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Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species

Nevcihan Gursoy [a,*], Cengiz Sarikurkcu [b], Mustafa Cengiz [c], M. Halil Solak [d]

[a] Cumhuriyet University, Faculty of Engineering, Department of Food Engineering, Sivas 58140, Turkey
[b] Mugla University, Faculty of Science and Literature, Department of Chemistry, Mugla 48000, Turkey
[c] Suleyman Demirel University, Faculty of Science and Literature, Department of Chemistry, Isparta 32260, Turkey
[d] Mugla University, Ula Ali Kocman Vocational School, Program of Fungi, Mugla 48100, Turkey

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ABSTRACT

Seven *Morchella* species were analyzed for their antioxidant activities in different test systems namely β-carotene/linoleic acid, DPPH, reducing power, chelating effect and scavenging effect (%) on the stable $ABTS^{\cdot+}$, in addition to their heavy metals, total phenolic and flavonoid contents. In β-carotene/linoleic acid system, the most active mushrooms were *M. esculenta* var. *umbrina* and *M. angusticeps*. In the case of DPPH, methanol extract of *M. conica* showed high antioxidant activity. The reducing power of the methanol extracts of mushrooms increased with concentration. Chelating capacity of the extracts was also increased with the concentration. On the other hand, in 40 μg ml^{-1} concentration, methanol extract of *M. conica*, exhibited the highest radical scavenging activity (78.66 ± 2.07%) when reacted with the $ABTS^{\cdot+}$ radical. Amounts of seven elements (Cu, Mn, Co, Zn, Fe, Ca, and Mg) and five heavy metals (Ni, Pb, Cd, Cr, and Al) were also determined in all species. *M. conica* was found to have the highest phenolic content among the samples. Flavonoid content of *M. rotunda* was also found superior (0.59 ± 0.01 μg QEs/mg extract).

1. Introduction

Vegetables and fruits are considered to be good sources of functional ingredients. Many studies have shown that antioxidants, present in plants at high levels, are the compounds responsible for these functionalities (Sugimura, 2002). Antioxidants or molecules with radical scavenging capacity are thought to exert a potential protective effect against free radical damage. These biomolecules contribute to prevention of coronary and vascular diseases and of tumor formation by inhibiting oxidative reactions (Kris-Etherton et al., 2002). This oxidative damage is the result of free radical action on, for instance, lipids or DNA (Vinson et al., 1998).

Methanol and/or water extracts from common button (*Agaricus bisporus*), shiitake (*Lentinus edodes*), straw (*Volvariella volvacea*), oyster [abalone mushrooms (*Pleurotus cystidiosus*) and tree oyster mushrooms (*P. ostreatus*)], winter (*Flammulina velutipes*), ear (*Auricularia* sp. and *Tremella* sp.) mushrooms (Mau et al., 2001; Yang et al., 2002; Cheung et al., 2003), *Agrocybe* aegerita (Lo and Cheung, 2005) and other edible mushrooms mostly consumed in Asian countries (*Dictyophora indusiata, Grifola frondosa, Hericium erinaceus, Tricholoma giganteum, Ganoderma lucidum*) (Mau et al., 2002; Wachtel-Galor et al., 2004) and in Turkey (*Lactarius deterrimus, Suillus collitinus, Boletus edulis, Xerocomus chrysenteron*) (Sarikurkcu et al., 2008) have shown important antioxidant (Fui et al., 2002) and antitumoral activities (Grube et al., 2001) and other beneficial bioactive capacities. Sarikurkcu et al. (2008) reported that *Lactarius deterrimus* and *Boletus edulis* showed the strongest activity patterns in β-carotene/linoleic acid and DPPH systems among edible mushrooms collected from Eskişehir, Turkey. In addition, Elmastas et al. (2006) found that ethanolic extracts of *Morchella vulgaris* and *M. esculenta* scavenged DPPH radicals by 95% and 94% at 180 μg/mL, respectively. At present, however, not many reports can be found describing the bioactive properties of wild edible mushrooms commonly found in European woods.

It is also known that, wild-growing mushrooms can accumulate great concentrations of toxic metallic elements and metalloids such as mercury, cadmium, lead, copper or arsenic and radionuclides (Gadd, 1993; Gaso et al., 1998; Kirchner and Daillant, 1998; Svoboda et al., 2000; Kalac, 2001; Falandysz et al., 2003; Vetter, 2004). There are many reports concerning the ability to take up and accumulate metals of wild-growing mushrooms from several

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxyltoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GAEs, gallic acid equivalents; QEs, quercetin equivalents.

* Corresponding author. Tel.: +90 346 219 10 10x2889; fax: +90 346 219 11 77.
E-mail addresses: ngursoy2@gmail.com, ngursoy@cumhuriyet.edu.tr, gursoy_gu@hotmail.com (N. Gursoy).

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countries such as France (Michelot et al., 1998), Czech Republic (Svoboda et al., 2002), Poland (Falandysz et al., 2003; Malinowska et al., 2004; Rudawska and Leski, 2005), Slovenia (Kalac et al., 1996; Svoboda et al., 2000), Spain (Garcia et al., 1998), Turkey (Demirbas, 2001; Tuzen et al., 2003; Mendil et al., 2005), and USA (Aruguete et al., 1998). The accumulation of metals in macrofungi has been found to be affected by environmental and fungal factors (Garcia et al., 1998). Environmental factors such as organic matter amount, pH, metal concentrations in soil and fungal factors such as species of mushroom, morphological part of fruiting body, development stages and age of mycelium, biochemical composition, and interval between the fructifications affect metal accumulation in macrofungi (Garcia et al., 1998; Demirbas, 2001; Tuzen et al., 2003; Mendil et al., 2005; Aruguete et al., 1998; Kalac and Svoboda, 2000).

Although the edible wild mushrooms command higher prices than cultivated mushrooms, people prefer to consume them due to their flavour and texture. Nevertheless, wild edible mushrooms are becoming more and more important in our diet for their nutritional and pharmacological characteristics (Manzi et al., 2001; Elmastas et al., 2006). Although there are many studies on cultivated and wild edible mushrooms in the northern hemisphere, there is little information available about the antioxidant properties of wild edible mushrooms of Turkey. The reason for this study is that antioxidant activities of the mushrooms collected from Mugla, Turkey have yet to be examined, although Anatolian people have been using them as food for a long time.

The aim of present work were to evaluate the antioxidant potentials of the methanol extracts of *Morchella rotunda* (Pers.: Fr.) Boud., *Morchella crassipes* (Ventenat) Pers., *Morchella esculenta var. umbrina* (Boud.) S. Imai, *Morchella deliciosa* (Fr.) Jct., *Morchella elata* Fr.: Fr., *Morchella conica* Pers.: Fr. and *Morchella angusticeps* Peck by five different antioxidant test systems; β-carotene/linoleic acid, DPPH, reducing power, chelating effect and scavenging effect (%) on the stable $ABTS^{\cdot+}$, and to determine their total phenolic and flavonoid contents, and to asses of the toxicity profile of these mushrooms as important food sources due to their functional mineral and heavy metal contents.

2. Materials and methods

2.1. Chemicals

Potassium ferricyanide, ferrous chloride, ferric chloride, Folin–Ciocalteu's reagent (FCR), methanol and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt. Germany). 1.1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and butylated hydroxyanisol (BHA) were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH. Sternheim. Germany). All other chemicals and solvents are of analytical grade.

2.2. Mushrooms

Fruiting bodies of edible mushrooms including *Morchella rotunda, M. crassipes, M. esculenta* var. *umbrina, M. deliciosa, M. elata, M. conica,* and *M. angusticeps* were collected from Mugla in 2005, Turkey and were authenticated based on their microscopic and macroscopic characteristics by M. Halil Solak, Program of Fungi, Ula Ali Kocman Vocational School, Mugla University, Mugla, Turkey. They were stored at the Mugla University Ula Ali Kocman Vocational School Herbarium Laboratory (*Morchella rotunda* MHS 106a, *M. crassipes* MHS 104, *M. esculenta* var. *umbrina* MHS 3, *M. deliciosa* MHS 82, *M. elata* MHS 46, *M. conica* MHS 1766, and *M. angusticeps* MHS 8). The fruiting bodies of mushroom samples were divided into parts and then air-dried in an oven for 48 h at 40 °C before analysis.

2.3. Preparation of the methanol extracts

The air-dried fruiting bodies of mushroom samples (5 g) were extracted by stirring them with 100 ml of methanol at 25 °C at 150 rpm for 24 h and filtering through filter paper. The residue was then extracted with two additional 100 ml of methanol as described above. The combined methanol extracts were then rotary evaporated at 40 °C to dryness and kept in the dark at + 4 °C until tested for a short period of time (1–2-days). Extract yields (% dry weight of mushroom) of the mushroom samples were 24.00%, 17.50%, 18.00%, 21.75%, 14.25%, 18.00%, and 11.25% (w/w), respectively.

2.4. Total antioxidant activity by β-carotene–linoleic acid method

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade). 25 μl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispersed to test tubes and 0.5 ml of various concentrations (0.5–4.5 mg ml^{-1}) of the extracts in methanol were added and the emulsion system was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive control BHT, BHA, quercetin and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared. Antioxidative activities of the extracts were compared with those of BHT, BHA and quercetin at 0.5 mg ml^{-1} and blank consisting of only 0.5 ml methanol.

2.5. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH. The effect of methanolic extracts on DPPH radical was estimated according to Hatano et al. (1988). One milliliter of various concentrations (0.5–4.5 mg ml^{-1}) of the extracts in methanol was added to a 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (*I*%) was calculated in following way:

$$I\% = 100 \times (A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}$$

where A_{Control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{Sample} is the absorbance of the test compound. BHT, BHA and quercetin were used as a control.

2.6. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (0.5–4.5 mg ml^{-1}) 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 ferricynide 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 (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. BHT, BHA and quercetin were used as a control.

2.7. Chelating effects on ferrous ions

The chelating effect was determined according to the method of Dinis et al. (1994). Briefly, 2 ml of various concentrations (0.05–0.25 mg ml^{-1}) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\text{Metal chelating effect}(\%) = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control} is the absorbance of control (The control contains $FeCl_2$ and ferrozine, complex formation molecules) and A_{Sample} is the absorbance of the test compound. EDTA was used as a control.

2.8. $ABTS^{\cdot+}$ radical cation decolorization assay

The spectrophotometric analysis of $ABTS^{\cdot+}$ radical scavenging activity was determined according to the method of Re et al. (1999). The $ABTS^{\cdot+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Before usage, the $ABTS^{\cdot+}$ solution was diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with phosphate buffer (0.1 M, pH 7.4). Then, 1 ml of $ABTS^{\cdot+}$ solution was added 3 ml of extract solution in methanol at different concentrations (8–40 μg/ml). After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance (methanol). The scavenging capability of $ABTS^{\cdot+}$ radical was calculated using the following equation:

$$ABTS^{\cdot+}\ \text{scavenging effect}(\%) = [(A_{Control} - A_{Sample}/A_{Control}) \times 100]$$

where $A_{Control}$ is the initial concentration of the $ABTS^{\cdot+}$ and A_{Sample} is absorbance of the remaining concentration of $ABTS^{\cdot+}$ in the presence of extract.

2.9. Determination of metal contents

The collected samples were cleaned, cut, dried at 105 °C for 24 h. Dried samples were homogenized using an agate homogenizer and stored in pre-cleaned polyethylene bottles until the analysis started. Deionized water (18.2 MΩ/cm) from a Milli-Q system (Human Power I Plus, Korea) was used to prepare all aqueous solutions. All mineral acids and oxidants (HNO_3 and H_2O_2) used were of the highest quality (Merck, Darmstadt, Germany). All the plastic and glassware were cleaned by soaking, with contact, overnight in a 10% nitric acid solution and then rinsed with deionized water. For the elemental analysis, a Perkin-Elmer Optima 2000 ICP-OES was used in this study.

For digestion, CEM Mars 5 microwave closed system was used in this study. Sample (0.25 g) was digested with 9 mL of HNO_3 (65%) and 1 ml of H_2O_2 (30%) in microwave digestion system for 7 min and finally diluted to 50 ml with deionized water. A blank digest was carried out in the same way. Digestion conditions for the microwave system applied were: The heat was run up to 180 °C in 5 min, and kept constant for 2 min. This process was repeated once more (Yamac et al., 2007). All sample solutions were clear.

2.10. Assay for total phenolics

Total phenolic constituent of the methanol extracts were determined by employing the methods given in the literature (Chandler and Dodds, 1983; Slinkard and Singleton, 1977) involving Folin–Ciocalteu reagent and gallic acid as standard. 1 ml of extract solution containing 2000 μg extract was added to a volumetric flask. 45 ml distilled water and 1 ml Folin–Ciocalteu reagent was added and flask was shaken vigorously. After 3 min, a 3 ml of Na_2CO_3 (2%) solution was added and the mixture was allowed to stand for 2 h by intermittent shaking. Absorbance was measured at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph:

$$\text{Absorbance} = 0.0167\ \text{gallic acid}\ (\mu g) + 0.0171 \quad (R^2 : 0.99)$$

2.11. Assay for total flavonoids

Total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). Briefly, 1 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the methanolic extracts (2000 μg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 mL extract solution with 1 ml methanol without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.0228\ \text{quercetin}\ (\mu g) - 0.0054 \quad (R^2 : 0.9979)$$

3. Results

3.1. Antioxidant activity

Using the β-carotene/linoleic acid method, methanolic extracts of four edible mushroom species showed different patterns of antioxidant activities. As can be seen from Table 1, the most active mushrooms were *M. esculenta* var. *umbrina* and *M. angusticeps* of which activity potentials were too close to each other at 4.5 mg ml^{-1} concentration (96.89 ± 0.34% and 96.88 ± 0.09%, respectively). This activity was followed by *M. conica* and *M. crassipes*, respectively. At this concentration value, the weakest activity was exhibited by *M. elata* extract (94.37 ± 0.35%).

M. conica methanol extract showed a high antioxidant activity, being able to scavenge more than 80% of the DPPH radical at concentration of 4.5 mg ml^{-1}. Other extracts from mushrooms scavenged less radical than *M. conica*. This activity was closely followed by *M. crassipes*, *M. esculenta* var. *umbrina* and *M. rotunda*, respectively (Table 2).

Table 3 shows the reducing power of mushroom methanolic extracts as a function of their concentration. The reducing power of the mushroom methanolic extracts increased with concentration. At 4.5 mg ml^{-1} concentration, the reducing power was higher than 0.50 for the all extracts. According to the results, the most active mushroom was *M. conica* with an absorbance value of 1.055 ± 0.025. At this concentration value, this mushroom was followed by the other in the order *M. esculenta* var. *umbrina* > *M. crassipes* > *M. elata* > *M. angusticeps* > *M. rotunda* > *M. deliciosa*. Reducing power of BHT, BHA and quercetin at 0.02 mg ml^{-1} were 0.163 ± 0.004, 0.321 ± 0.014 and 0.459 ± 0.004, respectively.

Table 4 shows the chelating effects of the methanolic extract of *Morchella* species compared with EDTA as standard on ferrous ions. As can be seen from the table, chelating capacity of the extracts

Table 1
Antioxidant activity (%) of the mushroom species measured by β-carotene–linoleic acid method[a].

Mushroom species	Sample concentration (mg ml^{-1})		
	0.5	2.0	4.5
M. rotunda	85.70 ± 0.93	93.91 ± 0.71	95.24 ± 0.03
M. crassipes	77.42 ± 0.01	94.28 ± 1.31	96.01 ± 0.15
M. esculenta var. umbrina	86.77 ± 0.38	95.46 ± 0.52	96.89 ± 0.34
M. deliciosa	86.33 ± 0.10	93.72 ± 0.05	95.63 ± 0.16
M. elata	63.18 ± 0.52	92.95 ± 0.19	94.37 ± 0.35
M. conica	82.38 ± 2.20	91.34 ± 3.69	96.55 ± 0.04
M. angusticeps	67.42 ± 0.44	93.04 ± 4.07	96.88 ± 0.09
BHT	96.51 ± 0.32	–	–
BHA	92.50 ± 0.17	–	–
Quercetin	96.22 ± 0.57	–	–

[a] Values expressed are means ± S.D. of three parallel measurements.

Table 2
Scavenging effect (%) of the mushroom species on 1.1-diphenyl-2-picrylhydrazyl[a].

Mushroom species	Sample concentration (mg ml^{-1})			
	0.1	0.5	2.0	4.5
M. rotunda	–	8.24 ± 0.34	33.94 ± 0.96	61.20 ± 1.52
M. crassipes	–	10.70 ± 0.73	34.82 ± 1.80	65.56 ± 2.00
M. esculenta var. umbrina	–	10.78 ± 0.56	33.68 ± 1.77	62.57 ± 1.83
M. deliciosa	–	3.96 ± 0.21	19.24 ± 1.50	40.63 ± 1.13
M. elata	–	4.60 ± 0.50	22.54 ± 1.05	48.07 ± 1.35
M. conica	–	13.91 ± 0.48	43.27 ± 0.48	85.36 ± 2.19
M. angusticeps	–	5.23 ± 0.34	22.90 ± 0.14	54.54 ± 0.48
BHT	72.74 ± 0.26	–	–	–
BHA	95.30 ± 0.18	–	–	–
Quercetin	98.75 ± 0.48	–	–	–

[a] Values expressed are means ± S.D. of three parallel measurements.

Table 3
Reducing power (absorbance at 700 nm) of the mushroom species[a].

Mushroom species	Sample concentration (mg ml)			
	0.02	0.5	2.0	4.5
M. rotunda	–	0.080 ± 0.006	0.271 ± 0.004	0.574 ± 0.009
M. crassipes	–	0.103 ± 0.001	0.389 ± 0.017	0.862 ± 0.033
M. esculenta var. *umbrina*	–	0.116 ± 0.001	0.454 ± 0.006	0.943 ± 0.036
M. deliciosa	–	0.073 ± 0.001	0.262 ± 0.012	0.563 ± 0.009
M. elata	–	0.095 ± 0.003	0.366 ± 0.013	0.831 ± 0.037
M. conica	–	0.145 ± 0.000	0. 531 ± 0.018	1.055 ± 0.025
M. angusticeps	–	0.062 ± 0.004	0.270 ± 0.005	0.639 ± 0.014
BHT	0.163 ± 0.004	–	–	–
BHA	0.321 ± 0.014	–	–	–
Quercetin	0.459 ± 0.004	–	–	–

[a] Values expressed are means ± S.D. of three parallel measurements.

Table 4
Chelating effect (%) of the mushroom species[a].

Mushroom species	Sample concentration (mg ml^{-1})		
	0.050	0.125	0.250
M. rotunda	82.33 ± 1.25	92.69 ± 0.22	95.44 ± 0.15
M. crassipes	88.08 ± 1.03	94.66 ± 1.69	96.68 ± 1.03
M. esculenta var. *umbrina*	87.25 ± 0.15	92.49 ± 0.37	94.87 ± 0.07
M. deliciosa	86.17 ± 0.81	87.77 ± 0.73	88.08 ± 0.00
M. elata	89.90 ± 0.81	95.54 ± 0.44	96.22 ± 0.37
M. conica	85.75 ± 2.56	93.01 ± 0.07	95.34 ± 0.44
M. angusticeps	88.81 ± 0.44	93.16 ± 0.88	96.01 ± 0.07
EDTA	96.22 ± 0.36	–	–

[a] Values expressed are means ± S.D. of three parallel measurements.

Table 5
Scavenging effect (%) of the mushroom species on the stable ABTS$^{\cdot+}$[a].

Mushroom species	Sample concentration (μg ml^{-1})		
	8	20	40
M. rotunda	50.16 ± 1.38	65.75 ± 0.92	76.06 ± 0.23
M. crassipes	49.35 ± 0.69	57.33 ± 1.84	71.50 ± 3.45
M. esculenta var. *umbrina*	52.44 ± 0.00	61.56 ± 2.76	76.38 ± 1.61
M. deliciosa	43.97 ± 0.92	48.21 ± 1.84	58.47 ± 2.99
M. elata	48.21 ± 0.46	54.23 ± 2.53	68.08 ± 2.76
M. conica	48.37 ± 2.53	57.00 ± 1.84	78.66 ± 2.07
M. angusticeps	51.30 ± 1.61	56.03 ± 0.92	70.36 ± 0.46

[a] Values expressed are means ± S.D. of three parallel measurements.

was increased with the increasing concentration. Except *M. deliciosa*, chelating effect of the methanol extracts was higher than 90% at 0.250 mg ml^{-1} concentration. The most active mushrooms were determined as *M. crassipes*, *M. elata* and *M. angusticeps*, respectively.

As can be seen from the Table 5, in 40 μg ml^{-1} concentration, the methanol extract of *M. conica*, exhibited the highest radical scavenging activity (78.66 ± 2.07%) when reacted with the ABTS$^{\cdot+}$ radicals. This activity was closely followed by the methanol extracts of *M. esculenta* var. *umbrina* and *M. rotunda*, respectively.

3.2. Determination of metal contents

Seven elements (Cu, Mn, Co, Zn, Fe, Ca, and Mg) and five heavy metals (Ni, Pb, Cd, Cr, and Al) were determined in seven *Morchella* species.

Element concentrations of the mushroom species are presented in Table 6. According to the results, the most abundant elements were calcium and magnesium, respectively. These are followed by Fe. On the other hand, cobalt was the lowest element presented in this table (ranged between 0.08 and 1.47 mg kg^{-1}).

In the case of heavy metals, the most abundant was aluminum (Table 7). Amount of this metal was ranged between 62.00 and 522.00 mg kg^{-1}. When compared with aluminum, the amount of nickel was much lower. It was ranged between 2.06 and 19.48 mg kg^{-1}among the mushroom species studied. The lowest heavy metal was determined as Pb ranged between 0.26 and 1.14 mg kg^{-1}.

3.3. Assay for total phenolics and flavonoids

As can be seen from the Table 8, *M. conica* found to have the highest phenolic content (25.38 ± 0.70 μg GAEs/mg extract) among the mushroom species evaluated. This is followed by *M. esculenta* var. *umbrina* with a value of 21.33 ± 1.40 μg mg^{-1}. In the case of total flavonoid content, *M. rotunda* found superior to the other mushrooms (0.59 ± 0.01 μg QEs/mg extract). The lowest flavonoid content was determined within *M. deliciosa* extract (0.15 ± 0.02 μg mg^{-1} extract).

4. Discussion

While wild mushrooms have potential beneficial properties, including antioxidant (Mau et al., 2002; Elmastas et al., 2006; Sarikurkcu et al., 2008), beta-glucans, cholesterol-lowering (Fukushima et al., 2001, 2002) activities to be used as functional foods, they have also potential hazards (e.g. heavy metals) (Gadd, 1993; Gaso et al., 1998; Kirchner and Daillant, 1998; Svoboda et al., 2000; Kalac, 2001; Falandysz et al., 2003; Vetter, 2004). Consecutive large consumption of *Tricholoma flavovirens* or *T. equestre* in mice (Bedry et al., 2001) and humans (Bedry et al., 2001; Nieminen et al., 2005) has been shown to cause an increase in plasma creatine kinase activities and tachypnea, reduction in motor activity, diarrhoea, and muscle fibre disorganisation visible in light microscopy. The potential toxicity of prolonged mushroom intake seems to be as likely as its potential for lowering of serum cholesterol values (Nieminen et al., 2009). Thus, the balance of detrimental and health promoting effects of mushroom consumption should be in general considered.

The free radical linoleic acid attacks the highly unsaturated β-carotene, and the presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralising the linoleate free radical and other free radicals formed in the system. The absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant the colour was retained for a long time.

M. esculenta is an edible and highly prized mushroom. Commercial cultivation of this mushroom has not been successful till now and hence its mycelium is extensively used as flavoring agent (Nitha et al., 2007). According to the literature records; antioxidant, anti-inflammatory and antitumoral activities of these species have previously been investigated (Mau et al., 2004; Elmastas et al., 2006; Nitha et al., 2007; Ramirez-Anguiano et al., 2007). Based on a detailed report on the antioxidant activity of *M. esculenta*, it has exhibited excellent activity patterns in β-carotene/lin-

Table 6
Element concentrations of the mushroom species[a].

Mushroom	Cu	Mn	Co	Zn	Fe	Ca	Mg
M. rotunda	26.00 ± 0.22[b]	34.80 ± 0.06	0.08 ± 0.02	75.80 ± 0.06	254.00 ± 1.00	2480.00 ± 6.00	1184.00 ± 2.00
M. crassipes	45.20 ± 1.24	38.00 ± 0.28	0.69 ± 0.05	88.80 ± 0.48	476.00 ± 4.00	3560.00 ± 14.00	1490.00 ± 8.00
M. esculenta var. *umbrina*	21.80 ± 0.18	22.60 ± 0.12	0.12 ± 0.00	153.00 ± 0.02	304.00 ± 2.00	2340.00 ± 18.00	1272.00 ± 1.00
M. deliciosa	18.94 ± 0.20	18.08 ± 0.17	0.35 ± 0.04	93.80 ± 1.28	96.00 ± 2.00	742.00 ± 5.00	974.00 ± 1.00
M. elata	38.20 ± 0.24	13.98 ± 0.08	nd[c]	120.60 ± 0.74	72.00 ± 2.00	1354.00 ± 11.00	1386.00 ± 5.00
M. conica	11.66 ± 0.03	25.00 ± 0.02	1.47 ± 0.03	126.00 ± 0.10	336.00 ± 1.00	1404.00 ± 7.00	1690.00 ± 28.00
M. angusticeps	11.20 ± 0.01	45.60 ± 0.10	1.27 ± 0.02	110.80 ± 0.58	594.00 ± 7.00	5180.00 ± 36.00	1662.00 ± 7.00

[a] mg/kg, dry weight basis.
[b] Mean ± standard deviation, $n = 5$.
[c] nd, not determined.

Table 7
Heavy metal concentrations of the mushroom species[a].

Mushroom	Ni	Pb	Cd	Cr	Al
M. rotunda	8.52 ± 0.02[b]	0.87 ± 0.20	1.89 ± 0.03	nd[c]	91.00 ± 1.00
M. crassipes	4.40 ± 0.04	0.26 ± 0.33	1.78 ± 0.03	nd	258.00 ± 0.00
M. esculenta var. *umbrina*	6.40 ± 0.07	0.70 ± 0.37	0.72 ± 0.01	1.21 ± 0.09	145.00 ± 2.00
M. deliciosa	2.06 ± 0.06	1.14 ± 0.11	1.04 ± 0.03	nd	62.00 ± 1.000
M. elata	6.58 ± 0.03	0.44 ± 0.41	0.74 ± 0.02	nd	84.00 ± 0.00
M. conica	15.74 ± 0.14	0.85 ± 0.04	0.74 ± 0.02	3.58 ± 0.23	159.00 ± 1.00
M. angusticeps	19.48 ± 0.08	0.96 ± 0.21	1.36 ± 0.01	4.92 ± 0.17	522.00 ± 2.00

[a] mg/kg, dry weight basis.
[b] Mean ± standard deviation, $n = 5$.
[c] nd, not determined.

oleic acid, reducing power, DPPH and metal chelating effect systems (85.4–94.7% at 25 mg ml^{-1} concentration; 0.97–1.02 at 25 mg ml^{-1} concentration; 78.8–94.1% at 10 mg ml^{-1} concentration; and 90.3–94.4% at 10 mg ml^{-1} concentration, respectively) (Mau et al., 2004). On the other hand, in another study on the same mushroom, results obtained from in β-carotene/linoleic acid has been determined as 80–87% at different experimental concentrations (Elmastas et al., 2006). In the light of the data given above, our results are parallel with them, except some minor differences. According to our literature research, there are limited numbers of reports on these mushroom species. Most of them have especially been focused on *M. esculenta* but no record is available for var. *umbrina*. Antioxidant activity of *M. conica* has also been investigated by Turkoglu et al. (2006). According to this report, inhibition capacity of this mushroom against the oxidative stress of oxygen has been determined as 96.9%, approximately. The antioxidant activity of this sample has been found as 82–96% at different concentration values in this study. Hereby, our results from this study are completely homologous with Turkoglu et al. (2006). Since the activity potentials of the other mushroom species evaluated here (*M. rotunda*, *M. crassipes*, *M. deliciosa*, *M. elata*, and *M. angusticeps*) are not available in the literature, data presented here could be assumed as the first records for them.

The radical scavenging of mushrooms extracts was tested using a methanolic solution of the "stable" free radical, DPPH. Unlike laboratory-generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition (Amarowicz et al., 2004). Table 2 summarizes the effective concentrations of each extract and reference compound required to scavenge DPPH radical, the scavenging values as percentage. The extracts prepared by methanol exhibited varying degrees of scavenging capacities. *M. conica* showed the strongest radical scavenging effect (85.36± 2.19%) in the case of 4.5 mg ml^{-1} which is better than those of the positive controls BHT (72.74 ± 0.26%) at 0.1 mg ml^{-1}. The methanolic extracts from other mushrooms showed a moderate increase in antioxidant activity, from 3.96 ± 0.21% at 0.5 mg ml^{-1} to 65.56 ± 2.00% at 4.5 mg ml^{-1}. At the previous studies, the same situations were reported for cold and hot water extracts of *Pleurotus citrinopileatus* (Lee et al., 2007) and methanolic extract of *Lactarius deterrimus* (Sarikurkcu et al., 2008).

Table 8
Total phenolics and flavonoids of the mushroom species[a].

Mushrooms	Phenolic content (μg GAEs/mg extract)[b]	Flavonoid content (μg QEs/mg extract)[c]
M. rotunda	16.98 ± 1.03	0.59 ± 0.01
M. crassipes	18.59 ± 0.70	0.47 ± 0.05
M. esculenta var. *umbrina*	21.33 ± 1.40	0.25 ± 0.03
M. deliciosa	12.36 ± 1.21	0.15 ± 0.02
M. elata	15.36 ± 0.05	0.30 ± 0.01
M. conica	25.38 ± 0.70	0.24 ± 0.01
M. angusticeps	16.55 ± 0.98	0.26 ± 0.04

[a] Values expressed are means ± S.D. of three parallel measurements.
[b] GAEs. gallic acid equivalents.
[c] QEs. quercetin equivalents.

Assay of reducing activity was based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the tested samples. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986). The reducing power of the mushroom methanolic extracts increased with concentration. At the concentration of 4.5 mg ml^{-1}, the reducing powers of *Morchella rotunda, M. crassipes, M. esculenta* var. *umbrina, M. deliciosa, M. elata, M. conica,* and *M. angusticeps* were higher than those of BHA (0.321 ± 0.014), BHT (0.163 ± 0.004) and quercetin (0.459 ± 0.004) at 0.02 mg ml^{-1}, and were 0.574 ± 0.009, 0.862 ± 0.033, 0.943 ± 0.036, 0.563 ± 0.009, 0.831 ± 0.037, 1.055 ± 0.025 and 0.639 ± 0.014, respectively. According to these results, *M. conica* was found as the better radical reducer for this system. Mau et al., 2004 found that the reducing power of methanolic extract from *M. esculenta* was 0.11 at 0.5 mg ml^{-1} and our results for *M. esculenta* var. *Umbrina* (0.116 ± 0.001) at the same concentration are strongly similar with them.

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Gordon, 1990). The catalysis of metal ions also correlates with incidents of cancer and arthritis (Halliwell et al., 1995). Ferrous ions, the most effective pro-oxidants, are commonly found in food systems (Yamaguchi et al., 1998). Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991). The chelating ability of the mushroom extracts toward ferrous ions was investigated. There was no significant difference between chelating capacity of methanolic extracts of mushrooms at all of concentration. At 0.05 mg ml^{-1} concentration, the percentage of metal chelating capacity of methanolic extract of *M.rotunda, M. crassipes, M. esculenta* var. *umbrina, M. deliciosa, M. elata, M. conica* and *M. angusticeps*, and EDTA were found as 82.33 ± 1.25%, 88.08± 1.03%, 87.25 ± 0.15%, 86.17 ± 0.81%, 89.90 ± 0.81%, 85.75 ± 2.56%, 88.81 ± 0.44%, and 96.22 ± 0.36%, respectively, and these results were higher than those of the ethanolic extracts of *Morchella vulgaris* and *M. esculenta* (Elmastas et al., 2006). Soares et al. (2009) found that the chelating abilities of the methanolic extracts of *Agaricus brasiliensis* (mature) (MB) were higher than those of extracts of *A. brasiliensis* (young) (YB) and at 10 and 20 mg ml^{-1}, MB chelated 62 ± 7.8% and 78 ± 6.1% of ferrous ions whereas YB chelated 46 ± 6.3% and 61 ± 6.9%, respectively. All of the extracts evaluated here showed

significantly higher chelating effects on ferrous ions than those of data reported by Soares above.

In some cases, the scientists can not establish a clear relationship between the results obtained from the complementary test systems. This fact could be explained by the following possibilities: (i) some of the phytochemicals available in the mushroom extracts may contain high molecular weight antioxidants or antioxidants bound to complex molecules such as dietary fibers (Manzi et al., 2004), beta-glucans (Chauveau et al., 1996) and other polysaccharides present in the methanol extracts; (ii) some of the phenolic compounds might not have antioxidant properties. In order to eliminate these speculations, the $ABTS^{\cdot+}$ scavenging capacities of the mushroom extracts were studied. At the concentration of 20 μg ml^{-1}, the $ABTS^{\cdot+}$ scavenging capacity was higher than 48% and in the order *M. rotunda* > *M. esculenta* var. *umbrina* > *M. crassipes* > *M. conica* > *M. angusticeps* > *M. elata* > *M. deliciosa*. In 40 μg ml^{-1} concentration, the methanol extract of *M. conica*, exhibited the highest radical scavenging activity (78.66% ± 2.07) when reacted with the $ABTS^{\cdot+}$ radicals.

Antioxidant properties of mushrooms are usually related to low-molecular-weight compounds, in particular to the phenolic fractions. Therefore, a wide range of these potentially beneficial phenolic compounds could be natural substrates of oxidative enzymes, such as peroxidases or polyphenol oxidases, which are present in high levels in mushrooms (Lee and Jang, 2004; Cheung and Cheung, 2005).

Some mechanisms are available for the mode of action of phenolic compounds in antioxidant activity test systems. One of them has been put forward by Ramirez-Anguiano et al. (2007). According to this group, the oxidation of diphenols to quinines is a very fast reaction, which might occur in seconds. Even when only a few quinones are formed before the preparation of the extract, they become to the low molecular weight compounds and might react spontaneously with other phenols, generating molecules like dopachrome, indolic compounds, catechol dimers and other higher polymers yielding radical scavenging degradation products. Therefore, it could be concluded that the phenolic compounds were highly involved in the antioxidant activity found in these mushroom extracts, but other compounds mainly having low molecular weight were also able to enhance or complement their activity.

Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities. These activities may be related to their antioxidant activity (Chung et al., 1998). Thus, the total phenolic and flavonoid contents of the mushrooms were also evaluated.

Polyphenolic compounds seem to have important role in lipid oxidation stabilization and to be associated with antioxidant activity when the results obtained from the total phenolic assay are compared with the literature, (Yen et al., 1993; Gulcin et al., 2003). The phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka et al., 1998).

The antioxidant activity of plant materials is well correlated with their content in phenolic compounds (Velioglu et al., 1998). More recently, several researchers have shown a correlation between total phenolic content and antioxidant activity in mushroom extracts (Cheung et al., 2003; Turkoglu et al., 2007). According to our results, considering the fact that different contents of phenolics were found in the methanolic extracts of mushrooms, but presented a similar antioxidant activity in β-carotene/linoleic acid system, chelating ability and $ABTS^{\cdot+}$ radical activity, it is acceptable to conclude that other factors may be involved in these properties. In this respect, data in the literature about the relation between concentration of phenolic compounds and antioxidant activity are contradictory. While some authors have observed high correlation (Cheung et al., 2003), others find no direct correlation or only a very weak one since the antioxidant action is raised by other substances such as tocopherols and β-carotene. Lindsay (1996) has been reported that compounds with structures containing two or more of the following functional groups –OH, –SH, –COOH, $–PO_3H_2$,–COO, $–NR_2$, –S– and –O– in a favourable structure–function configuration can show metal chelating activity.

Mushrooms with the fruits and vegetables are important sources of essential elements, ranking after animal tissues. Minerals play a vital role in the proper development and health of human body. However, high amounts of certain minerals are also toxic for most organisms (Savas et al., 1995). As far as our literature survey could as certain, there are several reports on the metal concentrations of *M. rotunda* (Konuk et al., 2007), *M. deliciosa* (Turhan, 2007) and *M. elata* (Isildak et al., 2004) in the literature. Although heavy metal concentration of *M. esculenta* has been determined by Yesil et al. (2004), no study has been made for var. *umbrina*. Data presented on the metal concentrations on *M. crassipes*, *M. conica* and *M. angusticeps* could be assumed as the first report on this topic.

According to the EU Scientific Committee for Food Adult Weight parameter, 60 kg of body weight was used for intake calculations as the weight of an average consumer. In addition, for intake calculations, usually a 300 g portion of fresh mushrooms per meal is assumed, which contains 30 g of dry matter (Kalac and Svoboda, 2000; Svoboda et al., 2000). The metal intakes by a normal (60 kg) consumer in mg/serving for *M.rotunda, M. crassipes, M. esculenta* var. *umbrina, M. deliciosa, M. elata, M. conica,* and *M. angusticeps* were calculated from table 7 as 0.057, 0.053, 0,022, 0.031, 0.022, 0.022 and 0,041 for Cd, and 0.026, 0.008, 0.021, 0.034, 0.013, 0.026 and 0.029 for Pb, respectively .

These results conform to EU Scientific Committee (2001) standards for Pb, Cd and As (toxic metals). Provisional tolerable weekly intake values for Pb, Cd and As for adults (of 60 kg) are 1.50, 0.42 and 0.90 mg, respectively (Council of Europe, 2001). These values correspond to 0.21, 0.06 and 0.13 mg of Pb, Cd and As, respectively, on a daily basis. Therefore, the intake of heavy metals (Pb, Cd, As) by consumption of 30 g dry weight of mushrooms daily poses no risk at all for the consumer.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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