Cytotoxic, Antioxidant, Antibiofilm, and Antimicrobial Activities of Mushroom Species from Turkey

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ABSTRACT: Mushrooms, which have been collected to meet the nutritional needs of the world for many years, have gained medical importance thanks to the bioactive compounds they produce. Thanks to studies carried out to determine mushroom diversity, the number of species identified is increasing year by year. Accordingly, in recent years, studies conducted to determine the biological activities of metabolites produced by fungi have been increasing. The present study was conducted to determine the cytotoxic, antioxidant, antibiofilm and antimicrobial activities of the seven different mushroom species (*Craterellus cornucopioides*, *Hymenopellis radicata*, *Lepista nuda*, *Pisolithus arhizus*, *Ramaria flava*, *Schizophyllum commune*, and *Tricholoma ustale*) collected from Tokat and Yozgat regions located in northern and central Turkey. Laboratory studies have demonstrated that mushrooms used in this study have different degrees of antibiofilm, antimicrobial, antioxidant and cytotoxic activities. At the end of the study, it is determined that *C. cornucopioides* and *L. nuda* species have the highest antimicrobial activity. In addition, mushroom species have biofilm inhibitory effects on indicator microorganisms at varying degrees ranging between 20.7 and 96.3%. As a result of antioxidant activity studies, it was determined that *T. ustale* has the highest free radical scavenging effect and *P. arhizus*, which has the highest polyphenol content, has the highest reducing power. Finally, it is determined that, among the mushrooms used in the present study, *H. radicata* showed higher selectivity on the MDA-MB-231 breast cancer cell line than on the normal cell line tested, while *C. cornucopioides* showed higher selectivity on the MCF-7 breast cancer cell line.

KEY WORDS: *Craterellus cornucopioides*, *Hymenopellis radicata*, *Lepista nuda*, *Pisolithus arhizus*, *Ramaria flava*, *Schizophyllum commune*, *Tricholoma ustale*, medicinal mushrooms, antimicrobial activity, antioxidant activity, cytotoxic activity, antibiofilm activity

ABBREVIATIONS: BIC, biofilm inhibition concentration; **CLSI**, Clinical and Laboratory Standards Institute; **DMEM**, Dulbecco's modified Eagle's medium; **DPPH**, 2,2-difenil-1-pikrilhidrazil; **ELISA**, enzyme-linked immunosorbent assay; **FAME**, fatty acid methyl ester; **FBS**, fetal bovine serum; **FRAP**, ferric reducing antioxidant power; **GAE**, gallic acid equivalent; **GC**, gas chromatography; **GC-FID**, gas chromatography flame ionization detector; **MHB**, Mueller Hinton broth; **MIC**, minimum inhibitory concentration; **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **OD**, optical density; **PBS**, phosphate-buffered saline; **RSA**, free radical scavenging activity; **TE**, trolox equivalent; **TSB**, tryptic soy broth

I. INTRODUCTION

Fungi are among the most diverse organisms in the world. Although 120,000 fungi species have been reported worldwide so far, given the few studied habitats, unidentified species in the tropics, and materials to be studied in collections, the number of fungi species is estimated to range between 2.2 and 3.8 million.[1,2]

Mushrooms belonging to Ascomycota and Basidiomycota that naturally grow in forest and grassland areas have attracted the public's interest greatly for many years throughout the world (especially in Asian regions) due to not only they're being a natural food source but also their medicinal properties and economic potential. Edible mushrooms are considered a healthy food source due to their high mineral, protein, fiber, unsaturated fatty acids and vitamin content, and low fat and calorie levels. They are an important option especially for people who eat vegetarian diet or want to eat a diet rich in protein. Many mushrooms contain

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high quality protein, essential amino acids, organic compounds such as thiamine, riboflavin, ascorbic acid, ergosterol, niacin, and mineral nutrients such as phosphorus and iron. Medicinal mushrooms have been used in traditional medicine for the treatment of various diseases from past to present due to their important therapeutic properties. In several studies, it has been shown that various compounds produced by naturally grown mushrooms and exhibiting biological activity have many pharmacological and medical effects such as antioxidant, anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, immunomodulatory, antidiabetic, antioxidant, anti-allergic and prebiotic properties. In recent years, mushrooms have also been used as food supplements called nutraceuticals and mycotherapy products.[3–8]

Antitumor properties of fungi are the leading medicinal effect of mushrooms and their metabolites attracting public attention. Various factors such as the relatively expensive cost of cancer treatment and its undesirable pharmacological effects encourage many scientists to seek natural products with anticancer activity. Many successful anti-cancer medications used today are natural products or their analogues. Experimental studies have shown that many natural products derived from mushrooms have anticancer potential in various bioassay systems and animal models.[9–13] Various mushroom-derived compounds are now increasingly used in Japan, Korea, and China as adjuvants to standard radiotherapy and chemotherapy. The most encouraging effect is that these mushroom-derived compounds significantly reduce the side effects of these treatments when they are administered before or during radiotherapy or chemotherapy.[14]

An important feature of mushroom species that has attracted the most attention and has been researched by scientists in recent years is that they contain antioxidant substances. Although most of the antioxidant compounds are produced in the body by various endogenous systems, studies have revealed the importance of exogenous antioxidant intake, especially with diet, in the fight against oxidative stress. The most common antioxidant compounds in foods are polyphenol derivatives. In addition, many foods are good sources of other non-enzymatic antioxidants. Studies have shown that bioactive substances produced by mushroom exhibit antioxidant activities.[15–17]

In some studies, conducted in recent years, mushrooms have been shown to have the potential to produce various compounds with antibiofilm activity.[18–20] Biofilms, which are 10 to 1000 times more resistant to antibiotics and cause serious clinical problems especially due to their potential to evade the host immune systems, are a major threat to humans and are the cause of 80% of bacterial infections. Biofilms lead to food spoilage in the food industry and cause persistent infections. The resistance of bacteria embedded in a biofilm to antimicrobial agents makes it vital to search for new agents able to kill these bacteria effectively.[21–26]

In the present study, we aimed to determine the biological activity of seven different mushroom species collected from different localities in Tokat and Yozgat. For this purpose; cytotoxic, antioxidant, antibiofilm and antimicrobial activities of samples were determined, and to contribute to studies to be conducted in this field. The mushroom species used in the study were chosen mainly because they are studied less in areas such as biofilms or cytotoxic activity.

II. MATERIALS AND METHODS

A. Mushroom Samples and Extraction Procedure

The mushroom samples used in our study were collected during routine field trips in different localities in the provinces of Tokat and Yozgat, especially during rainy seasons. Color photographs of the species were taken in their natural environments, and their morphological and ecological characteristics were noted down. Spore prints were taken from the fresh samples brought to the laboratory and they were dried at about 50°C with the help of an electric heater. After drying procedure, each sample assigned a collection number were given and samples transferred in polyethylene bags for further study (in other words samples were converted to fungarium material). Then, the microscopic properties of the dry samples were determined

using various chemicals and light microscopy. Identification of the samples whose macroscopic and microscopic features were revealed was carried out with the help of the existing literature.[27–34] Some characteristics of mushroom species are given in Table 1.

Samples whose were identified were pulverized with the help of a crusher for extraction. Soxhlet extraction method was preferred for the preparation of mushroom extracts, and absolute ethyl alcohol (400 mL) was used as solvent. After the extraction process was carried out for 24 hours, the alcohol was removed with the help of an evaporator and the substances obtained were completely dried in sterile Petri dishes. Then, the main stock was prepared by dissolving 0.2 g of the extracts obtained in 10 mL of ethanol. The prepared main stocks were sterilized with the aid of a 0.22-µm-diameter syringe filter. The main stock prepared until the analysis was performed was stored at –20°C, and the remaining substances were stored at 4°C.[35]

B. Determination of Antimicrobial Activity

Antimicrobial activity of fungi extracts was determined as *in vitro* using the micro-broth dilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Two Gram-positive (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213), two Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) indicator bacteria and one yeast (*Candida albicans* ATCC 10231) were used as indicator microorganisms in the present study. Indicator microorganisms were grown in the 10 mL of Mueller Hinton broth (MHB) for 16–24 hours. To determine the activity, serial dilutions of the mushroom extracts whose concentration ranged between 1000 and 31.25 µg/mL were prepared in MHB and 100 µL of the prepared dilutions were transferred to sterile enzyme-linked immunosorbent assay (ELISA) plates. Then, the densities of the indicator microorganisms were adjusted according to McFarland No. 0.5, and after they were diluted by 1/100, 100 µL was taken and added to the wells. Plates were incubated at 37°C for 24 h for indicator bacteria and 48 h at 30°C for indicator yeast. After their incubation period was completed, the absorbance values of the plates were read at 600 nm, and the well where the minimum inhibitory concentration (MIC) was observed, and the MIC values were determined. The analysis was repeated three times. In the study, 200 µL of indicator microorganism solution was used as negative control, and prepared dilutions of an antibiotic (ciprofloxacin and fluconazole) whose efficacy is known were used as positive control.[36,37]

C. Determination of Antibiofilm Activity

Antibiofilm activities of fungi extracts were determined by modifying the method which was first proposed by Christensen.[38] *C. albicans* ATCC 10231, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213 were used as indicator microorganisms. After the microorganisms

TABLE 1: Habitats of mushroom species

Mushroom species	Edibility	Fungarium no.	Localities/habitat
C. cornucopioides	Edible	ISIK 825	Tokat/oak forest, among the leaf litter
H. radicata	Inedible	ISIK 793	Tokat/beech forest, under a beech tree, among the leaf litter
L. nuda	Edible	ISIK 169	Yozgat/mixed forest, among the debris on the ground
P. arhizus	Inedible	ISIK 796	Tokat/roadside on the ground
R. flava	Edible	ISIK 170	Yozgat/pine forest among pine needles
Sch. commune	Inedible	ISIK 439	Yozgat/on the oak stump
T. ustale	Inedible	ISIK 812	Tokat/mixed forest, under leaf litter

were activated in MHB, they were transferred to tryptic soy broth (TSB) containing 2% glucose, and incubated at 37°C overnight. At the end of the incubation period, after the densities of the microorganisms were adjusted according to McFarland No. 0.5, 100 µL was taken, and 100 µL of extract prepared in the same medium was added to the wells containing (6 different concentrations in the range of 500–15.62 µg/mL). The plates were then incubated at 37°C for 24 hours. After the incubation period, the medium was poured, and the plates were washed three times with sterile phosphate-buffered saline (PBS) solution to remove planktonic cells and left to dry for 2 hours at room temperature. After drying, the plates were stained with 200 µL of 1% crystal violet solution for 15 min and then washed again with PBS solution three times to remove the dye that was not attached to the biofilm. Finally, methanol:acetone (80:20) solution was added to the wells and crystal violet was dissolved and optical density (OD) measurements were performed at 595 nm with the help of the ELISA reader. MHB containing 0.25% glucose was used as a positive control and 200 µL of pathogenic microorganism was used as a negative control. The process was repeated three times and the average values were taken as basis. Results were calculated as percentage biofilm inhibition concentration (BIC):

$$[(\text{OD negative control} - \text{OD experimental group})/\text{OD negative control}] \times 100.$$

D. Estimation of Antioxidant Activity

1. 2,2-Difenil-1-Pikrilhidrazil (DPPH) Radical Scavenging Activity

The free radical scavenging effect of mushroom extracts was determined by modifying the method proposed by Blois.[39] For analysis, 100 µL of 0.1 mM DPPH solution prepared freshly in ethanol and the 100 µL of mushroom extract at various concentrations (in the concentration range of 7.81 µg–1000 µg/mL) were mixed. After the prepared mixtures were kept in the dark at room temperature for 50 min, absorbance reading was performed at 517 nm wavelength. The process was repeated three times and the average values were taken as basis. Serial dilutions (200–1.562 µM) for ascorbic acid were prepared in distilled water for positive control and the process was repeated as described above. Two hundred µL of DPPH solution was used as negative control. The free radical scavenging activity (RSA) of the extracts was calculated with the following formula:

$$[(\text{OD negative control} - \text{OD experimental group})/\text{OD negative control}] \times 100.$$

2. Total Phenolic Content

Total phenolic contents of mushroom extracts were determined by modifying the Folin-Ciocalteu method suggested by Singleton and Rossi.[40] To analysis, 12.5 µL of mushroom extract at a concentration of 1 mg/mL and 623.5 µL of 1:10 Folin-Ciocalteu reagent and 125 µL of 20% sodium carbonate solution were mixed. The resulting mixture was incubated in the dark at room temperature for 30 min and absorbance was read at 700-nm wavelength. The process was repeated three times and the average values were taken as basis.

In the study gallic acid was used as a positive control. Gallic acid standard graph was prepared to determine the total amount of phenolic substance in the extracts as equivalent to gallic acid [as mg gallic acid

equivalents (GAEs)/100 g] by means of the absorbances obtained. In this process, the main stock of 10000 µg/mL gallic acid was obtained by dissolving 10 mg of gallic acid with 1 mL of distilled water. By taking samples from the main stock, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 µg/mL serial dilutions were prepared in distilled water, and the gallic acid standard graph calibration curve was created by repeating the process as described above.

3. Ferric Reducing Antioxidant Power (FRAP) Analysis

Iron (Fe III) reducing capacities of mushroom extracts were determined by modifying the Benzie and Strain method.[41] Twenty microliters of mushroom extracts at a concentration of 1 mg/mL were taken and the reaction was started by adding 50 µL of 0.2 M phosphate buffer and 50 µL of 1% $K_3Fe(CN)_6$ solution. After the mixture was incubated at 50°C for 20 min, 50 µL of sample was taken and transferred to a new ELIZA plate. Then, after 50 µL of 10% TCA solution, 50 µL of distilled water and finally 10 µL of 0.1% $FeCl_3$ solution were added to the mixture, it was incubated at room temperature for 5 min in the dark and absorbance was read at 700 nm wavelength. The process was repeated three times and the average values were taken as basis.

In the study, trolox was used as a positive control. The trolox standard graph was prepared to determine the obtained absorbances as equivalent to trolox [as micrograms of trolox equivalents (TEs)/100 g]. In this process, 10000 µg/mL trolox master stock was obtained by dissolving 0.001 g trolox with 1 mL of distilled water. By taking samples from the main stock, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 µg/mL serial dilutions were prepared in distilled water, and the calibration curve of the trolox standard graph was created by repeating the process as described above.

E. Determination of Cytotoxic Activity

The cytotoxic effects of mushroom extracts were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method proposed by Mosmann.[42] The following three different cell lines were used: MCF-7, MDA-MB-231, and L929. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin in a humidified incubator at 37°C in 5% CO_2. To analysis, after 1×10^5 cells were added to each well on 96 ELISA plates, serial dilutions of mushroom extracts whose concentration ranged between 100 µg/mL and 1.56 µg/mL were added. MTT solution (5 mg/mL PBS) was then added to each well and the plates were incubated at 37°C for 4 hours. After the incubation period was completed, dimethyl sulfoxide was added to the cells and formazan crystals were formed by incubating them again for 2 hours. At the end of the incubation period, absorbance values were determined at 545 nm wavelength with the ELISA reader. In the study cisplatinum was used as a positive control. The process was repeated three times for each cell line and the average values were taken as basis. Selectivity index ratios for MCF-7 and MDA-MB-231 breast cancer cell lines were calculated using the following formula: selectivity index = IC_{50} of healthy cell line/IC_{50} of cancer cell line, where IC_{50} is the half-maximal inhibitory concentration.

F. Fatty Acid Analysis

To prepare the fatty acid methyl esters (FAMEs) found in the mushroom samples, 35 mL of isopropanol hexane (3:2) solution were added to 5 g of powdered dried fungi samples and vortex-mixed for 5 min. Then the samples were kept in an ultrasonic water bath for 30 min. Then the samples were centrifuged at 1000 rpm for 5 min and the supernatant obtained was separated by filtration with filter paper. The solvent in the supernatant was removed with the evaporator and the resulting fat was dissolved in 5 mL of hexane and

transferred to a clean tube. Then, 1 mL of 2N KOH solution in methanol was added, the mixture was kept in the dark for 2 hours and at the end of the period the hexane phase was taken into the gas chromatography (GC) vial to be used in analysis.[43] Analysis was performed with the gas chromatography flame ionization detector (GC-FID) system (Shimadzu, QP2010). DB FastFame (30 m × 0.25 mm ID, 0.25 µm film thickness) capillary column was used for the analysis. The injection port was operated in split mode (split ratio: 50), at 250°C. Nitrogen was used as the carrier gas (at a flow rate of 3 mL/min). Total analysis time was 20 min. The temperature of the oven was initially kept at 50°C for 30 seconds. Then the temperature was raised to 194°C by increasing the temperature 30°C per minute, and the mixture was kept at this temperature for 3.5 min. Finally, the temperature was raised to 240°C by increasing the temperature 5°C per minute and the mixture was kept at this temperature for 2 min.[44]

III. RESULTS AND DISCUSSION

A. Antimicrobial Activity

The antimicrobial activities of mushroom species were investigated using the micro-broth dilution method and the results are given in Table 2 in detail. The results show that all of the mushroom species exhibited the lowest antimicrobial activity against *E. coli* and *P. aeruginosa* with an MIC value of 500 µg/mL. A general evaluation indicates that the antimicrobial activity against *E. faecalis* and *S. aureus* is stronger, although the MIC values vary according to the types of mushrooms used. In addition, it was determined that the highest antimicrobial activity was exhibited by *Craterellus cornucopioides* and *Lepista nuda* species against *S. aureus* with a MIC value of 62.5 µg/mL.

The fact that methods and solvents used in studies in which the antimicrobial activity of various mushroom species is determined are different may lead to different results for the same species. However, the analysis of the available data in the literature revealed that some results reported by various researchers are consistent with the results of our study. Dimitrijevic et al.[45] worked on the extracts obtained from the mushroom species used in our study and found low activity on *E. coli*, *P. aeruginosa*, and *E. faecalis*, and

TABLE 2: Minimum inhibitory concentrations (MIC, µg/mL) of mushroom species against indicator microorganisms

Mushroom species	Indicator microorganism				
	C. albicans **ATCC 10231**	***E. faecalis*** **ATCC 29212**	***E. coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**	***S. aureus*** **ATCC 29213**
C. cornucopioides	500	125	500	500	62.5
H. radicata	125	250	500	500	125
L. nuda	500	125	500	500	62.5
P. arhizus	500	250	500	500	250
R. flava	500	125	500	500	125
Sch. commune	125	250	500	500	125
T. ustale	125	250	500	500	125
Positive control					
Ciprofloxacin		< 1.9	< 1.9	< 1.9	< 1.9
Fluconazole	< 1.9				

Assay was performed three times.

strong activity on *S. aureus*. Kosanic et al.[46] reported that *C. cornucopioides* extracts had strong activity on *S. aureus* and *E. coli* and low activity on *C. albicans*. In our study, we observed that *C. cornucopioides*, *L. nuda*, *Pisolithus arhizus*, and *Ramaria flava* extracts had a low effect on *C. albicans*. Barros et al.[47] determined that the extracts obtained from *L. nuda* basidiocarps showed a strong effect on *S. aureus*, but not on *E. coli* and *C. albicans*. Similarly, Alves et al.[48] determined that *L. nuda* samples did not have an inhibitory effect on the growth of *E. coli*. Yamaç and Bilgili[49] reported that the extracts they obtained from the mycelial culture of *L. nuda* were effective on *S. aureus*, but not on *E. coli* and *C. albicans*. Khardziani et al.[50] reported that *Schizophyllum commune* has antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*.

B. Antibiofilm Activities

We determined the antibiofilm activities of mushroom species used in our study by using five different indicator microorganisms with known biofilm forming ability, and provide the results in detail in Table 3. We observed that ethanol extracts of seven different mushroom species collected from the natural environment had biofilm inhibitory effects on indicator microorganisms at varying degrees ranging between 20.7 and 96.3%. While *Hymenopellis radicata* extract had the highest inhibition effect on *P. aeruginosa* (96.3%), *R. flava* extract had the lowest inhibition effect on *S. aureus* (20.7%). Only *H. radicata* and *Sch. commune* were effective on *E. faecalis* with 28.1 and 51.2% rates, respectively. *P. arhizus* was effective only on *P. aeruginosa* with a rate of 69.1%. None of the mushroom species showed significant antibiofilm activity on *C. albicans*.

Biofilm structures formed by microorganisms are considered important structures because they especially allow pathogenic microorganisms to become more resistant to antibiotics. Although the antibiofilm activity of a wide variety of compounds has been investigated in recent studies, a limited number of studies have been conducted on the biofilm inhibitory activities of mushrooms in the current literature. Our review of the existing literature data demonstrated that data was available only on the antibiofilm activity of *L. nuda*, one of the mushrooms species we used in our study. To our knowledge, the antibiofilm activity of other mushroom species worked on in our study has never been investigated previously.

Alves et al.[51] reported that *L. nuda* extracts exhibited different degrees of antibiofilm activity on *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, and *Acinetobacter baumannii*. In that study, the highest effect was observed on *P. aeruginosa* with a rate of 55.99%. Similarly, in our study, *L. nuda* samples showed the highest

TABLE 3: Reduction in biofilm formation on MIC or sub-MIC values [concentration (µg/mL)/biofilm inhibition ratio (%)]

Mushroom species	Indicator microorganism				
	***C. albicans* ATCC 10231**	***E. faecalis* ATCC 29212**	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 27853**	***S. aureus* ATCC 29213**
C. cornucopioides	—	—	62.5/68.3 ± 0.3	62.5/60.6 ± 0.1	125/68.9 ± 0.9
H. radicata	—	31.25/28.1 ± 0.1	62.5/73.1 ± 0.4	62.5/96.3 ± 0.9	31.25/68.4 ± 0.1
L. nuda	—	—	500/78.01 ± 0.1	250/83.2 ± 0.4	125/30.3 ± 0.4
P. arhizus	—	—	—	62.5/69.1 ± 0.2	—
R. flava	—	—	125/78.3 ± 0.3	62.5/65.7 ± 0.2	125/20.7 ± 0.1
Sch. commune	—	31.25/51.2 ± 0.6	125/81.6 ± 0.6	62.5/95.2 ± 0.6	250/77.3 ± 0.3
T. ustale	—	—	125/70.9 ± 0.4	31.25/82.4 ± 0.4	31.25/63.6 ± 0.1

–, Biofilm reducing ability was not observed. Assay was performed three times and data are given as mean ± standard deviation of the three repetitions.

inhibition effect on *P. aeruginosa* with a rate of 83.2%. Karaca et al.[20] worked on mushrooms different from those used in our study and determined that five different mushroom species exhibited different degrees of biofilm inhibitory activities on some *Enterococcus* strains. Garcia et al.[52] found that *Boletus edulis* and *Neoboletus luridiformis* extracts had different degrees of antibiofilm effect on *E. coli* and *S. aureus*. In another study, it was determined that three different mushroom species had antibiofilm activity on *P. aeruginosa* and *Salmonella typhimirium*.[53] Although there are different types of mushroom species, the antibiofilm activity of mushrooms on *S. aureus*, *P. aeruginosa*, and *E. coli* was found remarkable in our study.

C. Antioxidant Activity

In the current study, we used the following three methods used most frequently to determine antioxidant activity in foods: determination of total phenolic content and determination of DPPH radical scavenging effect and FRAP analysis. At the end of the study, it was determined that the mushroom species with the highest free radical scavenging effect was *Tricholoma ustale*, and that the mushroom species with the highest reducing power was *P. arhizus* which also has the highest polyphenol content. All of the results obtained are summarized in Table 4.

Today, mushrooms are accepted as an important dietary component and their consumption as food is common both in our country and in the other countries of the world. Therefore, the number of studies in which the possible benefits of mushrooms including the species used in the present study such as antioxidant activities for human health are investigated is increasing. The comparison of the analyses performed to determine antioxidant activity with the existing literature data demonstrated that our results did not fully overlap with the results obtained by other researchers. Although the results reported on *C. cornucopioides*, *Sch. commune*, and *R. flava* species by various researchers[54,55] were similar to those obtained in our study, we observed that DPPH radical scavenging capacity determined in our study was lower in general.[56–58] However, the comparison of the polyphenol contents of the species worked on and those in the literature indicated that the results obtained for various species such as *P. arhizus*[57] and *L. nuda*[59,60] were similar. However, polyphenol content of some other species such as *Sch. commune* was lower than was that in the literature.[61] In conclusion, according to the analysis of the literature data on the antioxidant capacities of mushroom species, different researchers have reported different results. Although *in vitro* antioxidant activity determination methods are used very frequently, there is no single standardization for these methods and differences can be observed in the steps of the methods. Many factors, ranging from the way the extracts of the samples are prepared to the processing steps of the applied method, affect the results. Within this context, it is expected that the data obtained from studies on the same mushroom species in different researchers vary.

TABLE 4: Antioxidant capacity of mushroom extracts

Mushroom species	DPPH (mg/mL)	FRAP (µg TE/100 g)	Polyphenol (mg GAE/100 g)
C. cornucopioides	0.23 ± 0.0029	50.06 ± 0.0003	30.33 ± 0.003
H. radicata	0.25 ± 0.003	61.67 ± 0.0058	20.33 ± 0.007
L. nuda	0.48 ± 0.0019	14.15 ± 0.0004	24.91 ± 0.0085
P. arhizus	0.194 ± 0.0037	62.32 ± 0.0008	83.66 ± 0.004
R. flava	0.279 ± 0.0039	52.64 ± 0.0012	47 ± 0.003
Sch. commune	0.23 ± 0.0037	32.96 ± 0.0002	14.08 ± 0.0075
T. ustale	0.21 ± 0.001	20.27 ± 0.0704	18.66 ± 0.002

Assay was performed three times and data are given as mean ± standard deviation of the three repetitions.

D. Cytotoxic Activity and Fatty Acid Analysis

The cytotoxicity of mushroom species was studied in MCF-7 and MDA-MB-231 breast cancer and L929 (mouse fibroblast) cell lines. Cisplatinum was used as a positive control, and selectivity indices were calculated at the end of the study. As is seen in Table 5, the mushrooms used in the study were cytotoxic at the indicated concentrations on breast cancer cells (MDA-MB-231 and MCF-7) and normal fibroblast cells (L929). The selectivity index shows the selectivity of a particular compound between normal and cancer cells. The higher the level of the selectivity index is, the greater its selectivity level is. Therefore, according to the calculation made by considering the selectivity indexes, compared to other mushrooms, while *H. radicata* is more effective for the MDA-MB-231 cell line, *C. cornucopioides* is more effective for the MCF-7 cell line. According to the comparison of the two cancer cells in terms of cytotoxic concentration, the IC_{50} values of MDA-MB-231 are generally lower than are the values of MCF-7.

According to the fatty acid profiles of mushroom species determined by FAME analysis, while the amount of oleic acid was high in *C. cornucopioides*, *H. radicata*, and *P. arhizus* species, the amount of linoleic acid was high in *L. nuda*, *R. flava*, and *T. ustale* species (Table 6). In breast cancer cells, oleic acid induces an increase in cellular Ca^{2+} concentration and proliferation[62–64] and inhibits cancer cell growth and survival in low metastatic carcinoma cells, such as gastric carcinoma SGC7901 and breast carcinoma MCF-7 cell lines. Therefore, it ca be said that the results of the fatty acid analyses were consistent with the results of cytotoxic activity study. The present results indicate that the fact that the mushroom species were more effective in MCF-7 than the MDA-MB-231 cell line may be related to their oleic acid content. However, further analysis is required to reach a definitive conclusion. In addition, the fatty acid profiles obtained in our study are compatible with those in the existing literature.[65]

IV. CONCLUSIONS

Mushrooms, whether they are collected from the natural environment or obtained by culturing, have become an important alternative in terms of meeting the food needs of the increasing world population. In recent years, more and more studies have been conducted on the medical and pharmacological effects of mushrooms in many parts of the world. These studies indicate that primary and secondary metabolites (e.g., proteins, peptides, enzymes, and polysaccharides) produced by mushrooms have therapeutic effects in the prevention and treatment of many diseases. In the current study, it was determined that ethanol extracts

TABLE 5: IC_{50} values and selectivity ratios of mushrooms

Mushroom species	Cell lines and selectivity index				
	MCF-7	MDA-MB-231	L929	Selectivity index/MDA	Selectivity index/MCF
C. cornucopioides	33.74 ± 1.12	24.50 ± 0.60	65.37 ± 1.03	1.94	2.67
H. radicata	20.25 ± 0.62	22.25 ± 1.09	45.93 ± 1.89	2.27	2.06
L. nuda	33.01 ± 1.09	25.24 ± 0.40	57.37 ± 0.84	1.74	2.27
P. arhizus	28.48 ± 0.43	20.73 ± 1.32	49.99 ± 1.03	1.76	2.41
R. flava	36.50 ± 1.04	22.63 ± 1.23	49.86 ± 1.98	1.37	2.20
Sch. commune	32.73 ± 0.99	21.50 ± 0.75	52.02 ± 1.03	1.59	2.42
T. ustale	31.75 ± 0.68	25.24 ± 0.39	47.07 ± 0.02	1.48	1.86
Cisplatin	8.03 ± 0.23	10.39 ± 0.64	22.87 ± 0.38	2.85	2.20

Each concentration was worked on in MCF-7, MDA-MB-231, and L929 cell lines ($n = 3$).

TABLE 6: Fatty acid contents (%) of mushrooms species

Fatty acid	Mushroom species						
	C. cornucopioides	*H. radicata*	*L. nuda*	*P. arhizus*	*R. flava*	*Sch. commune*	*T. ustale*
Palmitic acid	10.25	8.28	15.80	27.40	12.43	38.95	14.04
Stearic acid	9.52	1.92	3.56	7.92	3.54	6.23	5.04
Oleic acid	50.15	63.44	26.30	41.76	36.43	8.75	31.22
Linoleic acid	28.00	18.77	54.33	22.91	46.77	36.34	47.05
Erucic acid	2.07	—	—	—	—	—	—
Lauric acid	—	1.73	—	—	—	—	—
Myristic acid	—	0.69	—	—	—	—	—
Palmitoleic acid	—	2.19	—	—	—	2.24	—
Linolelaidic acid	—	1.60	—	—	0.83	—	2.64
α-Linolenic acid	—	1.36	—	—	—	7.50	—

–, Not detected.

obtained from *C. cornucopioides*, *H. radicata*, *L. nuda*, *P. arhizus*, *R. flava*, *Sch. commune*, and *T. ustale* exhibited biological activity at various degrees. Determining the metabolites of these mushrooms with biological activity, revealing their chemical structures and effects in the *in vivo* environment, and adding them to the pharmacology literature will be realized by further analyses to be made.

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