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Antioxidative, Antimicrobial Activities and Fatty Acid Compositions of Four Agarics

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ABSTRACT

The edible mushrooms are valued by people because they possess a large variety of secondary metabolites with diverse beneficial effects on human health. The Agaricales order is one of the largest taxon in fungal systematics represented by a number of edible species in Türkiye. This study aimed to evaluate the antioxidant and antimicrobial activities, as well as the fatty acid composition, of four Agarics (*Agaricus bitorquis*, *Coprinopsis atramentaria*, *Coprinellus micaceus*, and *Leucoagaricus leucothites*) collected from Nigde Province, Türkiye. The antioxidant capability was determined using DPPH assay and the total phenolic content was measured by Folin-Ciocalteu's phenol reagent technique. Among the four species, *A. bitorquis* showed the highest DPPH radical scavenging activity (84.259±1.32%) and total phenolic content (1472.21±10.35µg GAE/mL). Gas chromatography-mass spectrometry (GC-MS) technique was performed for the analysis and characterization of the fatty acid compositions. Linoleic acid was the major fatty acid detected in all four species, with percentages ranging from 23.58% to 42.96%. The antimicrobial activity of the mushroom extracts was assessed using the disc diffusion method, and the ethanol extract of *C. atramentaria* showed the most significant effect on *E. coli* with a 29±0.6 mm inhibition zone diameter.

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Introduction

The kingdom Fungi is one of the largest taxa in systematics, with over 150000 species (Species Fungorum, October 2022) across diverse life forms (saprophytism, parasitism, and mutualism). It is estimated that over a million fungal species are reserved to be described (Hibbett et al., 2016; Nilsson et al., 2019).

Identification of mushroom species is highly dependent on the morphology of the sample, such as the size and shape of the cap, gills, stem, and spore structures. Moreover, the ecology of the mushroom sample reveals a lot about the species. However, the identification of mushrooms in terms of morphological features may need to be more accurate, particularly for relatively close species. Molecular techniques, including isolation, amplification, and sequencing of the conserved regions through the evolutionary period of nuclear DNA, help to identify mushroom samples more accurately by comparing the sequence data with molecular databases. In the case of identification of the mushroom species, the Internal Transcribed Spacer (ITS) region of the rDNA, which is approximately 700 bp long and a highly conserved fragment, is mainly used because of its high inter-species variation (Lücking et al., 2020; Giusti et al., 2021).

Free radicals are one or more non-paired electrons bearing unstable compounds that usually act as intracellular signals that regulate some cell functions. Reactive oxygen species (ROS) are oxygen-containing free radicals. The accumulation of ROS in cells, resulting in oxidative stress, can cause severe chronic illnesses like diabetes, Parkinson's, cardiovascular disorders, Alzheimer's, and infertility (Aytar et al., 2020; Emsen and Güven, 2020). The natural defense systems of the organisms depend on the endogenous antioxidants capable of capturing and stabilizing free radicals. In recent years, natural sources of antioxidants have been significant due to their fewer side effects than synthetic ones. Phenolic

compounds obtained from herbal sources are valuable antioxidants since they act as reducing agents, singlet oxygen scavengers, metal chelators, and hydrogen donors. Mushrooms are known to produce and accumulate various secondary metabolites with antioxidant properties, including polyketides, steroids, terpenes, polysaccharides, and polyphenols (Pizzino et al., 2017; Taviano et al., 2018; Özdal et al., 2019).

Fatty acids are organic molecules comprising carbon chains, a methyl group at one end and a carboxyl group at the other. They play a pivotal role in metabolic processes. Energy storage and transportation, insulation against mechanical, thermal, and electrical factors, signal transportation and gene regulation, and utilization as cell membrane constituents may be listed as some of the functions of fatty acids (Rustan and Drevon, 2001).

The studies on the fatty acids involved in the human diet indicate that low-density lipoprotein (LDL) cholesterol in plasma increases with saturated fatty acids, while it decreases with monounsaturated and polyunsaturated fatty acids (Mensink et al., 2003). It was also suggested by the Food and Agriculture Organization and the World Health Organization that total fat intake in dietary energy should be 20-35%, including <10% saturated fatty acids, <1% trans fatty acids, 3-13% monounsaturated fatty acids and 6-11% polyunsaturated fatty acids (Salter, 2013).

Antimicrobial resistance evolved by the microorganisms via misuse and/or overuse of antibiotics is a global concern that results in prolonged hospital stays, expensive and intensive health care, and even death from infections caused by the pathogen organisms. It has been estimated that approximately 25.000 deaths occur annually in Europe as a result of infections caused by antibiotic-resistant bacteria (Davies et al., 2013). Strains of *Pseudomonas aeruginosa, Escherichia coli, Streptococcus pneumonia, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella spp.* are the most commonly reported antibiotic resistant bacteria (MacGowan and Macnaughton, 2017). People's ability to prevent and treat bacterial infections factors is reduced by the pathogens, which recurrently acquire new resistance factors, although the flow of bacteria and genes is restricted by multiple natural barriers (Larsson and Flach, 2022). Thus, is important to identify novel antimicrobial agents capable of preventing and/or treating infections, as well as increasing society's awareness about the use of antibiotics.

Many non-toxic bioactive compounds naturally synthesized by mushrooms with anti-allergic, anti-inflammatory, anti-tumoral, antioxidant, antimicrobial, antidiabetic, and immune system boosting effects have attracted so many researchers to investigate mushroom species (Lindequist et al., 2005; Akata et al., 2019; Badalyan et al., 2019; Bal et al., 2019; Canpolat et al., 2021; Saridogan et al., 2021;) not only for describing new species but also in terms of biochemical constituents of these mushrooms (Ribeiro et al., 2009; Akata et al., 2012; Pereira et al., 2012; Günç Ergönül et al., 2013; Dogan et al., 2013; Yılmaz et al., 2013).

The objective of this study was to determine the total phenolic contents, antioxidant and antimicrobial activities, and fatty acid compositions of four Agarics collected from Niğde, Türkiye.

Materials and Methods

Materials

Fruiting bodies of the mushroom species were collected between 2019 and 2020 from Niğde, Türkiye. According to the current literature, all samples were identified due to morphological traits (Wasser, 1977; Breitenbach and Kränzlin, 1986; Redhead et al., 2001) by Prof. Dr Ilgaz AKATA, Ankara University. All samples were photographed in the locations where they were collected. Environmental conditions, altitudes, and phenological properties (size, shape, color, odor…etc.) belonging to samples were noted. Subsequently, the identified samples were subjected to freeze-drying and stored in the laboratory until further analysis.

Molecular Identification

Identification through the morphological traits of mushroom samples may need to be more accurate, particularly for relatively close species. Thus, molecular techniques based on the nuclear ribosomal ITS region, which is widely used for identifying fungal species (Schoch et al., 2012), were performed to prevent possible confusion at the species level.

Macherey-Nagel™ Nucleospin Plant II kit, was employed for the purpose of extracting DNA from the mushroom samples. ITS regions of the nuclear DNA were amplified by PCR using ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') primers developed by White et al. (1990). PCR mixture comprises 2 µL of each primer (10 pm/µL), 3 µL of the DNA sample, 25 µL of the DreamTaq master mix, and 18 µL of sterile distilled water, resulting in a final volume of 50 µL.

Following agarose gel electrophoresis sequence analyses were conducted by BM Labosis (Ankara, Türkiye) for the PCR products. The data obtained from sequence analyses were compared with the NCBI GenBank database using the BLAST to identify the mushroom samples.

Mushroom Extracts

Dried mushroom samples were weighed (10 g) and pulverized by a mortar and pestle. Powdered materials were combined with 100 mL of solvent, which was ethanol and methanol and the mixture was homogenized by WiseTis HG-15d homogenizer (DAIHAN Scientific) at 8000 rpm for 3 min. The homogenized samples were incubated for 24 h at 30 °C at 150 rpm. Subsequently, extracts were filtered via filter papers, and a rotary evaporator (HeiVap Value, Heidolph) was used to evaporate the solvents. The residues were used to prepare 200 mg/mL extracts, which were kept at +4°C for further use.

Antioxidant Activity Assay

The ethanolic extracts of the mushroom samples were tested for antioxidant activity using the DPPH scavenging technique. Dark-colored powder DPPH (2,2-diphenyl-1-picrylhydrazyl) has a maximum light absorption of 517 nm (Blois, 1958; Shimada et al., 1992). The mushroom extracts were serially diluted five times and a 0.1 mM DPPH solution was prepared with ethanol. After adding an aliquot of 100 µL extract and giving the 2.9 mL DPPH

solution a good shake, the mixture was left to incubate for 15 min at room temperature in the dark.

Following incubation, a spectrophotometer (MultiSkan GO, Thermo) was used to detect the mixture's absorbance at 517 nm. The following formula was used to evaluate the DPPH scavenging activity of the mushroom extracts;

$$\%\,SA = \frac{\text{ADPPH} - \text{ASample}}{\text{ADPPH}} \times 100$$

where %SA is the % DPPH scavenging activity of the extracts, ADPPH is the absorbance of 0,1 mM DPPH solution at 517 nm, and ASample is the absorbance of the sample at 517 nm.

Total Phenolic Compounds

Folin-Ciocalteu's reagent and previously prepared methanolic extracts were used for the determination of total phenolic components. A standard curve was established using gallic acid (GA) to determine the total phenolic contents of mushroom samples. 100 µL extract was added to 1 mL Folin–Ciocalteu's reagent and shaken. Subsequently, 1 mL of a 7.5% Na_2CO_3 solution was added to the mixture, which had been prepared five minutes previously. After that, the mixture incubated for 90 min at room temperature. The absorbance of the samples absorbance was determined at 765 nm using a spectrophotometre, and the total amount of phenolic compounds was represented in terms of µg GAE/mL extract (Singleton et al., 1999).

Antimicrobial Activity Test

The disc diffusion method was employed to ascertain the antibacterial activity of the mushroom extracts against specific microorganisms. The test microorganisms used were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* SL1344, *Pseudomonas aeruginosa* DSMZ 50071 and *Bacillus subtilis* DSMZ 1971. Luria-Bertani (LB) broth medium was used to inoculate bacterial strains, which were then incubated at 37°C for 24 h. The turbidity of the microbial cultures was adjusted to 0.5 McFarland standard.

For the disc diffusion method, 100 µL of bacterial inoculum was plated by a sterile drigalski spatula on an LB agar medium. Following a five-minute incubation period, sterile discs with a diameter of 6 mm were placed on LB agar medium. These discs included 15, 30, and 60 µL aliquots of mushroom extracts, and were incubated for 24 h at 37°C. Following incubation, the diameters of the inhibition zones around the paper discs were measured and expressed in mm. Antibiotic discs comprised of vancomycin (30µg/disc) and gentamycin (10µg/disc) were utilized as a positive control, while solvents (ethanol or methanol) and empty discs served as a negative control.

Fatty Acid Compositions

The fatty acid compositions of the mushroom samples were ascertained by GC-MS. For this purpose, dried powders of mushroom samples were extracted by hexane. 40 mL of hexane was added to 0.5 g of mushroom sample, homogenized by WiseTis HG-15d homogenizer (Daihan Scientific) for 3 min at 8000 rpm and incubated at 40 °C for 30 min in an ultrasonic bath (Sonorex, Bandelin). A rotary evaporator was used to remove the solvent after the extract was filtered through a filter paper.

The residue was re-suspended with 5 mL of hexane, and 0.2 mL of 2M KOH in methanol was added and shaken for 1 min. The mixture was incubated in the dark for 6 min. Then, 0.45 mL of 1N HCl was added to the mixture and incubated for an additional 20-30 min in the dark for the phase separation. After phase separation, the upper layer was dried with anhydrous Na_2SO_4, filtered through a syringe filter with a pore diameter of 0.45 µm, and injected into the GC-MS (Canpolat and İşlek, 2022).

Shimadzu QP2010-Ultra GC-MS system equipped with Restek Rxi-5MS column (30 m * 0.25 mmID * 0.25 µm df) was used to determine the fatty acid compositions of the mushroom samples. The analysis conditions were as follows: The injection port temperature was 240 °C. One µL of the sample was injected into GC with a split ratio of 1:40. Injections were performed by AOC2.0i autosampler. Helium was used as the carrier gas at a constant flow rate of 1.78 mL/min. The initial oven temperature was set to 40 °C and held for 1 min, increased to 160 °C with a rate of 5 °C/min and held for 3 min, and increased again to 250 °C with a rate of 5 °C/min and held for 11 min. The interface and the ion source temperatures were 270 and 200 °C, respectively. All mass spectra were acquired in Electron Impact (EI) mode. The mass ranged between 40 to 650 *m/z* with a scan rate of 5 scans/sec in full scan mode. Ionization was maintained for the first 5 min to avoid solvent overload (Ribeiro et al., 2009). The compounds were identified by comparing the mass spectra with those from the Wiley mass spectra library (W9N11) and Flavour and Fragrance Natural and Synthetic Compounds library (FFNSC 1.2).

Results and Discussion

Identification of Mushroom Samples

The mushroom samples investigated in this study were identified both in terms of morphological characteristics based on the available literature (Wasser, 1977; Breitenbach and Kränzlin, 1986; Redhead et al., 2001). The mushroom samples were identified due to BLAST results that indicate the interspecific variations occured in the sequence data. GenBank is the most widely attended database by researchers who want to compare molecular data with previous studies because it is online and easy to access.

In this study, the investigated mushroom species were identified as *Agaricus bitorquis* (Quél.) Sacc. (1887) (Accession number PQ164268), *Coprinopsis atramentaria* (Bull.) Redhead Vilgalys & Moncalvo (2001) (Accession number PQ164269), *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson (2001) (Accesion number PQ164275), *Leucoagaricus leucothites* (Vittad.) Wasser (1977) (Accession number PQ164271) , which had similarity rates of 99.93; 99.94; 100 and 100 %, respectively. (Photograph 1).

Total Phenolic Compounds

The total phenolic content amounts of mushrooms extracted with methanol are shown in Figure 1. The range of total phenolic content was extensive, from 328.42±3.45 to 1472.21±10.35 µg GAE/mL. When the total phenolic substance content results were examined, it was determined that the mushroom species with the highest

range was *A. bitorquis*. In contrast, the lowest total phenolic substance content was found in *C. atramentaria*.

While Heleno et al. (2012) found the total phenolic compounds in *C. atramentaria* to be 33.58 mg GAE/g our study revealed that the total phenolic compounds in *C. atramentaria* were 328.42 µg GAE/mL (Figure 1). In another study, researchers found that *C. atramentaria* had 449.93 mg GAE/g of total phenolic compounds (Kaya et al., 2021). In our investigation, the total phenolic compounds in the *A. bitorquis* species were determined to be 1472.21 µg GAE/mL, whereas Gasecka et al. (2018) stated the total phenolic compounds in *A. bitorquis* as 654.7±25.5 mg GAE/100 g DW. Selem et al. (2021) determined the total phenolic content of *C. micaceus* as 22.10 mg GAE kg^{-1} FW in the stem and 26.22 mg GAE kg^{-1} FW in the cap. Our study determined the total amount of phenolic compounds as 462.56 µg GAE/mL in the whole mushroom.

In our study, total phenolic compound measurements are consistent with some of the research described above but not others. The variety in the physicochemical characteristics of the fungi, such as sugar structure, molar weights, and polysaccharides in their structure, is assumed to be the cause of the discrepancy in the results obtained from the extracts.

Antioxidant Activity

The scavenging activity of free radicals was evaluated using the DPPH assay, and the results are presented in Figure 2. Among the tested species, *A. bitorquis* (84.259±1.32%) showed the highest DPPH scavenging activity; on the other hand, *C. micaceus* (35.351±0.37%) showed the lowest DPPH scavenging activity. The increased amount of phenolic compounds in *A. bitorquis* mushroom is thought to be related to its antioxidant activity. Phenolic compounds act as hydrogen donors and effective antioxidants. The correlation between mushroom phenolics and antioxidant activity is well documented (Tsai et al., 2007; Kim et al., 2008; Gan et al., 2013; Düşgün et al., 2021). A significant positive correlation was observed between antioxidant activity, as indicated by the percentage inhibition of the DPPH radical, and total phenolics. This suggests that phenolic compounds act as scavengers of free radicals, potentially providing an antioxidative function. Several studies on various mushroom species have demonstrated a strong correlation between scavenging activity and total phenolics (Kim et al., 2008; Heleno et al.,2015). Researchers proposed that the antioxidant capabilities of phenolics may also be assigned to their ability to donate hydrogen and eliminate DPPH (Dubost et al., 2007; Gąsecka et al.,2018).

It is known that the different components responsible for antioxidant activity differ according to mushroom species. The concentration of functional groups in mushrooms can also be affected by their developmental status (Kaya et al.,2021). The meteorological conditions in which the mushrooms grow, the conditions of the extraction, and the variations in drying procedures are assumed to be the causes of the discrepancies between the obtained data and literature data. The ethanolic extract may possess compounds other than phenolics that contribute to its antioxidant properties, which can enhance the effects of phenolics. This may be due to the Folin-Ciocalteu assay, which potentially yields elevated results due to ascorbic acid, carbohydrates, and specific amino acids such as tryptophan or tyrosine (Palacios et al., 2011).

Antimicrobial Activity

The incidence of diseases caused by microorganisms has considerably increased in recent years (Mancuso et al., 2021). Due to the increase in resistant microorganisms and the side effects of antibiotics used against microorganisms, studies have been directed toward the discovery of novel natural products. In this context, researchers are trying to identify antimicrobial agents, especially by investigating novel natural products. (Sevindik and Bal, 2021; Sarıdoğan et al., 2021).

Photograph 1: Mushroom samples used in this study: *Agaricus bitorquis* (A), *Coprinopsis atramentaria* (B), *Coprinellus micaceus* (C) and *Leucoagaricus leucothites* (D)

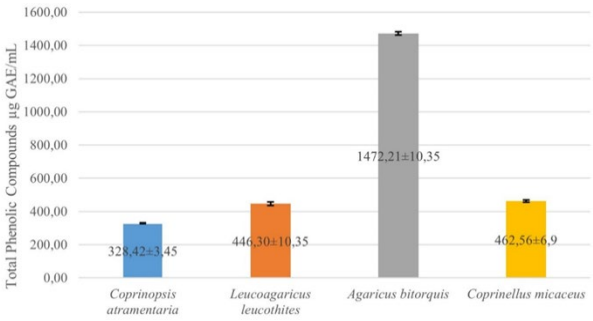

Figure 1. The content of total phenolic compounds in mushroom samples

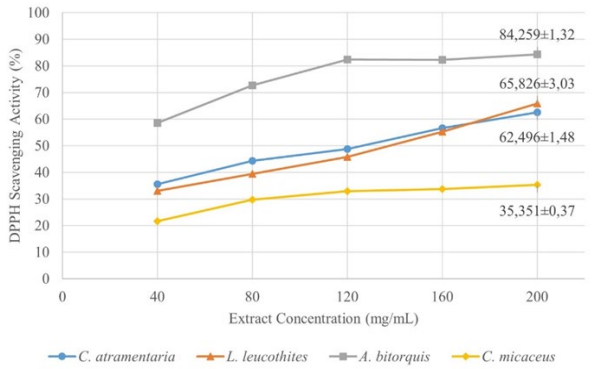

Figure 2. DPPH scavenging Activity (%) of Mushroom Species

Table-1. Antimicrobial activity of ethanolic extracts against test microorganisms

Test Microorganisms	CN	VA	Inhibition Zone Diameters (mm)											
			A. bitorquis			*C. atramentaria*			*C. micaceus*			*L. leucothites*		
			15	30	60	15	30	60	15	30	60	15	30	60
P. aeruginosa	20	10	14±0.6	19±0	26±1.2	13±0.6	19±1.2	26±2.3	12±0	19±0	25±0.6	11±1.2	15±1.5	21±1.2
B. subtilis	21	8	11±2.1	13±.0.2	16±1.0	nd	nd	15±0.6	nd	nd	12±0.6	nd	nd	15±0.6
S. aureus	21	8	13±1.5	18±0	22±1.2	13±0.6	17±1.2	23±1.5	13±0.6	14±1.5	22±2.1	11±1.0	15±1.0	23±0.6
E. coli	20	12	14±0	20±0.6	28±1.5	13±1.5	19±1.0	29±0.6	12±1.2	17±0.6	24±0.6	11±1.2	16±0.6	23±1.5
S. typhimurium	22	17	15±1.2	20±1.2	25±0.6	13±1.2	19±1.5	24±1.0	13±0.6	15±1.2	16±0.6	11±0	14±0.6	22±1.2

* CN: Gentamycin (10µg/disc), VA: Vancomycin (30µg/disc), nd: not detected

Table-2. Antimicrobial activity of methanolic extracts against test microorganisms.

Test Microorganisms	CN	VA	*Inhibition Zone Diameters (mm)*											
			A. bitorquis			*C. atramentaria*			*C. micaceus*			*L. leucothites*		
			15	30	60	15	30	60	15	30	60	15	30	60
P. aeruginosa	20	10	9.7±1.2	15±0.6	18±0.6	10±1.2	13±0.6	17±0.6	11±0.6	14±0.6	19±1.2	11±1.2	14±0.6	18±0.6
B. subtilis	21	8	11±0	12±1.2	18±0	9.7±0.6	13±0	17±1.0	7.7±0.6	9±0	15±0.6	7±0	12±0	14±1.2
S. aureus	21	8	10±0.6	15±0.6	18±0.6	10±1	13±1.0	20±0.6	11±0	15±0	21±0.6	9.3±0.6	14±0	19±1.5
E. coli	20	12	10±12	14±1.5	17±0.6	11±0.6	13±0.6	17±0	11±0.6	15±0.6	20±0.6	9.3±0.6	12±0.6	18±0.6
S. typhimurium	22	17	8.7±0.6	12±0	17±0.6	9.7±0.6	14±1.7	20±1.5	10±1.0	14±0.6	18±1.2	9.7±0.6	14±1.2	17±0.6

* CN: Gentamycin (10µg/disc), VA: Vancomycin (30µg/disc), nd: not detected

In this study, the antimicrobial effects of ethanol and methanol extracts of *A. bitorquis*, *C. atramentaria, C. micaceus,* and *L. leucothites* against various test microorganisms were investigated using the disc diffusion method. Blank and solvent bearing sterile discs used as negative control did not show any activity.

The results obtained from disc diffusion test demonstrated that the largest inhibition zone was formed by ethanolic extract of *C. atramentaria* (60 µL) against *E. coli* with 29±0.6 mm while ethanolic extract of *A. bitorquis* (15 µL) exhibited the lowest inhibition effect against *B. subtilis* with 11±2.1 mm (Table 1). On the other hand methanolic extract of *C. micaceus* (60 µL) showed the highest inhibition against *S. aureus* with 21±0.6 mm while the lowest inhibition was exhibited by *L. lecucothites* methanol extract (15 µL) with 7±0 mm against *B. subtilis* (Table 2). The results also indicated that both ethanol and methanol extracts of all mushroom samples with different volumes demonstrated antimicrobial activities against test microorganisms at varying levels.

In their 2020 study, Canlı et al. observed a significant positive correlation between the antimicrobial efficacy of *L. leucothites* extracts and the volume of extract utilized. With inhibitory zones of 7 mm, 50 µL ethanol extract of *Leucothites* exhibited antibacterial activity against most of the test microorganisms, including *P. aeruginosa* and *E. coli.* With inhibition zones of either 7 or 10 mm, 70 µL ethanol extract of *L. leucothites* shown antibacterial efficacy against pathogen microorganisms. The 180 µL ethanol extract, equivalent to 58.50 mg/mL extract, showed the highest activity against *S. infantis* (10 mm inhibition zone) and *E. coli* (11 mm inhibition zone). It can be postulated that an increase in the volume of the extracted material may result in an enhancement of the observed activity. Purifying the active compound and utilizing it against microorganisms would demonstrate better activities. In this context, our results are comparable to those of previous studies which indicated the antimicrobial activity of *L. leucothites*. It has been reported that the antimicrobial activity levels of fungal organisms are due to secondary metabolism products (Calvo et al., 2002). So, additional experiments are required for a better understanding of the mode of action of active substances in detail.

In another study, the antimicrobial activity of extracts prepared from three different fungi using three different solvents was tested against different pathogenic species. Gram-positive bacteria (*S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212), Gram-negative bacteria (*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922), and one fungal species (*C. albicans* ATCC 10231) were used as test microorganisms. Ethanol extracts of *G. lucidum* and *C. micaceus* were more effective than the extracts prepared with other solvents. Although the antimicrobial activity of ethanol and chloroform extracts against the test microorganisms was determined at different levels using both disc diffusion and agar-well diffusion methods, it was much lower in distilled water extracts of *L. edodes* (Avci et al., 2014). It was also stated that *C. micaceus* ethanol extract showed minimum inhibition against *E. faecalis* ATCC 29212 with 6.0 mm zone diameter while highest inhibition was demonstrated against *P. aeruginosa* ATCC 27853 with a zone diameter of 11.0 mm.

In a study in which nutritional values, hydrophilic and lipophilic compound contents, and biological activities of four different *Agaricus* sp. (*Agaricus bitorquis*, *A. bisporus*, *A. macrosporus* and *A. campestris*) were evaluated, ethanolic extracts of all investigated fungal samples exhibited higher antibacterial activity than methanolic ones, except for *L. monocytogenes*. The best antibacterial effect was obtained with *A. macrosporus* extracts against all bacteria, except *L. monocytogenes*, while *A. bitorquis* extracts showed the strongest effect against this bacterium. The antibacterial activity of the extracts was found to be lower than the antibiotics used as control (Glamočlija et al., 2015).

Fatty Acid Compositions

Although mushrooms are known to contain low amounts of fats, having high essential fatty acids and high protein, vitamin, and mineral content, they are regarded as healthy food sources for the human diet.

In this context, the fatty acid composition of the four mushroom samples was investigated using GC-MS. The majority of the fatty acids in the mushroom samples were found to be unsaturated, as shown in Table 3. Linoleic acid was one of the major unsaturated fatty acids, with 42.96; 39.07; 28.98 and 23.58% in *A. bitorquis, L. leucothites, C. micaceus* and *C. atramentaria,* respectively. Another major unsaturated fatty acid was oleic acid, with 34.65; 6.82; 6.37 and 3.65% in *C. atramentaria, C. micaceus, L. leucothites* and *A. bitorquis*, respectively. The beneficial effects of unsaturated fatty acids in the human diet are well documented (Barcelli et al., 1986; Kang and Leaf, 1996; López-Miranda et al., 2006; Yang et al., 2022).

Palmitic acid was one of the primary saturated fatty acids found in the mushroom samples, with 11.54; 11.13; 10.13; and 9.97% in *C. atramentaria*, *A. bitorquis*, *C. micaceus* and *L. leucothites*, respectively. Another saturated fatty acid was stearic acid, with 39.51; 31.26; 16.19 and 16.93% of the fatty acid found in *C. micaceus, L. leucothites, A. bitorquis* and *C. atramentaria*, respectively. Lauric, myristic, and palmitic fatty acids are considered to be responsible for the increase in plasma LDL cholesterol (Williams, 2000), while it was reported that stearic acid did not affect the increase of the total cholesterol or LDL cholesterol concentrations (Bonanome and Grundy, 1988).

Table-3. Fatty acid composition of mushroom species.

No	Compound	RT(min)	MW+CF	*A. bitorquis*		*C. atramentaria*		*C. micaceus*		*L. leucothites*	
				Con.	SI	Con.	SI	Con.	SI	Con.	SI
1	Phthalic acid, diethyl ester	26.796	222 (C12H14O4)	5.25	98	0.77	98	2.11	97	1.46	97
2	Myristic acid, methyl ester	30.927	242 (C15H30O2)	0.38	89	0.15	88	nd		0.32	87
3	Pentadecanoic acid, methyl ester	33.724	256 (C16H32O2)	0.37	96	0.37	97	nd		0.31	94
4	Palmitoleic acid, methyl ester	35.725	268 (C17H32O2)	nd		0.33	95	0.41	91	0.25	91
5	Palmitic acid methyl ester	36.238	270 (C17H34O2)	11.13	98	11.54	98	10.13	98	9.97	98
6	3,5-Bis(1,1-dimethylethyl)-4-hydroxy-benzenepropanoic acid methyl ester	36.677	292 (C18H28O3)	1.86	86	0.77	86	2.5	85	2.53	85
7	Margaric acid, methyl ester	38.513	284 (C18H36O2)	1.25	96	nd		nd		nd	
8	Linoleic acid, methyl ester	39.968	294 (C19H34O2)	42.96	96	23.58	97	28.98	97	39.07	97
9	Oleic acid, methyl ester	40.084	296 (C19H36O2)	3.65	97	34.65	96	6.82	96	6.37	96
10	11-Octadecenoic acid, methyl ester	40.2	296 (C19H36O2)	0.59	93	2.58	97	0.54	90	0.39	89
11	Stearic acid, methyl ester	40.62	298 (C19H38O2)	26.19	96	16.93	96	39.51	96	31.26	96
12	Linoleic acid, ethyl ester	41.314	308 (C20H36O2)	0.52	94	0.47	93	0.43	87	1.4	94
13	Oleic acid, ethyl ester	41.428	320 (C20H38O2)	nd		0.45	93	nd		nd	
14	Stearic acid, ethyl ester	41.938	312 (C20H40O2)	0.26	92	nd		nd		nd	
15	Hexadecadienoic acid, methyl ester	43.449	266 (C17H30O2)	nd		0.45	85	nd		nd	
16	Arachidic acid methyl ester	44.419	326 (C21H42O2)	1.16	95	0.63	84	0.81	91	0.5	93
17	Linolenic acid, methyl ester	44.66	292 (C19H32O2)	nd		0.23	80	nd		nd	
18	Behenic acid, methyl ester	48.083	354 (C23H46O2)	0.42	94	0.19	94	0.3	87	nd	

RT: Retention Time, MW: Molecular weight, CF: Chemical formula, Con.: Concentration (%), SI.: Similarity Index (%), nd: not detected

Conclusion

In this study molecular identification, amount of total phenolic compounds, antioxidant and antimicrobial activities, and fatty acid composition of four Agaric mushroom species were investigated. Among these samples, A. bitorquis exhibited significant antioxidant and antimicrobial activity. GC-MS analyses also showed that this mushroom was mostly rich in unsaturated fatty acids regarded as healthy components of the human diet. The findings of this study suggest that this mushroom may be considered as a valuable natural food source that should be widely promoted. Additional research into the chemical properties of the oxidative components and phytochemicals in the extracts is recommended for developing mushrooms in the industry, given the documented antioxidant activity of the mushroom extracts.

Declarations

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