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Antioxidant Potential of *Lactarius deliciosus* and *Pleurotus ostreatus* from Amanos Mountains

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Abstract

Background: Edible mushrooms are considered as the significant source of minerals and vitamins. *Lactarius deliciosus* and *Pleurotus ostreatus* are the two known edible mushroom species because of their distinctive taste as well as nutritional properties.

Methods: *L. deliciosus* and *P. ostreatus* were collected from the native flora of Amanos Mountains in Turkey. The methanol (MeOH) extract of the *L. deliciosus* and *P. ostreatus* was obtained to assess antioxidant potential, Total phenolic, flavonoid, β -carotene and lycopene content.

Results: Total phenolic (mg/kg), flavonoid (mg/kg) and β -carotene (mg/100 ml) contents of the MeOH extracts of *L. deliciosus* and *P. ostreatus* were 34.55, 6.03, 1.15 and 15.66, 0.16, 0.02, respectively. Lycopene was only detected in *L. deliciosus* with a content of 0.25 (mg/100 ml). At 5 mg/ml, DPPH (%), RP (Abs.), H_2O_2 (%), NO (%) and FRAP (mmol Fe^{2+}/L) activities of *L. deliciosus* and *P. ostreatus* were 45.33, 0,790, 88.30, 55.51, 0.57 and 17.42, 0,530, 73.77, 33.64, 0.28, respectively.

Conclusion: Pearson correlation among the antioxidant results was found to be well correlated ($r \geq 0.90$). Antioxidant results were highly correlated ($r \geq 0.93$) with total phenolic compounds and lycopene. As of date, there has no previous work not only the NO but also H_2O_2 radical scavenging capabilities of *L. deliciosus* and *P. ostreatus*.

Keywords:

Lactarius, *Pleurotus*,
antioxidant, scavenging,
Amanos

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Introduction

The combination of substances with oxygen causes losing electrons and atoms and yield energy, which also forms a significant source of fuels for the fundamental process in the livings to sustain their life [1]. Some illness e.g. cancer, diabetes, rheumatism, cirrhosis, arteriosclerosis and etc. could be resulted from a variety of free radicals and reactive oxygen species generated in the cell nature [1-4]. In a daily life, edible food has the significant sources of natural constituents might be helpful for living things to reduce damages derived from oxidation [5-7]. Edible mushrooms are considered as the significant source of minerals and vitamins [8-10]. Today, mushroom consumption is steadily increasing due to developments in cultivation techniques [11]. Antioxidant activities of these group of organisms were previously investigated by many authors [11-19]. Turkey has a great potential in terms of natural edible mushrooms and is becoming a major exporter in the world [20]. Amanos Mountains, also known as Nur or Gavur, are located in South of Turkey. Amanos Mountains have been surrounded by Osmaniye (Duzici) and Kahramanmaraş in North, Gaziantep (Islahiye) in East, Hatay (Samandag) in South. The highest point of Amanos Mountains is Boztepe (2240 m). Amanos, is the place important for bio diversification, which include 1580 plant species and 251 of those are endemics.

In the present work, phenol, flavonoid, β -caroten and lycopene contents, antioxidant activities including DPPH, RP, NO, FRAP and H_2O_2 potential of the MeOH extract prepared from *L. deliciosus* and *P. ostreatus* collected from the native flora of Amanos Mountains were analyzed. Antioxidant potential of *L. deliciosus* and *P. ostreatus*, could be the first investigation in the Amanos region.

Methods

Fungal Samples and Extraction

Lactarius deliciosus and *Pleurotus ostreatus* were collected from Cebel (37°01'17" N, 36°22'10" E, 988 m) and Türkü (37°02'01" N, 36°28'18" E, 1189 m) regions of Amanos Mountains (Yarpuz-Osmaniye, Turkey, on 15 November 2014). After collection, mushroom samples were dried in a dehydrator for 48 h and then powdered. Dried samples were pulverized in a blender (Waring Blender, HGB2WTS3). Extraction step was carried out using the powder (50 g) in 400ml of the MeOH solvent

at 65 °C for 72 h and this followed by removing the solvent in a rotary evaporator at 40 °C and then preserved at refrigerated state until use. The yield (% w/w) was 10.62 in *Lactarius deliciosus* and 6.30 in *Pleurotus ostreatus*, respectively.

Total Phenolics (TP)

Folin-Ciocalteu (FC) technique was used to determine the phenolic compounds of the samples [13]. Distilled water and FC reagent (45: 1 ml, 2 N) were transferred into flask including the test extract (1 ml) from the stock solution (2 mg/ml), respectively. After 5 minutes, Na_2CO_3 solution (3.0 ml, 2.0%) was pipetted and incubated (2 h) in the dark. At the wavelength of 760 nm in a spectrophotometer (UV 1800 Shimadzu). As a standard, gallic acid ranged from 0 to 100 mg/l was used for preparing the slope.

Total Flavonoids (TF)

The modification of Dowd technique used by Gursoy *et al.* was employed [13]. As described in total phenolic contents, aliquot from the stock solution (1 ml): equal amount of aluminium trichloride solution (2%, $AlCl_3$) was kept for 10 min. Blank had only extract solution and MeOH (1:1, ml). Following the incubation, reaction was assessed versus MeOH blank, at the wavelength of 415 nm. Quercetin at varies concentrations were prepared to compare the results (mg QE/kg).

β -Carotene and Lycopene Content

The method and the formula used by Barros *et al.* were employed for these chemical parameters in this assay [21]. 0.1 g of the extract: 10 ml of acetone: hexane solution (4:6) was thoroughly shaken for 60 sec. The solution was filtered and then filtrate was read in comparison to blank (acetone: hexane solution), at four different wavelengths (453, 505, 645 and 663 nm). The results were calculated on the basis of the following equations:

$$\begin{aligned} \text{Lycopene (mg/100 ml)} &= 0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453} \\ \beta\text{-Carotene (}\frac{\text{mg}}{100}\text{ ml)} &= 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453} \end{aligned}$$

Antioxidant Assays

In all assays, BHT was tested as the standard to check the potential of the tested substances.

Reducing Power Assay

Oyaizu method was employed for testing the reducing power activity of the extract solution [22]. In this

method, equal volumes (2.5) of the extract solution, buffer (0.2 M sodium phosphate, pH 6.6) and tripotassium hexacyanoferrate (III) (1%) in a tube was vortexed and kept for incubation (50 °C/ 20 min). After addition of the same amount of trichloroethanoic acid (10%, w/v), tube was spun (1000 rpm/8 min). Supernatant and distilled water in equal volume (5:5, ml) and iron chloride (1 ml, 0.1%) was added to a tube and then vortexed. To measure the abs. value of the reaction was employed at 700 nm.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

Aliquot from each concentration of the test substance and DPPH solution in MeOH (60 mM) (0.1:3.9 ml) in a test tube was vortexed and then maintained without light exposure for 1 h. Measurement of the abs. in the solution was performed at 515 nm in comparison to blank (MeOH). % inhibition of the DPPH was assessed using the equation as below: A_{sample} is the abs. value of tested substance and A_{control} is the abs. value of the DPPH [23].

$$\% \text{ Scavenging DPPH} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP)

A fresh reagent before each testing was made and consisted of 1 ml of TPTZ solution (10 in 40 mmol/l HCl), 1 ml of FeCl_3 (20 mmol/l) and 10 ml of acetate buffer (0.1 mol/l, pH 3.6) at 37 °C/10 min [24]. Test substance in MeOH and the reagent (0.3: 2, ml) was subsequently added into a flask and then adding distilled water up to 10 ml of final volume. After incubation period (10 min), blue colour appeared in the solution. The measurement of the Abs. value of the solution versus blank (reagent solution only) was done at 593 nm. A curve was made with different concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at ranging from 0.2 to 2 mmol/l.

Nitric Oxide (NO) Scavenging Activity

Nitric oxide scavenging activity was carried out with the Griess Illosvoy reaction [25]. Test solution: Phosphate buffer (0.1M, pH 7.0): sodium nitroprusside solution (10mM) (0.5:0.5:2, ml) was subsequently added into test tube and then vortexed. This was then followed by incubating the solution at room temperature for 2.5 h. Equal volumes of the reacting solution and Griess reagent (1.25:1.25 ml) was maintained for incubation

(30 min). Abs. value of the reacting solution versus distilled water as the control was done at 548 nm.

NO (%) was based on the following equation:

$$\% \text{ Scavenging NO} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

H_2O_2 Scavenging Activity

In this test, varying concentrations of the test substance (0.0156 to 0.125 mg/ml) were assayed [26]. Test solution: H_2O_2 solution (40 mmol/l in phosphate buffer): phosphate buffer (pH 7.4) at 1:0.6:3.4 ml was added to test tube. Abs. of the reacting solution versus blank including extract solution plus phosphate buffer (1:4, ml) was checked spectrophotometrically at 230 nm. The control consisted of phosphate buffer: H_2O_2 solution (3.4:0.6, ml). The equation was used for % H_2O_2 inhibition.

$$\% \text{ Inhibition } \text{H}_2\text{O}_2 = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Statistical Analysis

Data were analysed by Pearson correlation method using SPSS statistics software version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

In the present study, the results of total phenolic (mg/kg), flavonoid (mg/kg) and β -carotene (mg/100 ml) contents of *L. deliciosus* and *P. ostreatus* in Amanos Mountains were determined as 34.55, 6.03, 1.15, and 15.66, 0.16, 0.02, respectively (Table 1). Unlike *P. ostreatus*, *L. deliciosus* had lycopene (mg/100 ml) content of 0.25. As a result, it was found that total phenolic, flavonoid, β -carotene and lycopene contents of *L. deliciosus* were higher than that of *P. ostreatus*.

Antioxidant Activities of Mushrooms

Results of the antioxidant activity of the MeOH extract obtained from each mushroom are shown in Figure 1.

DPPH Scavenging Activity (DSA)

DPPH activities of MeOH extracts of *L. deliciosus* and *P. ostreatus* in Amanos Mountains were tested at 1.25, 2.5 and 5 mg/ml. At 5.0 mg/ml, *L. deliciosus* and *P. ostreatus* revealed highest DPPH activity with 45.33% and 17.42%, respectively. DPPH activities of BHT had 96% at the same concentration.

Reducing Power (RP)

RP of *L. deliciosus* and *P. ostreatus* had concentration-dependent activities. RP (Abs.) of MeOH extracts at 1.25, 2.5 and 5 mg/ml were 0.28, 0.54, 0.79 in *L. deliciosus* and 0.15, 0.27, 0.54 in *P. ostreatus*, respectively.

Nitric Oxide Scavenging Activity (NOSA)

In this study, NOSA (%) of *L. deliciosus* and *P. ostreatus* were 55 and 33 at 5.0 mg/ml, respectively, whereas NOSA (%) of BHT was 51 at 5.0 mg/ml. It appeared that NOSA of *L. deliciosus* was higher activity than those of *P. ostreatus* and BHT.

Hydrogen Peroxide (H₂O₂) Scavenging Activity (HPSA)

HPSA (%) of *L. deliciosus*, *P. ostreatus* and BHT were 88.30, 73.77 and 93.76 at 5.0 mg/ml, respectively.

Ferric-Reducing Antioxidant Power (FRAP)

FRAP (mmol Fe²⁺/L) activities of *L. deliciosus* and *P. ostreatus* in Amanos Mountains revealed concentration dependent activities. As per results, FRAP activities at 1.25, 2.5 and 5.0 mg/ml were 0.10, 0.26, 0.57 in *L. deliciosus* and 0.03, 0.11, 0.28 in *P. ostreatus*, respectively.

The antioxidant activities as well as antioxidant content of *L. deliciosus* and *P. ostreatus* are given in Table 1. A good correlation ($r \geq 0.90$) was observed among the antioxidant results when using different methods. In the present study, antioxidant results were also well-correlated ($r \geq 0.93$) with total phenolic compounds and lycopene. In addition, β -carotene content was correlated with DPPH, RP, NO and FRAP. Flavonoid contents were also correlated with DPPH, RP and NO.

| | <i>L. deliciosus</i> | <i>P. ostreatus</i> |
|------------------------------|----------------------|---------------------|
| Total Phenol (mg/kg) | 34.55 | 15.66 |
| Total Flavonoid (mg/kg) | 6.03 | 0.16 |
| β -Carotene (mg/100ml) | 1.15 | 0.02 |
| Lycopene (mg/100ml) | 0.25 | nd |

nd: not detected

Table 1: Total Phenol, Flavonoid, β -Carotene and Lycopene Contents in MeOH Extract of *Lactarius deliciosus* and *Pleurotus ostreatus*

Discussion

Phenolic compounds are important compounds that contribute to coloration and organoleptic properties of plants and fruits [27]. Flavonoids are low molecular weight polyphenolic compounds [28]. Phenolic compounds inhibit the oxidation *in vitro* LDL (low-density lipoprotein) and play important roles on the elimination effects of hypertension, absorption of glucose and the growth of tumors by neutralizing the free radicals [29-33]. β -carotene is a pigment and soluble in oil, which is a precursor of vitamin A in mammals [34]. The lycopene is a well known compound as the isomer of β -carotene, which is synthesized to absorb light during photosynthesis by plants and microorganisms [35]. β -carotene and lycopene function as the antioxidants by scavenging free radicals [36].

Total phenolic and flavonoid contents were expressed as equivalent to gallic acid and quercetin (mg/kg). In previous studies, total phenolic and flavonoid contents of various extracts of *L. deliciosus* and *P. ostreatus* varied in different habitats. It was reported that total phenolic content (mg/g) of *L. deliciosus* was 1.5 in Spain [11], 51.27 in Bolu region of Turkey [37] and 17.25, 24.0 in Bragança and Trás-os-Montes regions of Portugal [38,39]. In addition, flavonoid content (mg/g) of *L. deliciosus* was 3.0 in Spain [11]. Total phenolic and flavonoid contents (mg/g) in the MeOH extract of *P. ostreatus* were 1.6 and 1.0 in Spain [11], 1.44 and 0.37 in Poland, respectively [40]. In Korea, total phenolic contents (mg/g) of yellow, pink and dark grey strain of *P. ostreatus* were 39.3, 30.1, 21.2 and flavonoid contents were 1.96, 1.21, and 2.16 respectively [41]. Total phenolic contents (mg/g) of *P. ostreatus* MeOH extracts were 1.32 in India [42], 12.1 in Blacksea region of Turkey [7], and 42.47 in Thailand [43].

There have been limited reports on the presence of β -carotene and lycopene contents of mushroom species. In the first report, β -carotene and lycopene contents (mg/g) in MeOH extract of *P. ostreatus* were determined as 0.317, 0.195, respectively [40]. In the second report, β -carotene was not determined in *P. ostreatus* collected from Black Sea region of Turkey [7].

DPPH is one of the mostly accepted methods for assessing the antioxidant activity [44]. DPPH radical has an advantage because of not affected by side reactions. Antioxidant agents neutralize the DPPH radical by donating an electron or the hydrogen atom and radical

| | Total Phenolics | Flavonoid content | β -carotene | Lycopene | DPPH | RP | NO | FRAP |
|-------------------------------|-----------------|--------------------|--------------------|----------|--------|-------|--------|--------|
| Flavonoid content | 0.92* | | | | | | | |
| β -carotene | 0.95* | 0.99** | | | | | | |
| Lycopene | 0.97* | 0.82 ^{ns} | 0.86 ^{ns} | | | | | |
| DPPH | 0.99** | 0.95* | 0.97* | 0.95* | | | | |
| RP | 0.99** | 0.94* | 0.96* | 0.97* | 0.99** | | | |
| NO | 0.98* | 0.90* | 0.93* | 0.98* | 0.98* | 0.98* | | |
| FRAP | 0.98* | 0.87 ^{ns} | 0.90* | 0.99** | 0.97* | 0.98* | 0.99** | |
| H ₂ O ₂ | 0.93* | 0.79 ^{ns} | 0.83 ^{ns} | 0.97** | 0.90* | 0.93* | 0.98** | 0.98** |

ns: not significant. *:significant at p<0.05 and **: significant at p<0.01

Table 2: Pearson's correlation coefficients of antioxidant contents and antioxidant activities

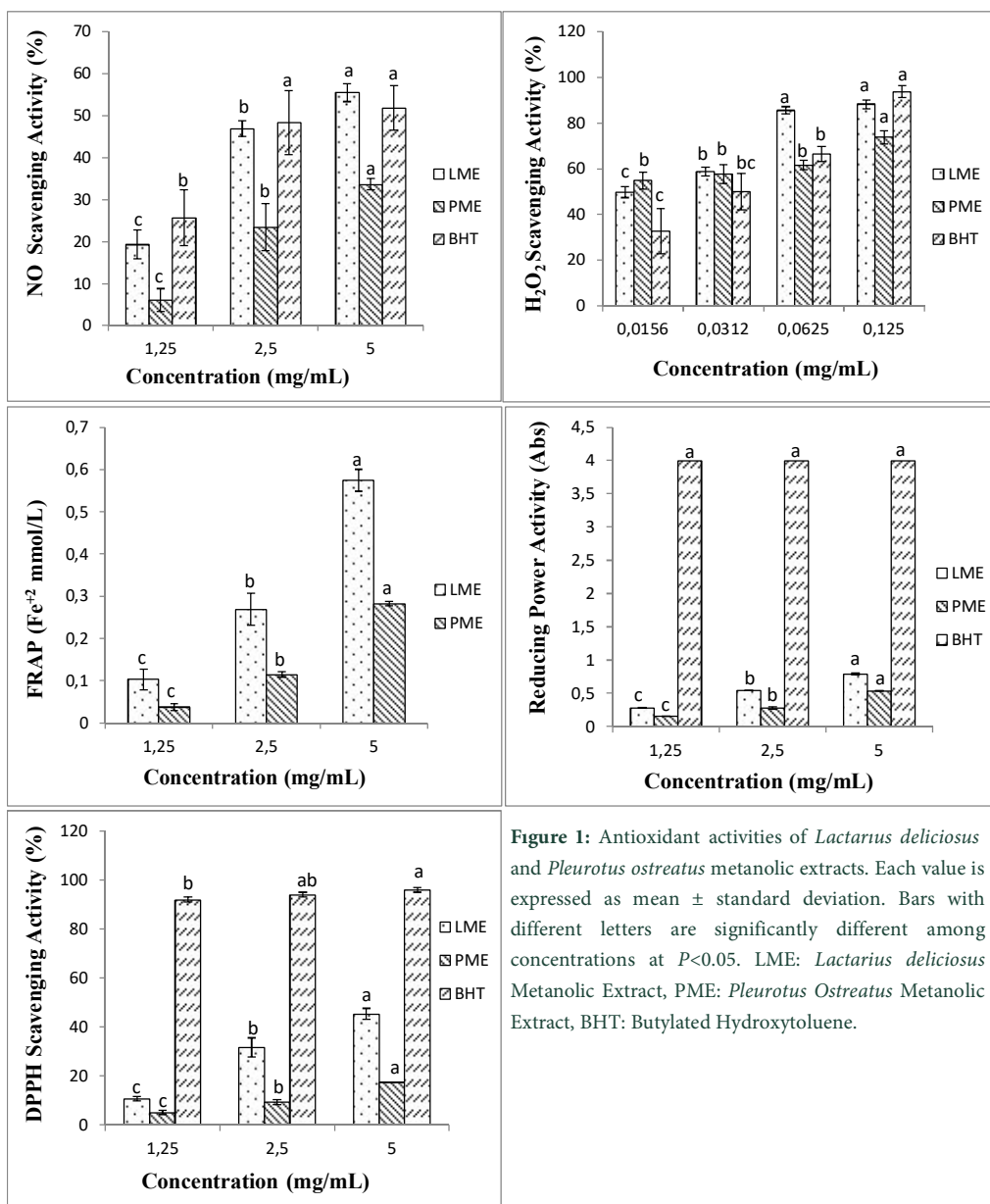


Figure 1: Antioxidant activities of *Lactarius deliciosus* and *Pleurotus ostreatus* metanolic extracts. Each value is expressed as mean \pm standard deviation. Bars with different letters are significantly different among concentrations at $P < 0.05$. LME: *Lactarius deliciosus* Metanolic Extract, PME: *Pleurotus Ostreatus* Metanolic Extract, BHT: Butylated Hydroxytoluene.

scavenging capacity of DPPH could be determined by reading absorbance at 517 nm by spectrophotometry with a colour change from purple to yellow [45].

The present results showed some differences than that of Akata *et al.* [16] who reported that DPPH activity of *L. deliciosus* at 2.56 mg/ml was 92.02%, and *P. ostreatus* at 2.72 mg/ml was 96.16%. DPPH activity of MeOH extract at 50 mg/ml of *L. deliciosus* was 84.3% in Portugal [38] and ethanolic extract at 5 mg/ml was 62.41% in Turkey. DPPH activity of MeOH extract of *P. ostreatus* was 29.0% at 2 mg/ml in Korea [37], 87.4% at 20 mg/ml in India [46] and 82.8% at 180 µg/ml in Turkey [7]. And also, fresh and steamed samples of *P. ostreatus* (1 mg/ml) showed the highest DPPH activity with 44.7% and 94.4%. It seemed that DPPH activities of *L. deliciosus* and *P. ostreatus* in this study were lower than those previous studies. The differences might be due to extraction methods, solvent types, used concentrations, localities and etc.

A good interaction between antioxidant capacity and reducing power is commonly accepted [47]. Antioxidants' reduction capacities are usually associated with the presence of compounds which is breaking the free radical chain via contributing either electrons or hydrogen atom [21,48]. Antioxidants facilitate the conversion of Fe^{+3} to Fe^{+2} . Higher the abs. value at the appropriate wavelength means the higher reducing power [21].

The results of the present study showed differences when compared with previous studies with regard to RP of *L. deliciosus* and *P. ostreatus*. RP of MeOH extract of *L. deliciosus* collected from Bragança region of Portugal was 2.41 at 50 mg/ml [38]; MeOH extract of *P. ostreatus* was 0.40 at 2 mg/ml in Korea [41], 1.97 at 10 mg/ml in India [46], 0.75 at 0.6 mg/ml in Black Sea region of Turkey [7], also ethanolic extract showed 1.367 absorbance at 10 mg/ml [49].

Based on these findings, *L. deliciosus* was higher RP in Bragança, Portugal [38] than that of Amanos Mountains, Turkey. Also, RP of *P. ostreatus* in this study was similar to Korean findings, whereas RP was lower than that of an Indian finding [46,49] and a Turkish study in the Black Sea region of Turkey [7]. It could be suggested that differences in RP might be due to different extracts, concentrations and localities.

NO is one of the free radicals synthesized by nitric oxide synthase from L-arginine [50] and can be toxic at

high concentrations [51,52]. However, preventing the excessive production of nitric oxide is an important process [14]. In a previous report, it was reported that NOSA (%) of MeOH extract of *Pleurotus squarrosulus* and cultivated *Pleurotus florida* in India were 80 and 81.8 at 1 mg/ml, respectively [45,51]. The findings of the present study seemed to lower than *Pleurotus squarrosulus* and cultivated *Pleurotus florida* in India.

Hydrogen peroxide occurring in tissues with oxidative processes is a relatively stable and type of the non-radical oxidant [18]. H_2O_2 is produced in the cytoplasm, plasma membrane and extracellular matrix of plants. In the cytoplasm, electron transport chain associated with the endoplasmic reticulum is known as the main source of H_2O_2 [53]. H_2O_2 accumulation in plant tissues has been acted as a signal between cells. Moreover, it also stimulates proteins related with stress responses as alternative oxidase catalase, peroxidase along with many genes [54,55]. It was reported that H_2O_2 damaged any tissue exposed to oxidative stress and caused the cancer [56]. Ozyurek *et al.* [18] determined that HPSA (%) of MeOH extract of *Lactarius volemus*, which purchased from local markets in different regions of Turkey, was 78.7% at 15 mg/ml. This assay depends on measuring the reducing capacity from Fe^{3+} to Fe^{2+} of materials and also widely accepted technique for antioxidant activity of different substances [23,57,58]. FRAP activities of *Lactarius* and *Pleurotus* genus have been reported in previous studies.

In Turkey, ethanolic extracts of *L. deliciosus* (Bolu-Turkey) were 0.229 at 250 µg/ml and 0.590 at 500 µg/ml [37]. In Thailand (Nakhon Ratchasima), FRAP activities of water and MeOH extracts of *P. ostreatus* were 4.38 and 1.61 at 20.0 mg/ml, respectively [43]. The significant correlations between antioxidant activities and antioxidant content were also reported in relation to phenolics, lycopene and carotenoids of tomato [59]. *L. deliciosus* and *P. ostreatus* collected from the natural habitats of the Amanos Mountains exerted notable antioxidant capacities; however, further studies are required to isolate and identify the specific compounds that are forming the antioxidant properties of the tested species.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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