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\*Corresponding author: Abdurrahman Dundar, Department of Medicinal Promotion and Marketing, Health Services Vocational High School, Mardin Artuklu University, Mardin 47000, Turkey  
E-mail: [anzdundar@gmail.com](mailto:anzdundar@gmail.com)

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Fatih Yildiz, Middle East Technical University, Turkey

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## FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

# Determination of cytotoxic, anticholinesterase, antioxidant and antimicrobial activities of some wild mushroom species

Abdurrahman Dundar<sup>1\*</sup>, Veysi Okumus<sup>2</sup>, Sadin Ozdemir<sup>2</sup>, Kadir Serdar Celik<sup>3</sup>, Mehmet Boğa<sup>4</sup> and Eren Ozcagli<sup>5</sup>

**Abstract:** In the performed study of methanol extraction of wild edible mushroom species; *Agaricus arvensis*, *Agaricus campestris*, *Armillaria mellea*, *Fomes fomentarius*, *Coprinus micaceus*, *Coriolus versicolor* and *Lactarius deliciosus* were examined for screening their cytotoxic, anticholinesterase, antioxidant and antimicrobial capacity. Phenolic acid composition of mushrooms was also analysed. *L. deliciosus* and *F. fomentarius* were generally showed the highest activities at antioxidant test systems (metal chelating, superoxide anion radical scavenging, total antioxidant, 2,2-diphenyl-1-picrylhydrazyl radical scavenging and reducing power activity tests). The highest activities at antimicrobial activity displayed by *A. arvensis* and as  $18 \pm 0.8$  against

### ABOUT THE AUTHORS

Abdurrahman DÜNDAR works as an associate professor at Mardin Artuklu University, Vocational School of Health Services, Medical Promotion and Marketing Program, Mardin Turkey. He is expertise area is in vitro antioxidant and antimicrobial activities of mushroom extracts.

Veysi Okumus works as an associate professor at Siirt University Science and Arts Faculty Department of Biology, Siirt Turkey. He is expertise area is biosorption, enzyme technology, antioxidant and antimicrobial activity.

Sadin Ozdemir works as an associate professor at Siirt University Science and Arts Faculty Department of Biology, Siirt Turkey. He is expertise area is biosorption, enzyme technology, antioxidant and antimicrobial activity.

Kadir Serdar Celik works as an expert at Faculty of Science and Arts, Department of Chemistry, Batman University, Batman, Turkey. He is expertise area is analytical chemistry.

Mehmet Boga works as an assistant professor Faculty of Pharmacy, Department of Pharmaceutical Technology, Dicle University, Diyarbakir 21280, Turkey. He studies cytotoxic, anticholinesterase, antioxidant activities of natural products.

Eren Ozcagli works as an lecturer at Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul University, Beyazit, Istanbul 34116, Turkey. She studies cytotoxic, anticholinesterase, antioxidant activities of natural products.

### PUBLIC INTEREST STATEMENT

The paper emphasises, anticancer, anticholinesterase, antioxidant and antimicrobial capacity and phenolic acid composition of wild mushrooms collected from nature. Especially nowadays, antibiotic resistance threatens effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. So, this event triggers to searching alternative therapeutic medicines. Our study results showed us that mushrooms have got promising potential at this area. But further studies should be done to characterise specifically effective molecules.

to *Staphylococcus aureus*. The best  $IC_{50}$  values of mushroom methanol extracts at anticancer activities on HeLa and NRK-52E were 7.09 and 18.23 mg/mL exhibited by *C. micaceus* and *A. campestris*, respectively. The highest butyrylcholinesterase activity exhibited by *L. deliciosus*. Total amount of phenolic acids were found as 1,224.70 mg/kg at *L. deliciosus*.

**Subjects:** Bioscience; Environment & Agriculture; Food Science & Technology

**Keywords:** cytotoxic; anticholinesterase; antioxidant; antimicrobial; mushroom

## 1. Introduction

The properties of matchless flavour and relish and appealing delicacy made mushrooms indispensable for early civilisations. At the same time countless health benefits of some edible mushrooms afford also dates back several centuries. In fact, several thousand years ago in eastern cultures, many edible and non-edible mushrooms were known for their potential health utility. In Chinese medicine, the dietary supplements and nutraceuticals made from mushroom-derived extracts are broadly used, along with various combinations of other herbal preparations, to cure a number of disease conditions. Many of these are used as immunomodulators in cancer therapy (Diyabalanage, Mulabagal, Mills, DeWitt, & Nair, 2008). Reactive oxygen and nitrogen species formed when an imbalance occurred in oxygen metabolism. These entail many pathogenesis in human such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer as well as they trigger the oxidative deterioration of food products by chain reactions. These reactive oxygen and nitrogen species are inactivated by antioxidants (Halliwell & Gutteridge, 1996). Mushrooms are rich sources of antioxidant compounds such as phenolic compounds (phenolic acids and flavonoids) and tocopherols (Ferreira, Barros, & Abreu, 2009; Heleno, Barros, Sousa, Martins, & Ferreira, 2010; Sun, Zhuang, & Bai, 2011). The antioxidant activity of mushrooms is well correlated with their phenolic content and profile (Cheung, Cheung, & Ooi, 2003). Most recent studies have intensively placed major emphasis on the identification of health beneficial components, especially anticancer, antioxidants and antimicrobials in mushrooms and their mechanism of action in humans (Dundar, Okumus, Ozdemir, & Yildiz, 2013; Dundar et al., 2012; Orhan & Üstün, 2011; Ramirez-Anguiano, Santoyo, Reglero, & Soler-Rivas, 2007). In particular, mushrooms are useful against cancers of the stomach, oesophagus, lungs, etc. are known in China, Japan, Korea, Russia, the United States and Canada. Both cellular components and secondary metabolites of a large number of mushrooms have been shown to affect the immune system of the host and therefore could be used to treat a variety of diseases (Ferreira, Baptista, Vilas-Boas, & Barros, 2007). The antioxidative and free radical scavenging properties of the phenolic content of mushroom methanolic extracts have been reported, suggesting possible protective roles of these compounds, due to their ability to capture metals, inhibit lipoxygenase and scavenge free radicals (Mau, Chang, Huang, & Chen, 2004). In the current study, anticancer, anticholinesterase, antioxidant and antimicrobial capacity and phenolic acid composition of mushrooms were analysed.

## 2. Results and discussion

The phenolic compound of mushroom methanol extracts were determined by HPLC technique. These phenolics are, protocatechuic, syringic, caffeic, vanillic, *o*-coumaric, *p*-coumaric, catechin, quercetin and rutin (Table 1). Total amount of phenolic compound of *Lactarius deliciosus* was at the highest level (1,224.70 mg/kg). This is followed by *Fomes fomentarius* as 986.23 mg/kg. None of the mushroom contains all analysed phenolic compounds.

The antibacterial activity of extracts was examined against six micro-organisms. The range of inhibition zones obtained from the study was  $1 \pm 0.75$ – $18 \pm 0.8$  mm as shown in Table 2. The highest antimicrobial activity was exhibited by *Agaricus arvensis* against *Staphylococcus aureus* and inhibition zone was  $18 \pm 0.8$  mm and the nearest activity to this activity was observed as  $16 \pm 0.7$  by *L. deliciosus* against *S. aureus*.

**Table 1. Phenolic compounds of mushroom species (mg/kg)**

Phenolic acids	<i>L. deliciosus</i>	<i>F. fomentarius</i>	<i>A. campestris</i>	<i>C. micaceus</i>	<i>A. mellea</i>	<i>A. arvensis</i>	<i>C. versicolor</i>
Protocatechuic	18.89	5.45	Nd	6.45	5.13	13.10	5.45
Catechin	76.39	75.02	101.15	28.02	72.14	112.84	16.87
Coffeic	8.63	6.06	3.58	Nd	Nd	3.21	Nd
Syringic	78.64	32.58	Nd	15.69	65.73	12.54	Nd
Vanilic	65.87	13.45	13.54	2.81	1.21	Nd	32.13
<i>p</i> -coumaric	27.96	5.45	6.24	3.47	2.14	66.41	56.98
<i>o</i> -coumaric	497.45	361.76	Nd	Nd	32.84	1.20	315.42
Rutin	Nd	Nd	Nd	Nd	3.47	0.59	1.42
Quercetin	450.87	486.46	219.27	4.87	5.07	130.02	156.48
Total	1,224.70	986.23	343.78	61.31	182.28	339.91	584.75

Note: Nd: not deducted.

**Table 2. Antimicrobial activity of mushroom methanol extracts<sup>a</sup> (mm)**

Micro-organisms	<i>L. deliciosus</i>	<i>F. fomentarius</i>	<i>A. campestris</i>	<i>C. micaceus</i>	<i>A. mellea</i>	<i>A. arvensis</i>	<i>C. versicolor</i>
<i>S. aureus</i>	16 ± 0.7	4 ± 0.3	NA	1 ± 0.7	NA	18 ± 0.8	12 ± 0.6
<i>E. coli</i>	12 ± 0.3	NA	10 ± 0.7	6 ± 0.3	5 ± 0.8	4 ± 0.3	11 ± 0.4
<i>M. luteus</i>	NA	13 ± 0.5	6 ± 0.3	5 ± 0.1	4 ± 0.6	NA	4 ± 0.1
<i>E. hirae</i>	5 ± 0.4	6 ± 0.7	5 ± 0.2	NA	2 ± 0.7	NA	2 ± 0.6
<i>P. aeruginosa</i>	11 ± 0.2	8 ± 0.7	NA	3 ± 0.2	NA	9 ± 0.16	NA
<i>B. subtilis</i>	NA	NA	9 ± 0.41	6 ± 0.2	3 ± 0.5	5 ± 0.67	5 ± 0.7

Note: NA: not active.

<sup>a</sup>Values expressed are means ± S.D of three parallel measurements.

In the study edited to determine the antioxidant capacity of mushroom methanol extracts, only the highest activities and concentrations are given in Table 3. The highest activities at DPPH scavenging, reducing power, total antioxidant, superoxide anion radical and metal chelating activity exhibited by *L. deliciosus* (89.69%), *F. fomentarius* (2.09), *L. deliciosus* (68.45%-48th h), *F. fomentarius* (86.03%) and *L. deliciosus* (88.68%), respectively. At all the tests studied all of the extracts increased their antioxidant activity when the concentration increased.

None of the methanol extracts of the mushrooms possessed anticholinesterase effect, but all of the extracts exhibited butyrylcholinesterase inhibitory activity at 200 µg/mL (Table 4). At the study, galantamine was used as standard which is used for Alzheimer's disease.

To specify cytotoxic activity of the extracts, HeLa and NRK-52E cell lines were used, which are most suitable and common to perform cytotoxicity studies. As can be seen in the Table 5, *L. deliciosus*, *Coriolus versicolor*, *A. arvensis* and *Armillaria mellea* were not showed cytotoxic activity for both cells. However, *F. fomentarius*, *Agaricus campestris* and *Coprinus micaceus* were disturbed on mitochondrial activity of cell. The extracts were not displayed selectivity to the cancer and normal cell line. The highest cytotoxic activity was exhibited by *C. micaceus* with 7.09 and 15.22 mg/ml of IC<sub>50</sub> values for HeLa and NRK-52E cells, respectively.

Phenolics (flavonoids, phenolic acids and tannins) possess a wide spectrum of biochemical activities such as antioxidant, antimicrobial, antimutagenic, anticarcinogenic as well as ability to modify

**Table 3. Antioxidant activities of mushroom methanol extracts\***

Mushroom extracts	DPPH scavenging activity % (20 mg/mL)	Reducing power activity (10 mg/mL)	Metal chelating activity % (4 mg/mL)	Total antioxidant activity % (0.1 mg/mL-48th h)	Super oxide anion radical activity % (2 mg/mL)
<i>A. arvensis</i>	65.73	1.47	78.12	52.24	72.65
<i>A. campestris</i>	84.72	1.65	75.23	53.54	62.81
<i>A. mellea</i>	73.48	1.58	68.41	56.73	50.45
<i>F. fomentarius</i>	64.47	2.09	82.78	64.78	86.03
<i>C. micaceus</i>	81.45	1.25	55.17	34.72	56.58
<i>C. versicolor</i>	87.78	1.21	73.56	59.92	65.54
<i>L. deliciosus</i>	89.69	1.95	88.68	68.45	80.12

\*Only the results of the highest concentrations and activities are given.

**Table 4. Anticholinesterase activity of the methanol extracts of mushrooms at 200 µg/mL<sup>a</sup>**

Mushroom extracts	Inhibition % against AChE	Inhibition % against BChE
<i>A. arvensis</i>	NA	52.25 ± 0.4
<i>A. campestris</i>	NA	25.24 ± 0.1
<i>A. mellea</i>	NA	32.45 ± 0.4
<i>F. fomentarius</i>	NA	67.54 ± 0.1
<i>C. micaceus</i>	NA	23.26 ± 1.1
<i>C. versicolor</i>	NA	33.45 ± 0.4
<i>L. deliciosus</i>	NA	62.21 ± 0.9
Galantamine <sup>b</sup>	87.08 ± 0.7	81.83 ± 0.2

Note: NA: not active.

<sup>a</sup>Values expressed are means ± S.D of three parallel measurements.

<sup>b</sup>Standard drug.

**Table 5. Anticancer activity of mushroom methanol extracts (IC<sub>50</sub> values)\***

Mushroom extracts	Hela cells	NRK-52E cells
<i>A. mellea</i>	NA	NA
<i>L. deliciosus</i>	NA	NA
<i>C. versicolor</i>	NA	NA
<i>C. micaceus</i>	7.09	8.65
<i>A. arvensis</i>	NA	NA
<i>A. campestris</i>	18.23	19.87
<i>F. fomentarius</i>	22.45	24.61

Note: NA: not active.

\*Concentration causing proliferation inhibition by 50% compared to the control.

the gene expression. Numerous epidemiological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk (Marinova, Ribarova, & Atanassova, 2005; Moein & Moein, 2010). It is a well-documented fact that some flavonoids, mostly quercetin and its derivatives and catechin, possess scavenging (or quenching) abilities against free radicals and active oxygen species (Hanasaki, Ogawa, & Fukui, 1994; Nagata, Takekoshi, Takagi, Honma, & Watanabe, 1999). *L. deliciosus* and *F. fomentarius* were

generally showed higher antioxidant activity than the other mushrooms. This can be explained by higher phenolic ingredient of mushrooms. Palacios et al. (2011) have reported *L. deliciosus* phenolic compounds. In the study, catechin and *p*-coumaric were not detected and amount of caffeic and protocatechuic were found 15.51 and 18.64 mg/kg, respectively. Turkoglu, Duru, Mercan, Kivrak, and Gezer (2007) found 12, 8, 9, 10 and 6 mm inhibition zones against *B. cereus*, *Bacillus subtilis*, *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively, using *Laetiporus sulphureus* ethanol extracts. Phenolic compounds are related with many biologic functions included antimicrobial and antioxidant activity (Penna et al., 2001). From this point of view it can be said that antimicrobial activity of extracts concludes from their phenol content. Orhan and Üstün (2011) found 62.41% DPPH scavenging activity from *L. deliciosus* at the concentration of 5 mg/ml. *L. deliciosus* showed 1.65 activity at 50 mg/ml (Ferreira et al., 2007). As can be seen from Table 3, our antioxidant activity findings are higher than the authors mentioned. According to our literature survey, no studies were detected about the anticholinesterase and anticancer activities of the mushroom species we studied. Only Orhan and Üstün (2011) studied anticholinesterase activity of *L. deliciosus* and they reported 24.04% anticholinesterase activity at a concentration of 500 µg/ml. In anticancer activity only, similar to our results, Tong et al. (2009) have reported that the growth of Hela cells could be inhibited by *P. ostreatus* extracts at a concentration as low as 50 µg/ml. As a result, we obtained high antioxidant, moderate antimicrobial and low anticancer and anticholinesterase activities from mushroom methanol extracts. When the results of the study take into consideration no correlations can be installed between the activities we studied. While *L. deliciosus* and *F. fomentarius* methanol extracts showed higher antioxidant activities than the other mushrooms, the activities of these mushrooms at anticholinesterase and anticancer tests were not appreciable.

### 3. Experimental

#### 3.1. Mushrooms

*A. arvensis*, *A. campestris*, *A. mellea*, *F. fomentarius*, *C. micaceus*, *C. versicolor* and *L. deliciosus* mushrooms were collected during March and April 2010. All mushrooms were collected from Eruh-Siirt region located the south-east region of Turkey.

#### 3.2. Preparation of the methanol extracts of mushrooms

Methanol extraction of mushroom samples was prepared as follows. Mushrooms were cut into small pieces and dried in an oven at 40°C. Dried mushroom samples (10 g) were powdered in a blender and then extracted by stirring with 100 ml of methanol at 30°C at 150 rpm for 24 h and filtering through Whatman No. 4 filter paper. Two additional 100 ml portions of methanol were used for re-extracting the residue as described above. The combined methanol extracts were then evaporated at 40°C and extraction yields were calculated for each mushroom. For preparing stock solution (25 mg/ml) crude extracts were re-dissolved in methanol and stored at 4°C for further use.

#### 3.3. Anticholinesterase activity

A spectrophotometric method developed by Ellman, Courtney, Andres, and Featherstone (1961) was established to indicate the acetyl- and butyryl-cholinesterase inhibitory effects.

#### 3.4. Cytotoxic activity

##### 3.4.1. Cell lines, culture treatments

The cytotoxic effect of mushroom extracts were assessed using MTT test on human cervix cancer (HeLa) and rat kidney epithelium cell (NRK-52E) lines. NRK-52E (ATCC CRL-1571) and HeLa (ATCC CCL-2) were cultured according to manufacturer's protocols. Following steps were carried out for both cells. Cells were seeded at 10<sup>4</sup> cells/100 µl into each well of 96-well plates. After 24 h of incubating period, culture medium was removed. Then, the extracts were added to wells in various concentrations. The exposure concentrations were determined as µg/ml for the extracts. After 24 h of incubation with the extracts, MTT cytotoxicity test was performed to assess anticancer activity of mushroom extracts.

### 3.4.2. MTT cytotoxicity test

The test principle is that MTT, formed 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide and yellow coloured water-soluble tetrazolium salt, is reduced to an insoluble purple formazan product by the mitochondrial succinate dehydrogenase, which belongs to the mitochondrial respiratory chain and is only active in viable cells, in the presence of an electron-coupling reagent. The protocol was performed according to the method of Alley et al. (1998). Absorbance was read at 590 nm using microplate spectrophotometer system (BioTek® Epoch Microplate Spectrophotometer, Winooski-USA). In every test, negative (untreated, culture medium) and solvent (1% DMSO) controls were used. For each extract, four concentrations were tested in triplicates and each test was repeated twice. Fifty per cent inhibition concentration ( $IC_{50}$ ) was used for cytotoxic activities.  $IC_{50}$  value was expressed as the concentration of sample caused an inhibition of 50% in enzyme activities in cells. In calculation, the absorbance values of samples were compared with the absorbance values of solvent controls after all absorbance values were corrected by subtracting the absorbance of blank. In MTT test, a dose-response curves was constructed and  $IC_{50}$  calculated according to the below formula as the percentages of solvent controls;

$$\% \text{ inhibition} = 100 - (\text{corrected mean } A_{\text{sample}} \times 100 / \text{corrected mean } A_{\text{solvent control}})$$

$IC_{50}$  values are defined as the concentrations of test compounds required to reduce the absorbance to 50% of the control values.

## 3.5. Determination of phenolic compounds of mushrooms

### 3.5.1. Sample preparation

Each mushroom sample (~3 g) was extracted using an acetone:water (80:20; 30 ml) mixture at  $-20^{\circ}\text{C}$  for 6 h. After treated 15 min in an ultrasonic bath the extracts were centrifuged at 4,000 g for 10 min (5430 R model, Eppendorf) and filtered through Whatman No. 4 paper. For extraction of the residue two additional 30 ml portions of the acetone:water mixture were used. The combined extracts were evaporated to remove acetone at  $40^{\circ}\text{C}$  by a nitrogen evaporator (TAB-40 WEL model, Teknosem). The aqueous phase was washed with *n*-hexane and then submitted to a liquid-liquid extraction with diethyl ether ( $3 \times 30$  ml) and ethyl acetate ( $3 \times 30$  ml). At  $40^{\circ}\text{C}$  the organic phases were evaporated and re-dissolved in water:methanol (80:20). At finally prepared solutions were filtered through a 0.22- $\mu\text{m}$  disposable LC filter disc for HPLC analysis.

### 3.5.2. Determination of phenolics by HPLC analysis

The phenolic extracts of mushroom species were determined using HPLC equipment consisting of an integrated system with an Agilent 1260 Infinity HPLC-DAD. Data were analysed using Agilent Chem Station revision B.04.01 software (Agilent). The chromatographic separation was achieved with an Agilent ZORBAX ( $250 \times 4$ , 6–5  $\mu\text{m}$ ) thermostated at  $35^{\circ}\text{C}$ . For gradient elution, two solvents were used: one consisted of acetic acid–water (2:98 v/v) and the other consisted of only methanol. Injection volume was 20  $\mu\text{l}$ . Detection was carried out in a DAD, using 280 nm as the preferred wavelength.

## 3.6. Determination of antimicrobial activity of mushroom extracts

### 3.6.1. Test micro-organisms

For determining antimicrobial activity of mushroom methanol extracts *E. coli* (ATCC 10536), *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6051), *Enterococcus hirae* (ATCC 10541), *Micrococcus luteus* (ATCC 9341) and *P. aeruginosa* (ATCC 9027) were used. Nutrient broth was used for culturing of test bacteria. All strains were regenerated twice before use in the antimicrobial test.

### 3.6.2. Disc diffusion method

Antimicrobial activity of mushroom methanol extracts was examined by the method of Kalemba and Kunicka (2003). About 100  $\mu\text{l}$  of bacterial cultures were inoculated to Petri plates which contain nutrient broth agar media and dealt with a Drigalski spatula. The blank discs (7.0 mm, Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK) were impregnated with 100  $\mu\text{l}$  of extracts (10 mg/

ml concentration), the same volume (100 µl) of methanol was used as a control. The Petri plates were incubated for at 37°C for 24 h. After incubation, the diameter of the inhibition zone was measured with calipers. The measurements were done basically from the edge of the inhibition zone to the edge of the discs.

### 3.7. Determination of antioxidant activity of mushroom methanol extracts

#### 3.7.1. Scavenging activity of DPPH radical cation

The scavenging activity of DPPH radical was applied according to the method described by Chu, Chang, and Hsu (2000) with some modifications. An aliquot of 1.0 ml of 0.1 mM DPPH radical solution dissolved in methanol was mixed with 0.5 ml of sample extract of various concentrations (0.05–2 mg/ml) or negative control (methanol). The reaction mixture was mixed and its absorbance at 520 nm was measured. The DPPH radical scavenging activity (%) was calculated by the following equation:

$$I\% = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

where  $A_{\text{control}}$  is the absorbance of the control reaction (containing all reagents except the test compound) and  $A_{\text{sample}}$  is the absorbance of the test compound. Tests were carried out in triplicate. BHA and BHT were used as positive control.

#### 3.7.2. Reducing power

The reducing power of methanolic extracts of mushroom spices was determined according to the method of Oyaizu (1986). Each extract (1, 2.5, 5, 7.5 and 10 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 g for 10 min. About 2.5 ml of deionised water and 0.5 ml of 0.1% ferric chloride was mixed with the upper layer (2.5 ml) of the solution. Finally, the absorbance was measured at 700 nm against a blank. Trolox and ascorbic acid were used as control.

#### 3.7.3. Metal chelating ability

The chelating ability of methanol extraction of mushrooms was determined according to the method of Dinis, Madeira, and Almeida (1994). Each extract (0.1–4 mg/ml) in methanol (1 ml) was added to 3.7 ml of methanol and mixed with 0.1 ml of 2 mmol/L ferrous chloride. The reaction was started by adding of 0.2 ml of 5 mmol/L ferrozine. The mixture was left for standing 10 min at room temperature and then the absorbance was measured at 562 nm against blank. The results were expressed as percentage of inhibition of the ferrozine-Fe<sup>2+</sup> complex formation. EDTA was used as a positive control. The percentage inhibition of the ferrozine-Fe<sup>2+</sup> complex formation was calculated using the formula given below:

$$\text{Chelating ability (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

#### 3.7.4. Superoxide anion radical scavenging activity

The method of Zhishen et al. (1999) was applied for determining the scavenging activity of superoxide anion radicals of mushroom extracts. All solutions were added to 0.05 M phosphate buffer (pH 7.8). To start the anion radical formation in reactants by photoinduced reactions, 4,000 lx of illumination intensity was adjusted in a closed box with fluorescent lamps. The total volume of reactant was 5 ml and the concentration of riboflavin, methionine and nitro blue tetrazolium (NBT) were 4.0, 2.0 and 3.0 M, respectively. The reactant was illuminated at 25°C for 25 min. The photochemically reduced riboflavins generated anion radicals, which reduced NBT to form blue formazan. The un-illuminated reaction mixture was used as a blank. Absorbance (A) was measured at 560 nm. Methanolic extract of mushroom species and standards were added to the reaction mixture, in which anion radical was scavenged, thereby inhibiting the NBT reduction. Absorbance ( $A_1$ ) was measured and decrease in anion radical was represented by  $A - A_1$ . The degree of scavenging was calculated by the following equation:

$$\% \text{ Scavenging} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

### 3.7.5. Total antioxidant activity by the ferric thiocyanate method

Ferric thiocyanate method (Osawa & Namiki, 1981) was used for settling the antioxidant capacity of mushroom extracts. A volume of 100 µl of each extracts was mixed with 0.05 M phosphate buffer at pH 7.4 and 0.07 M linoleic acid was solved in Tween 20 to obtain 4 ml of solution. The final solutions were incubated at 37°C in prosody caps. About 100 µl of aliquot from each extract were removed periodically, and added to FeCl<sub>2</sub>-ammonium thiocyanate solution. During the linoleic acid oxidation, peroxides formed which oxidised Fe<sup>2+</sup> to Fe<sup>3+</sup>. The Fe<sup>3+</sup> ions form a complex with SCN<sup>-</sup>, and this complex has maximum absorbance at 500 nm. This step was repeated every 24 h until the control (phosphate buffer, linoleic acid mixture) reached its maximum absorbance value. Therefore, high absorbance values indicated high levels of linoleic acid oxidation. Phosphate buffer was used as the reaction blank. The total antioxidant activity was expressed as the average of three independent determinations carried out in duplicate. The percentage inhibition of lipid peroxidation of linoleic acid was calculated by applying the following equation: inhibition of lipid peroxidation

$$I\% = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

where  $A_{\text{control}}$  is the absorbance of the control reaction (phosphate buffer plus linoleic acid) and  $A_{\text{sample}}$  is the absorbance obtained in the presence of the extracts or positive control of antioxidant activity (BHA and BHT).

## 4. Conclusion

From this point of view to deeply understand which compound is responsible from which activity, further chromatographic fractionations and structural lighting need to be made. Beside to this, *in vivo* studies must be made to observe how effective the mushrooms on live organisms. After these studies we are in the opinion that these potentials of mushrooms may be useful for pharmaceutical and food industry to decrease utilising synthetic compounds. The results obtained from study are significant as they also provide new substructure knowledge for further studies about the mushrooms studied.

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### Competing interests

The authors declare no competing interests.

### Author details

Abdurrahman Dundar<sup>1</sup>

E-mail: [anzdundar@gmail.com](mailto:anzdundar@gmail.com)

Veysi Okumus<sup>2</sup>

E-mail: [veysiok@hotmail.com](mailto:veysiok@hotmail.com)

Sadin Ozdemir<sup>2</sup>

E-mail: [sadin77@hotmail.com](mailto:sadin77@hotmail.com)

Kadir Serdar Celik<sup>3</sup>

E-mail: [kscelik@hotmail.com](mailto:kscelik@hotmail.com)

Mehmet Boğa<sup>4</sup>

E-mail: [mehmet.boga@dicle.edu.tr](mailto:mehmet.boga@dicle.edu.tr)

Eren Ozcagli<sup>5</sup>

E-mail: [eren.ozcagli@istanbul.edu.tr](mailto:eren.ozcagli@istanbul.edu.tr)

<sup>1</sup> Department of Medicinal Promotion and Marketing, Health Services Vocational High School, Mardin Artuklu University, Mardin 47000, Turkey.

<sup>2</sup> Faculty of Science and Arts, Department of Biology, Siirt University, Siirt 56100, Turkey.

<sup>3</sup> Faculty of Science and Arts, Department of Chemistry, Batman University, Batman, Turkey.

<sup>4</sup> Faculty of Pharmacy, Department of Pharmaceutical Technology, Dicle University, Diyarbakir 21280, Turkey.

<sup>5</sup> Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul University, Beyazit, Istanbul 34116, Turkey.

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