista, P., Sousa, M.J., Meireles, T., & Pais, M.S. (2002). Edible mycorrhizal fungi associated with *Castanea sativa* Mill. trees in the north-east of Portugal. In I. Hall, Wan Yun, E. Danell, A. Zambonelli (Eds.). Proceedings of the second international workshop on edible mycorrhizal fungi. ISBN 0-478-10828-X.

Mokrani, A., Krisa, S., Cluzet, S., Da Costa, G., Temsamani, H., Renouf, E., ... & Richard, T. (2016). Phenolic contents and bioactive potential of peach fruit extracts. *Food Chemistry,* 202, 212-220. https://doi.org/10.1016/j.foodchem.2015.12.026

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E., & Nyirenda, C. (2020). Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African.*, 9, e00481.https://doi.org/10.1016/j.sciaf.2020.e00481

Nagy, M., Socaci, S., Tofană, M., Biris-Dorhoi, E.S., Țibulcă, D., Salanță, L., & Petruț, G. (2017). Chemical composition and bioactive compounds of some wild edible mushrooms. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology,* 74(1), 1-8. DOI: 10.15835/buasvmcn-fst:12629

Niedzielski, P., Szostek, M., Budka, A., Budzyńska, S., Siwulski, M., Proch, J., ... & Mleczek, M. (2023). *Lactarius* and *Russula* mushroom genera–Similarities/differences in mineral composition within the Russulaceae family. *Journal of Food Composition and Analysis,* 115, 104970. https://doi.org/10.1016/j.jfca.2022.104970

Oyetayo, V.O. (2009). Free radical scavenging and antimicrobial properties of extracts of wild mushrooms. *Brazilian Journal of Microbiology,* 40(2), 380-386. https://doi.org/10.1590/S1517-83822009000200031

Özen, T., Darcan, C., Aktop, Ö., & Turkekul, İ. (2011). Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea Region of Turkey. *Combinatorial Chem. High Troughput Screening,* 14, 72-84. https://doi.org/10.2174/138620711794474079

Özen, T., Darcan, C., Kaygusuz, Ö., & Türkekul, İ. (2016). The Chemical content, antioxidant and antimicrobial assays of *Lactarius controversus* and *Lactarius*

musteus: two edible wild mushrooms from Giresun province of Turkey. *Annals of Food Processing and Preservation,* 1(1), 1001.

Ozen, T., Kizil, D., Yenigun, S., Cesur, H., & Turkekul, I. (2019). Evaluation of bioactivities, phenolic and metal content of ten wild edible mushrooms from Western Black Sea Region of Turkey. *International Journal of Medicinal Mushrooms,* 21(10), 979-994. DOI: 10.1615/IntJMedMushrooms.2019031927

Pancu, D.F., Scurtu, A., Macasoi, I.G., Marti, D., Mioc, M., Soica, C., ... & Dehelean, C. (2021). Antibiotics: conventional therapy and natural compounds with antibacterial activity—a pharmaco-toxicological screening. *Antibiotics,* 10(4), 401. https://doi.org/10.3390/antibiotics10040401

Petrović, J., Papandreou, M., Glamočlija, J., Ćirić, A., Baskakis, C., Proestos, C., Lamari, F., Zoumpoulakis, P., & Soković, M. (2014). Different extraction methodologies and their influence on the bioactivity of the wild edible mushroom *Laetiporus sulphureus* (Bull.) Murrill. *Food & Function,* 5(11), 2948-2960. https://doi.org/10.1039/C4FO00727A

Proskura, N., Podlasińska, J., & Skopicz-Radkiewicz, L. (2017). Chemical composition and bioaccumulation ability of *Boletus badius* (Fr.) Fr. collected in western Poland. *Chemosphere*, 168, 106-111.https://doi.org/10.1016/j.chemosphere.2016.10.003

Puttaraju, N.G., Venkateshaiah, S.U., Dharmesh, S.M., Urs, S.M.N., & Somasundaram, R. (2006). Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural and Food Chemistry*, 54(26), 9764-9772. https://doi.org/10.1021/jf0615707

Rosa, G.B., Sganzerla, W.G., Ferreira, A.L.A., Xavier, L.O., Veloso, N.C., da Silva, J., de Oliveira, G.P., Amaral, N.P., de Lima Veeck, A.M., & Ferrareze, J.P. (2020). Investigation of nutritional composition, antioxidant compounds, and antimicrobial activity of wild culinary-medicinal mushrooms *Boletus edulis* and *Lactarius deliciosus* (Agaricomycetes) from Brazil. *International Journal of Medicinal Mushrooms*, 22(10), 931-942. DOI: 10.1615/IntJMedMushrooms.2020036347

Santoyo, S., Ramírez-Anguiano, A.C., Reglero, G., & Soler-Rivas, C. (2009). Improvement of the antimicrobial activity of edible mushroom extracts by inhibition of oxidative enzymes. *International Journal of Food Science & Technology,* 44(5), 1057-1064.https://doi.org/10.1111/j.1365-2621.2008.01896.x

Sarikurkcu, C., Andrade, J.C., Ozer, M.S., de Lima Silva, J.M.F., Ceylan, O., de Sousa, E.O., & Coutinho, H.D.M. (2020). LC-MS/MS profiles and interrelationships between the enzyme inhibition activity, total phenolic content and antioxidant potential of *Micromeria nervosa* extracts. *Food Chemistry*, 328, 126930. https://doi.org/10.1016/j.foodchem.2020.126930

Sarikurkcu, C., Tepe, B., Semiz, D.K., & Solak, M.H. (2010). Evaluation of metal concentration and antioxidant activity of three edible mushrooms from Mugla, Turkey. *Food and Chemical Toxicology*, 48, 1230-1233. https://doi.org/10.1016/j.fct.2009.12.033

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. *Journal of Toxicology and Environmental Health, Part A.,* 85(17), 699-714. https://doi.org/10.1080/15287394.2022.2075502

Su, S.Y., Ding, X., Fu, L., & Hou, Y.L. (2019). Structural characterization and immune regulation of a novel polysaccharide from Maerkang *Lactarius deliciosus* Gray. *International Journal of Molecular Medicine,* 44(2), 713-724. https://doi.org/10.3892/ijmm.2019.4219

Suleiman, W.B., Shehata, R.M., & Younis, A.M. (2022). In vitro assessment of multipotential therapeutic importance of *Hericium erinaceus* mushroom extracts using different solvents. *Bioresources and Bioprocessing,* 9(1), 99. https://doi.org/10.1186/s40643-022-00592-6

Todorović, J.D., Petrović, N.N., & Kosanić, M.M. (2023). Selected mushrooms as potential sources of antimicrobials and antioxidants. *Kragujevac Journal of Science,* 45, 245-256. doi: 10.5937/KgJSci2345245T

Vieira, V., Barros, L., Martins, A., & Ferreira, I.C. (2014). Expanding current knowledge on the chemical composition and antioxidant activity of the genus

Lactarius. *Molecules*, 19(12), 20650-20663.https://doi.org/10.3390/molecules191220650

Volcao, L.M., Halicki, P.B., Bilibio, D., Ramos, D.F., Bernardi, E., & Da Silva, F.M.R. (2021). Biological activity of aqueous extracts of Southern Brazilian mushrooms. *International Journal of Environmental Health Research,* 31(2), 148-159. https://doi.org/10.1080/09603123.2019.1634798

Wright, J.S., Johnson, E.R., & DiLabio, G.A. (2001). Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *Journal of the American Chemical Society,* 123, 1173-1183. https://doi.org/10.1021/ja002455u

Yılmaz, H.C., & Bengü, A.S. (2018). The investigation of fatty acids and mineral profiles of some edible Lactarius species (*L. delicious, L. deterrimus, L. salmonicolor, L. sanguifluus, L. semisanguifluus*) in the Usak/Turkey province of Aegean Region. *Biological Diversity and Conversation,* 11, 95-104.

Zeng, X., Suwandi, J., Fuller, J., Doronila, A., & Ng, K. (2012). Antioxidant capacity and mineral contents of edible wild Australian mushrooms. *Food Science and Technology International,* 18(4), 367-379. https://doi.org/10.1177/1082013211427993

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