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# Fatty acid composition and antioxidant capacity of some medicinal mushrooms in Turkey

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**Abstract** Turkey has a very rich fungal flora due to its phytogeographical position. The screening of chemical content and active substances of mushrooms becomes an important subject not only for Turkey but also for all over the world. In the last decade, Analyses on phytochemical and biological activity of fungi have gradually increased as a result of improvement in the number and quality of facilities. In the scope of the present research, four medicinal mushrooms; *Morchella elata, Lactarius volemus, Cantharellus cibarius* and *Tricholoma terreum* were analyzed for their fatty acid compositions and antioxidant capacities. The fungal species have been found with unsaturated fatty acid/saturated fatty acid ratio of 6.73 for *Morchella elata*, 4.12 for *Lactarius volemus*, 5.21 for *Cantharellus cibarius*, 3.73 for *Tricholama terrum*. In addition, the concentration of malondialdehyde which was an indicator of lipid peroxidation was also determined in these species. According to the results, free radical scavenging activity of *Morchella elata* and *Lactarius volemus* were found higher than the other species. Any of the mushroom species investigated were found having very high metal chelating activity. The results showed that the extract of *Morchella elata* and *Lactarius volemus* exhibited significant antioxidant activities. Hence, the mushrooms have a potential to be a natural antioxidant in food industries as antioxidant agent.

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**Keywords** Antioxidant Capacity · Fatty Acid · Malondialdehyde Medicinal Mushroom

## Introduction

Wild edible mushrooms are preferred by many people due to their flavour and texture (Sanmee et al. 2003). Some of them have important medicinal properties as well. They can be used in preventive medicine against various diseases such as hypertension, hypercholesterolemia, antidiabetic and cancer (Manzi et al. 2001). Some of them can be evaluated as candidates for development of novel medicines and nutraceuticals due to their chemical compositions (Elbatrawy et al. 2015). Fruit body of mushrooms accumulates a variety of bioactive metabolites with immunomodulatory, cardiovascular, liver protective, anti-fibrotic, anti-inflammatory, antidiabetic, antiviral, antioxidant, antitumor and antimicrobial properties (Vaz et al. 2012; Qin et al. 2015).

Medicinal mushrooms with biologically active substances are currently under human clinical trials and some promising results were obtained on their usage in conventional therapies (Money 2016). Morels (*Morhella spp.*) are reported to be used as a source of immunostimulants, antitumor agents and medicinal adaptogens (Nitha et al. 2007). Some compounds extracted from *Tricholoma terreum* were found to have cytotoxic effects against five human cancer cell lines (Yin et al. 2013). *Cantharellus cibarius* is a beneficial nutrient for human health with high levels of vitamins B and C (Muszynska et al. 2013). *Lactarius volemus* is a source of critical nutraceuticals, such as essential fatty acids and phenolic bioactive compounds (Reis et al. 2011).

Natural antioxidants can be used as an effective tool against oxidative damage which can result in ageing and degenerative diseases (Muka et al. 2015). Some mushrooms are an important source of natural antioxidants and essential fatty acids. Many studies have been conducted individually on fatty acids, antioxidant activities, mineral and trace elements of mushrooms (Ebrahimzadeh

et al. 2010; Falandysz, Drewnowska 2015; Goyal et al. 2015). But malondialdehyde (MDA), antioxidant properties and fatty acid composition of mushrooms have not been evaluated altogether. Our objective was to evaluate the antioxidant capacity, fatty acid composition and MDA contents of four wild edible medicinal mushroom species (*Lactarius volemus, Morchella elata*, *Tricholoma terreum*, and *Cantharellus cibarius*) collected from Turkey.

## Materials and Methods

### Chemicals

2,2-diphenyl-1-picryl-hydrazyl (DPPH·), ferrous chloride, α-tocopherol and trichloracetic acid (TCA) were purchased from Sigma-Aldrich Company (Sternheim, Germany). Butylated hydroxyl toluene (BHT), thiobarbituric acid (TBA), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (Ferrozine) and ethylenediaminetetraacetic acid (EDTA) were purchased from Merck.

### Collection and extraction of the samples

Mushrooms were collected from the Black Sea region of Turkey and identified. They have been stored at the Mycology Herbarium Laboratory of Gaziosmanpasa University (Turkey). Table 1 shows the details including herbarium number of mushroom samples used in this study.

Prior to the analyses, fresh mushrooms from each species were air-dried in an oven at 40 °C constant weight. Extraction procedure for antioxidant assays was performed according to the literature. Methanol extracts of dried mushroom samples (5 g) were individually grinded into fine powders in a mill and was mixed with 50 mL of methanol at room temperature with vigorous mixing at 150 rpm for 24 h. Extracts were obtained after solvent removal under vacuum using rotary evaporator at 40 °C.

### Extraction procedure and GC-FID condition for fatty acid content determination

Crude oil was obtained from mushroom samples by light petroleum ether (b.p. 40–60 °C) extraction. The solvent was removed by rotary evaporator and the extracted oil was used for fatty acid analysis.

The fatty acid methyl esters (FAMEs) of oil were obtained by transmethylation (Cantellops et al. 1999). The temperature of the injector and detector were 250 and 260 °C, respectively. Fatty acid analyses were performed by Gas Chromatography equipped Flame Ionization Detector (GC-FID), Perkin Elmer Clarus 500 Series GC system, with an apolar capillary column (30 m×0.25 mm and 0.25 μm ID). Helium was used as carrier gas. Initial column oven temperature of 100 °C was elevated to 220 °C at a rate of 2 °C/min and held there for a 10 min. Identification of fatty acid components was accomplished based on the comparison of their retention times with the authentic standards (Supelco Company, Fatty acid Mix, Bellefonte, PA, USA). The peak area percentages of compounds were calculated based on the FID data.

### Determination of malondialdehyde content

Ohya's method was used to determine the concentration of lipid peroxidation products in the mushroom (Ohya 1993). Samples (0.5 g each) were homogenized in 5 mL of 0.1 % (w/v) TCA, and then centrifuged at 10,000 g for 20 min. 0.5 mL of the supernatant was added to 1 mL TBA in 20 % TCA and the mixture was incubated in boiling water for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath. Then, they were centrifuged at 10,000 g for 5 min, and the absorbance of supernatant was read at 532 nm and corrected for non-specific turbidity by subtracting the absorbance value at 600 nm. An extinction coefficient of $1.55\times10^5$ $mM^{-1}$ $cm^{-1}$ was used to quantify lipid peroxides and it was expressed as nmol MDA $g^{-1}$ dry weight (DW).

### Determination of reduction power

The reducing power of methanolic extract of mushroom species was determined according to Oyaizu's method (Oyaizu 1988) with some modification (Canabady-Rochelle et al. 2015). Absorbance was read at 700 nm. The higher the absorbance represents the higher the reductive capability of samples.

### Free radical scavenging activity

The free radical scavenging activities of mushroom species were measured by DPPH method with some modifications (Brandwilliams et al. 1995). The absorbance was read at 517 nm by UV-VIS spectrophotometer.

Scavenged percentage of DPPH radical was calculated using the following equation:

$$\text{Scavenging of DPPH (\%)}=((A_0-A_1/A_0)\times100)$$

where $A_0$ was the absorbance of the negative control as $A_1$ was the absorbance of the extract of mushroom species.

**Table 1** Habitats, edibility and herbarium numbers of mushroom species

| Species of mushrooms | Habitat and Herbarium Number | Edibility |
|---|---|---|
| *Morchella eleta* Fr. | In conifer woods or on chalk soil (No: 3620) | Edible |
| *Tricholoma terreum* (Schaeff.: Fr.) | In woods, especially with conifers (No: 1847) | Edible |
| *Cantharellus cibarius* Fr. | In all kinds of woodlands (No: 2163) | Edible |
| *Lactarius volemus* Fr. | Under bothe coniferous and broad-leaved trees (No: 2630) | Edible |

### Chelating effect on ferrous Ions

The chelating of ferrous ions by mushroom species was calculated by Dinis' method (Dinis et al. 1994) with a positive control, EDTA. The percentage of inhibition of ferrozine-$Fe^{2+}$ complex formation was calculated with the following equation:

$$\text{Percentage Inhibition}=((A_0-A_1)/A_0)\times 100$$

where $A_0$ was the absorbance of the negative control as $A_1$ was the absorbance of extract and standards of mushroom species.

### Statistical analysis

The experimental results were mean ± SD of at least three independent measurements. Data were subjected to analysis of variance (ANOVA) and the group means were compared through Paired-Samples T Test or Duncan's Multiple-Range Test using SPSS 20 statistical package. $P<0.05$ were regarded as significant and $p<0.01$ as very significant.

## Results and Discussion

### Determination of fatty acids

In the present study, fatty acid compositions of four medicinal mushroom species: *Lactarius volemus, Morchella elata, Tricholoma terreum, and Cantharellus cibarius* were analyzed. Results of fatty acid composition analyses in the mushroom samples were demonstrated in Table 2. According to the Table 2, it is clearly seen that the mushroom species comprised of myristic, pentha-decanoic, penthadecenoic, palmitic, oleic, linoleic, linoelaidic and eicosanoic acids. Fatty acid composition of mushroom samples shows differences between the species. Total unsaturated fatty acid (UFA) levels were ranged from 78 to 86 % as the total saturated fatty acid (SFA) ranged from 12 to 21 % in all four species (Table 2). Linoleic acid was observed at the highest amount except for *Morchella elata* which contains high amount of oleic acid (Table 2). In the earlier studies on fatty acid compositions of some mushroom species, the amount of UFA was found at the highest value. When the individual fatty acid compositions were compared along the mushroom species, it has been showed that linoleic and oleic acids percentages are dominant. The results obtained in this study are consistent with the previously reported results in the literature (Yilmaz et al. 2006; Goyal et al. 2015). In addition to linoleic acid, the other fatty acids such as oleic acid, palmitic acid, penthadecenoic acid and linoelaidic acids were also found in the mushroom samples (Table 2).

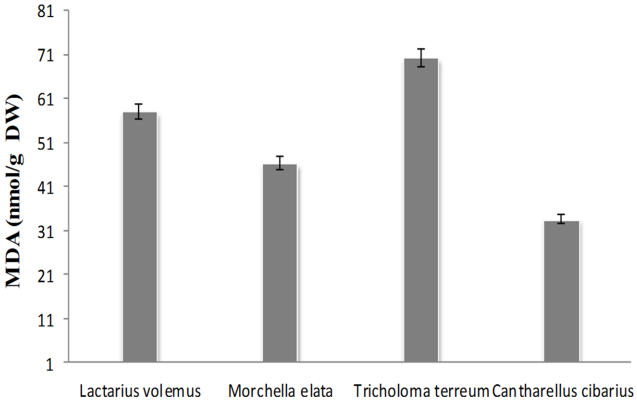


**Fig. 1** MDA content of *Morchella elata*, *Lactarius volemus, Cantharellus cibarius, Tricholoma terreum*

### Determination of malondialdehyde

Lipid peroxidation was the process that involved the chain reactions of free radicals attacked to polyunsaturated fatty acid (PUFA). Result of the reaction leaded to lipid breakdown to yield products such as MDA. Therefore inhibition of lipid peroxidation has great importance in storage of PUFA rich foods like mushrooms.

**Table 2** Fatty acid compositions of medicinal mushroom samples

| Fatyy acid | Percentage of fatty acids (%) | | | | *p* value |
|---|---|---|---|---|---|
| | *L.volemus* | *M. elata* | *T. terrum* | *C. cibarius* | |
| Myristic acid (C14:0) | | | 0.37 | | 0.01 |
| Penthadecanoic acid (C15:0) | 0.70 | $0.11^{c}$ | $1.18^{c}$ | $0.34^{c}$ | 0.05 |
| Cis-10-penthadecenoic Acid | 0.65 | $0.26^{c}$ | $11.93^{b}$ | $0.43^{c}$ | 0.05 |
| Palmitic acid (C16:0) | $18.76^{b}$ | $12.73^{b}$ | $14.33^{b}$ | $20.69^{b}$ | 0.05 |
| Oleic acid (C18:1n9c) | | $77.57^{a}$ | °° | $15.04^{b}$ | 0.01 |
| Linoleic acid (C18:2n6c) | $78.93^{a}$ | $7.24^{d}$ | $66.25^{a}$ | $50.54^{a}$ | 0.01 |
| Linoelaidic acid (C18:2n6t) | $0.16^{d}$ | $1.36^{c}$ | $4.63^{c}$ | $12.39^{b}$ | 0.01 |
| Cis-11-eicosanoic acids. | 0.35 | | | | 0.01 |
| ΣSFA | $19.46^{b}$ | $12.84^{b}$ | $15.88^{b}$ | $21.03^{b}$ | 0.05 |
| ΣUSSFA | $80.08^{a}$ | $86.19^{a}$ | $82.81^{a}$ | $78.4^{a}$ | 0.05 |
| ΣMUFA | $1^{d}$ | $77.83^{a}$ | $11.93^{b}$ | $15.47^{b}$ | 0.01 |
| ΣPUFA | $79.09^{a}$ | $8.36^{d}$ | $70.88^{a}$ | $62.93^{a}$ | 0.05 |
| ΣUSSFA/SFA | $4.12^{c}$ | $6.73^{d}$ | $5.21^{c}$ | $3.73^{c}$ | 0.05 |

[a,b]Means within a rpw with no common supercript differ significantly (Duncan, $p<0.05$)

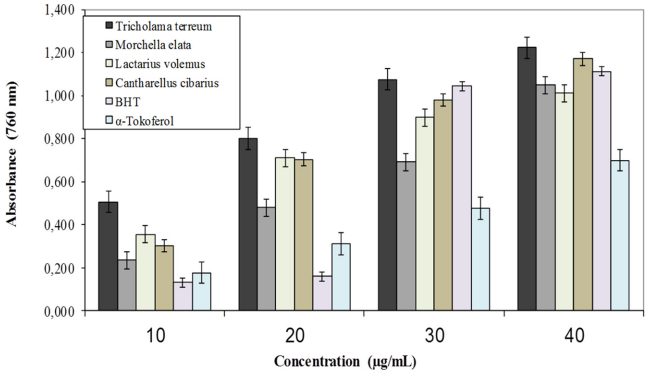


**Fig. 2** Comparision of reducing power of different concentrations of *Morchella elata*, *Lactarius volemus, Cantharellus cibarius, Tricholama terreum*, BHT, and α-tocopherol by spectrophotometric detection of the $Fe^{+3}$-$Fe^{+2}$ transformations at 700 nm (BHT: Butylated hydroxytoluene)

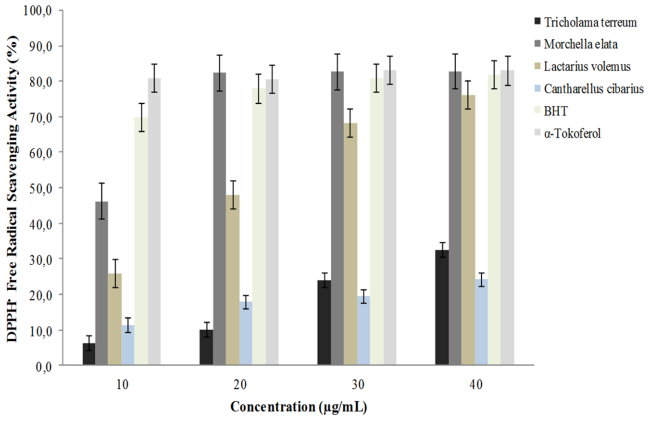


**Fig. 3** Free radical scavenging activity of different concentrations of *Morchella elata*, *Lactarius volemus, Cantharellus cibarius, Tricholoma terreum* BHT, and α-tocopherol by 1,1-diphenyl-2-picrylhydrazyl radicals (BHT: Butylated hydroxytoluene)

MDA analysis results were summarized in Fig. 1. It is clearly seen in the Fig. 1 that MDA contents were observed as 33.58 nmol/g DW in *C. cibarus*, 46.21 nmol/g DW in *M. elata,* 58.01 nmol/g DW in *L. volemus,* and 70.22 nmol/g DW in *T. terreum.* MDA level could effectively reflect the content of free radicals produced by lipid peroxidation (Layali et al. 2015). In the present study, we observed that increase in the MDA content was associated with USFA/SFA ratio in *C. cibarus*, *L. volemus*, and *T. terreum* mushroom species. On the contrary, the ratio of USFA/SFA of *M. elata* was found high despite low MDA content (Fig. 1 and Table 2). This could be due to high fat-soluble vitamins content or high antioxidant capacity of *M. elata*. Obtained results in this study obviously showed that free radical scavenging activity of *M. elata* is higher than the other mushroom species (Fig. 2).

**Determination of reducing power**

Ferric reduction power of a compound or an extract can be used with one of the indicators of antioxidant capacity (Demirtas et al. 2013). Ferric reduction power of mushroom species in this study were shown in Fig. 2 and results were compared to BHT and α-tocopherol. The mushroom species activities had higher than α-tocopherol but lower than BHT. The differences were statistically significant ($p$ <0.05). Reducing power of methanol extract of all of mushroom species in this study have been found higher than alpha-tocopherol but lower than BHT. The activity of *Tricholoma terreum* has been found higher than the other mushroom species (Fig. 2).

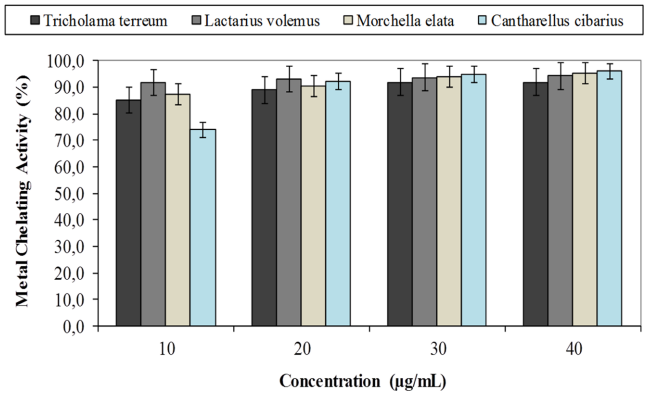


**Fig. 4** Metal chelating effects of different concentrations of *Morchella elata*, *Lactarius volemus, Cantharellus cibarius, Tricholoma terreum,* BHT, and α-tocopherol on ferrous ions

**Free radical scavenging activity**

Scavenging of DPPH radicals was determined by the decrease of the absorbance at 517 nm as a result of the interactions between DPPH radicals and antioxidants. Fig. 3 illustrates a significant ($p$ <0.05) decrease in the concentration of DPPH radical due to the scavenging ability of mushroom species with respect to a positive control. Methanol extract of the mushroom species, except for *T. terreum*, showed strong DPPH scavenging activity at 10 µg/mL concentration. The scavenging activity of the samples and the standards on the DPPH radical followed the order of *Morchella elata* > α-tocopherol > BHT > *Lactarius volemus* > *Cantharellus cibarius* > *Tricholoma terreum* with inhibition percentages of 82, 80, 77, 48 and 17 at 20 µg/mL concentrations, respectively. These results indicated that methanol extract of *Morchelal elata* has better effect on scavenging free radicals among the all four species.

**Chelating effect on ferrous ions**

As shown in Fig. 4, the formation of the $Fe^{2+}$-ferrozine complex was prevented by the extracts of mushroom samples. The metal chelating capacity of 30 µg/mL concentration of *Tricholama terreum, Lactarius volemus*, *Morchella elata*, and *Cantharellus cibarius* were found as 92, 93, 94 and 95 %, respectively. The differences were accepted as statistically insignificant between the samples ($p$ >0.05).

The data obtained from Fig. 4 reveals that the mushrooms used demonstrate marked capacity of iron binding which could be explained by their action as peroxidation protector related to probable iron binding capacity.

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