

## COMPARATIVE EVALUATION OF THE ANTIOXIDANT POTENTIAL OF *Hericum erinaceus*, *Hericum americanum* AND *Hericum coralloides*

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### ABSTRACT

The aim of this work was to determine the total phenolic content (TPC) and the antioxidant activity of methanol extracts of *Hericum erinaceus*, *Hericum americanum* and *Hericum coralloides*, including free radical scavenging method (DPPH), ferric reducing antioxidant power (FRAP) and radical cation scavenging method (ABTS). *Hericum* spp exhibited moderate to high antioxidant activity. The highest TPC ( $3.27 \pm 0.01$  mg GAE  $g^{-1}$ ) and antioxidant activity values ( $17.0 \pm 0.68$  mmol TE  $g^{-1}$  in FRAP;  $EC_{50} = 4.12 \pm 0.12$  mg  $mL^{-1}$  in DPPH;  $EC_{50} = 2.83 \pm 0.10$  mg  $mL^{-1}$  in ABTS<sup>+</sup>) were found for methanol extracts of *H. coralloides*. The TPC and antioxidant activity of *H. erinaceus* isolates varied from strain to strain. *H. americanum* possessed considerably lower total phenolic content ( $2.31 \pm 0.01$  mg GAE  $g^{-1}$ ) and antioxidant activity ( $10.5 \pm 0.59$  mmol TE  $g^{-1}$  in FRAP;  $EC_{50} = 7.82 \pm 0.09$  mg  $mL^{-1}$  in DPPH;  $EC_{50} = 6.36 \pm 0.12$  mg  $mL^{-1}$  in ABTS<sup>+</sup>) than *H. coralloides* and *H. erinaceus*. A high correlation was determined between TPC and ABTS<sup>+</sup> ( $r^2 = 0.855$ ), DPPH<sup>•</sup> ( $r^2 = 0.969$ ) and FRAP ( $r^2 = 0.942$ ). According to results obtained in the present study, *Hericum* spp., especially *H. coralloides* and some of *H. erinaceus* isolates, might be promising natural source of antioxidants for food and pharmaceutical industry.

**Key words:** *Hericum* spp., antioxidant activity, Folin–Ciocalteu, ABTS<sup>+</sup>, DPPH<sup>•</sup>, FRAP

### INTRODUCTION

Normal essential metabolic processes or external sources such as exposure to pollution, alcohol, tobacco smoke, heavy metals, pesticides, certain drugs and radiation cause the formation of free radicals in the organisms [Phaniendra and Periyasamy 2015]. Antioxidants inhibit free radical reactions and cellular damage in living organism [Nimse and Pal 2015]. Although human body is protected against free radical damage by antioxidant defense and repair systems, the balance between the antioxidant defenses and the production of free radicals may be disrupted. If there is an imbalance between the antioxidant and oxidant in the

human body, called oxidative stress, may cause many diseases such as rheumatoid arthritis, arteriosclerosis, cirrhosis and cancer may occur [Reuter et al. 2010].

Antioxidants are both natural and synthetic compounds. Artificial antioxidants [butylated hydroxyanisole (BHA), propyl gallate (PG), tert-butylated hydroxyquinone (TBHQ), butylated hydroxytoluene (BHT), etc] have been used in the stabilization of foods, however, the BHA and BHT have been observed to act as carcinogens in animals [Hocman 1988] and humans [Botterweck et al. 2000]. For that reason, there is an increasing interest in research on

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**Table 1.** Origin and isolate cods of *Hericium erinaceus* isolates tested in the study

Species	Isolate Cod	Origin
<i>Hericium erinaceus</i>	He-1	Turkey
<i>Hericium erinaceus</i>	He-2	Turkey
<i>Hericium erinaceus</i>	He-3	Turkey
<i>Hericium erinaceus</i>	He-4	Turkey
<i>Hericium erinaceus</i>	He-5	Turkey
<i>Hericium erinaceus</i>	He-6	USA
<i>Hericium coralloides</i>	Hc	Turkey
<i>Hericium americanum</i>	Ha	USA

natural additives such as fruit, vegetable, herbs and mushroom to protect human body from effect of oxidative damage.

Mushrooms are considered to be nutraceutical foods since they are high protein, dietary fiber, vitamins, minerals and low in essential fatty acids, calories and fats [Reis et al. 2012]. Moreover, edible mushrooms accumulate various bioactive molecules, which are very efficient scavengers of peroxy radicals, including phenolic compounds, terpenes and steroids [Barros et al. 2007]. *Hericium erinaceus*, monkey head, lion's mane or pom-pom mushroom, is one of the mushrooms cultivated worldwide. Its popularity is increasing day by day due to its pleasant taste, aroma and medical properties. Its fruiting bodies and the fungal mycelia exhibit various pharmacological activities.

The antioxidant capacity of edible mushrooms is of importance because this highly correlated to their medical properties. So, antioxidant capacity is extensively used a parameter for nutraceutical potential. Until now, research has tended to focus on the antioxidant activity of *H. erinaceus* [Wong et al. 2009, Koutrotsios et al. 2016], however, there is relatively a little information available about the phenolic content and antioxidant activity of *H. coralloides* [Heleno et al. 2015] and no information about that of *H. americanum*. The purpose of this study was to evaluate the total phenolic content (TPC) and the antioxidant properties of methanol extracts of *Hericium erinaceus*, *H. americanum* and *H. coralloides*, including free radical scavenging method (DPPH<sup>•</sup>), ferric reducing anti-

oxidant power (FRAP) and radical cation scavenging method (ABTS<sup>•+</sup>). Within the framework of this study, the antioxidant activity and TPC of *H. americanum* has been evaluated comparatively with *Hericium* spp. for the first time.

## MATERIALS AND METHODS

### Cultures

Three species and eight strains were examined in the study: (i) *Hericium erinaceus* (He-1, He-2, He-3, He-4, He-5, He-6), (ii) *Hericium coralloides* and (iii) *Hericium americanum*. The isolates were obtained from the mushroom culture collection of Ahi Evran University, Kırşehir, Turkey. The country of origin and isolate code of the *Hericium* species studied are shown in Table 1.

### Spawn production and mushroom cultivation

Grain spawn was produced in 500 mL glass bottles following the standard methods of Pieckenstein et al. [1999]. Oak sawdust and wheat bran were used as substrates for macrofungi production. Wheat bran was added to sawdust in a ratio of 8 : 2 and mixed. Water was added until the moisture reached 70%. 1 kg of substrates were filled to autoclavable polypropylene bags (25 × 45 cm) and then sterilized in an autoclave at 121°C for 90 min. Cooled substrates were inoculated in a laminar flow chamber using 3% grain spawn. The bags were incubated in the dark at a controlled temperature of 25°C. After the spawn running peri-

od, the temperature was dropped to  $18 \pm 2^\circ\text{C}$ , relative humidity (RH) maintained at 80–85% and light was provided 8 h of daily by fluorescent bulbs. The  $\text{CO}_2$  concentration was maintained below  $1000 \text{ mg kg}^{-1}$  by sufficient air changes.

Fruitbody samples were obtained from the first flushes. Freshly harvested whole fruitbodies were dried in an oven at  $40^\circ\text{C}$ . Then dried mushrooms were grinded (20 mesh) using a rotary mill.

#### Preparation of the mushroom extracts

Methanol (100 mL) was used as solvent for extraction of dry mushroom powder (10 g). The mixture was homogenized using the Ika Ultra-Turrax homogenizer. The homogenates were kept in the dark at  $4^\circ\text{C}$  for 14–16 h and then filtered using Whatman No. 4 filter paper. The supernatants were stored at  $-20^\circ\text{C}$  [Thaipong et al. 2006].

#### Total phenolic content (TPC)

The TPC in the mushroom methanol extracts was estimated via the Folin–Ciocalteu assay, described by Swain and Hillis [1959] with some modifications. The absorbance of the samples was recorded at 725 nm via spectrophotometer (Bio 100, Varian, Australia). The analysis were performed in triplicate and the results expressed as gallic acid equivalents (GAEs) in  $\text{mg g}^{-1}$  dry weight (d.w.) of extract.

#### Antioxidant capacity

The antioxidant activity of the methanolic extracts of *Hericium* spp. was assayed using the FRAP, DPPH and ABTS methods.

**Ferric reducing antioxidant power (FRAP).** The ferric ion reducing antioxidant power (FRAP) was carried out using the spectrophotometric method, as described by Benzie and Strain [1996]. The absorbance of the resulting supernatants was measured at 593 nm via spectrophotometer (Bio 100, Varian, Australia). Trolox was used as the standard. The analysis were performed in triplicate. FRAP values were expressed as Trolox equivalents ( $\text{mmol TE g}^{-1}$  d.w.) and were presented as mean values  $\pm$  standard deviations

**DPPH free radical scavenging activity.** The measurement of DPPH radical scavenging activity of the fruitbody extracts on radicals was measured accord-

ing to the method reported by Shimada et al. (1992). Various concentrations (1–10 mg mL) of methanol extracts of *Hericium* spp. were mixed with 1 ml of DPPH solution (0.2 mmol) (Sigma). Then, the mixture was vortex and kept for 30 min in the dark. The absorbance was recorded at 517 nm. Methanol was used as the blank. The scavenging ability was calculated as shown in Equation (1).

$$\text{DPPH scavenging effect (\%)} = \frac{[(A_{\text{DPPH}} - A_s) / A_{\text{DPPH}}] \cdot 100}{(1)}$$

$A_{\text{DPPH}}$  – the absorbance of the DPPH' solution,  
 $A_s$  – the absorbance of the solution when the sample extract has been at a particular level.

The analysis were performed in triplicate. The DPPH radical scavenging ability of the fruitbody extracts was expressed as the mean of  $\text{EC}_{50}$  value ( $\text{mg mL}^{-1}$ )  $\pm$  standard deviation. The  $\text{EC}_{50}$  value ( $\text{mg mL}^{-1}$ ) defined as the efficient concentration of extracts required to scavenge of DPPH' radicals by 50%.

**ABTS radical cation scavenging activity.** The radical cation scavenging activity of the methanol extracts of *Hericium* spp. against the ABTS was determined using the method of Re et al. [1999] with some modifications. The absorbance was recorded by ultraviolet–visible spectrophotometer at 734 nm after 6 min reaction in the spectrophotometer set at  $30^\circ\text{C}$ , with the methanol used as a blank. The radical scavenging activity was calculated as the percentage of ABTS discoloration using Equation (2):

$$\text{ABTS scavenging effect (\%)} = \frac{[(A_{\text{ABTS}} - A_s) / A_{\text{ABTS}}] \times 100}{(2)}$$

$A_{\text{ABTS}}$  – the absorbance of the ABTS solution without sample,  
 $A_s$  – the absorbance of the solution containing the sample.

The assay of the ABTS radical scavenging activity was carried out in triplicate. The result was expressed as the  $\text{EC}_{50}$  value ( $\text{mg mL}^{-1}$ )  $\pm$  standard deviation.  $\text{EC}_{50}$  value ( $\text{mg mL}^{-1}$ ) is the concentration needed to reduce ABTS by 50%.

### Statistical analysis

The Social Sciences (SPSS) version 16 was used to carry out Statistical analysis. Statistically significant differences among the means were determined by Tukey Test. The relationship between TPC and antioxidant attributes were analyzed by Pearson's correlation.

## RESULTS AND DISCUSSION

### Total phenolic content (TPC) of *Hericium* spp.

Phenolic compounds play important roles in the protection of oxidation processes. The fruit bodies and mycelia of mushrooms have shown high phenolic content and good antioxidant properties [Reis et al. 2012, Islam et al. 2016]. A high correlation was found between antioxidant activity and phenolic contents in several works [Mau et al. 2002, Koutrotsios et al. 2016].

In the study, significant differences were observed among the *Hericium* spp. in terms of TPC ( $P < 0.01$ ). The TPC in the methanolic extracts of *H. coralloides* were  $3.27 \pm 0.01$  mg GAE  $g^{-1}$ , while the *H. americanum* extracts contained only  $2.31 \pm 0.01$  mg GAE  $g^{-1}$ . The TPC of *H. coralloides* was almost 29.4% higher than that of *H. americanum*. The levels of phenolic compounds in the methanolic extracts of *H. erinaceus* isolates ranged between  $2.34 \pm 0.05$  and  $3.15 \pm 0.05$  mg GAE  $g^{-1}$ . The present findings are line with the results of Heleno et al. [2015], who reported that the TPC of *H. coralloides* was higher than that of *H. erinaceus*. The TPC of *H. erinaceus* varied from strain to strain. Methanolic extracts for He-6 showed a significantly higher phenolic content than that of the other *H. erinaceus* isolates. Although the He-2 and He-6 isolates were both included in the *H. erinaceus* species, there was a 25.7% difference between their phenolic content. Koutrotsios et al. [2016] reported a greater content of total phenols in *H. erinaceus* (3.24–5.04 mg GAE  $g^{-1}$  d.w.), while Wong et al. [2009] found a total phenol content of 2.37 mg GAE  $g^{-1}$  d.w. in the same species. However, Heleno et al. [2015] determined TPC values of 16.29 mg GAE  $g^{-1}$  d.w. and 13.41 mg GAE  $g^{-1}$  d.w. for *H. coralloides* and *H. erinaceus*, respectively. The values found in the present study were also lower compared to that reported by Abdullah et

al. [2012] (10.20 mg GAE $^{-1}$  g). The TPC differences seen by researchers among the same species may have been due to the varied isolates, extraction conditions, harvesting time, climatic conditions, growing substrate or drying methods used to prepare the mushroom samples.

The level of phenolics analyzed in the *H. erinaceus* isolates was lower than the TPC obtained from some other edible mushrooms (poplar mushroom, matsutake and porcino nero, as 4.23, 7.05 and 7.67 mg GAE  $g^{-1}$ , respectively) reported by Islam et al. [2016]. On the other hand, the results determined in the present study are similar to those of commonly consumed mushrooms such as shiitake, lingzhi, shimenji, golden oyster, nameko and truffles (1.35–3.00 mg GAE  $g^{-1}$ ), as reported in the above-mentioned study. Koutrotsios et al. [2017] evaluated the fruitbodies of sixteen strains of *Pleurotus ostreatus* and determined a TPC of 1.27–8.62 mg GAE  $100 g^{-1}$  d.w. The results in the present study indicate that the *Hericium* species are a moderate source of phenolics when compared to the values obtained for other mushroom species in previous studies.

### Antioxidant activity of *Hericium* spp.

A single assay cannot accurately reflect the action mechanisms of or antioxidants different oxidant sources in a complex system [Prior et al. 2005]. For the reason, three different methods, including the ABTS $^{+}$  and DPPH $^{\cdot}$  scavenging activities and FRAP, were carried out for a comparative evaluation of the *in vitro* antioxidant potential of *Hericium* spp.

**Ferric reducing antioxidant power (FRAP).** FRAP assay is based on the reduction of ferric tripyridyltriazine (Fe (III) –TPTZ) complex to tripyridyltriazine complex to form (Fe (III) –TPTZ) at low pH. The reaction causes a formation of blue colored ferroustripyridyltriazine (Fe $^{2+}$ –TPTZ) complex, which absorbs at 593 nm [Benzie and Strain, 1996]. The FRAP values of the samples of *Hericium* spp. were expressed in mmol TE  $g^{-1}$  d.w. (Tab. 2). There was a significant difference ( $P < 0.01$ ) between the FRAP values of the different *Hericium* species.

A higher FRAP value indicates the higher antioxidant activity of a mushroom. Among the methanolic extracts from the *Hericium* spp., *H. coralloides* exhibited higher reductive activities (17.0 mmol TE  $g^{-1}$  d.w.)

**Table 2.** Total phenolic content, reducing power and scavenging activity of the tested *Hericium* spp.

Species	Isolate	Total phenolic content	Reducing power	Scavenging activity	
		Folin–Ciocalteu assay (mg GAE g <sup>-1</sup> )	FRAP (mmol TE g <sup>-1</sup> )	DPPH scavenging activity assay (EC <sub>50</sub> , mg mL <sup>-1</sup> )	ABTS (EC <sub>50</sub> , mg mL <sup>-1</sup> )
<i>Hericium erinaceus</i>	He-1	2.88 ± 0.07 <sup>**c</sup>	14.6 ± 0.33 <sup>**b</sup>	5.13 ± 0.11 <sup>**c</sup>	3.32 ± 0.07 <sup>**de</sup>
<i>Hericium erinaceus</i>	He-2	2.34 ± 0.05 <sup>e</sup>	11.8 ± 0.16 <sup>c</sup>	6.74 ± 0.15 <sup>b</sup>	4.35 ± 0.07 <sup>b</sup>
<i>Hericium erinaceus</i>	He-3	2.49 ± 0.06 <sup>d</sup>	11.1 ± 0.32 <sup>c</sup>	6.73 ± 0.16 <sup>b</sup>	4.58 ± 0.06 <sup>b</sup>
<i>Hericium erinaceus</i>	He-4	3.01 ± 0.05 <sup>bc</sup>	15.7 ± 0.68 <sup>ab</sup>	4.27 ± 0.08 <sup>e</sup>	3.58 ± 0.12 <sup>cd</sup>
<i>Hericium erinaceus</i>	He-5	2.99 ± 0.07 <sup>c</sup>	14.8 ± 0.50 <sup>b</sup>	4.67 ± 0.07 <sup>d</sup>	3.86 ± 0.09 <sup>c</sup>
<i>Hericium erinaceus</i>	He-6	3.15 ± 0.05 <sup>b</sup>	14.4 ± 0.78 <sup>b</sup>	4.36 ± 0.09 <sup>de</sup>	3.23 ± 0.08 <sup>e</sup>
<i>Hericium coralloides</i>	Hc	3.27 ± 0.01 <sup>a</sup>	17.0 ± 0.68 <sup>a</sup>	4.12 ± 0.10 <sup>e</sup>	2.83 ± 0.08 <sup>f</sup>
<i>Hericium americanum</i>	Ha	2.31 ± 0.01 <sup>e</sup>	10.5 ± 0.59 <sup>c</sup>	7.82 ± 0.09 <sup>a</sup>	6.36 ± 0.12 <sup>a</sup>

Asterisks indicate significance at \**P* < 0.05, \*\**P* < 0.01, ns not significant; values within the same column followed by the same letter are not significantly different by Tukey's test (n = 3)

**Table 3.** Correlations between total phenolic content and antioxidant activities of *Hericium* spp.

	FRAP	EC <sub>50</sub> of DPPH	EC <sub>50</sub> of ABTS
Total phenolic content	(+)0.942 <sup>**</sup>	(-)0.969 <sup>**</sup>	(-)0.855 <sup>**</sup>

\*\* Correlation is significant at the 0.01 level

than the other mushroom extracts, followed by the *H. erinaceus* isolates. The measurements for reducing power exhibited a great variation among the *H. erinaceus* isolates. The ferric reducing antioxidant power of the *H. erinaceus* isolates ranged from 11.1 to 14.85 mmol TE g<sup>-1</sup> d.w., whereas He-4 had the highest FRAP value, followed by He-5, He-1, He-6, He-2 and He-3, in descending order. The lowest FRAP value was observed in *H. americanum*.

The FRAP value in extracts of *H. erinaceus* were reported earlier by Koutrotsios et al [2016] as having 3.56–5.34 mmol TE g<sup>-1</sup> dw, depending on the growing substrates. The results obtained from the present study were dramatically higher than those findings. Moreover, the FRAP value of *H. erinaceus* methanol extracts was reported previously as 13.72 mol

of FeSO<sub>4</sub>·7H<sub>2</sub>O equivalents/g by Wong et al. [2009], however, it is difficult to make a comparison due to the different units used to express the data. When it comes to some other mushroom species, the FRAP values of protein extracts from *Ganoderma lucidum* were between 1.73 ± 0.01 and 2.62 ± 0.01 μmol trolox/μg protein, respectively [Saard et al. 2015] and those of *P. ostreatus* were reported by Chirinang and Intarapichet [2019] as 4.38–1.61 at 20.0 mg mL<sup>-1</sup>, respectively.

**DPPH free radical scavenging activity.** The DPPH<sup>•</sup> procedure provides a rapid and easy way to evaluate the antioxidative activity of different extracts. DPPH method has been used to evaluate the antioxidant activity of various mushrooms extracts as antioxidants [Ferreira et al. 2007, Abdullah et al.

2012]. The method was used to determine the radical scavenging capacities of the methanol extracts of the *Hericium* species in the study. The ratio percentage of sample absorbance decrease and the absorbance of DPPH<sup>•</sup> solution in the absence of extract at 517 nm were measured. The results are shown in Figure 1. Moreover, the efficient concentration of extracts required to scavenge of DPPH<sup>•</sup> radicals by 50% (IC<sub>50</sub> values) were shown in the fifth column of Table 2. A lower IC<sub>50</sub> value shown a higher ability of the extracts to behave as DPPH<sup>•</sup> radical scavengers.

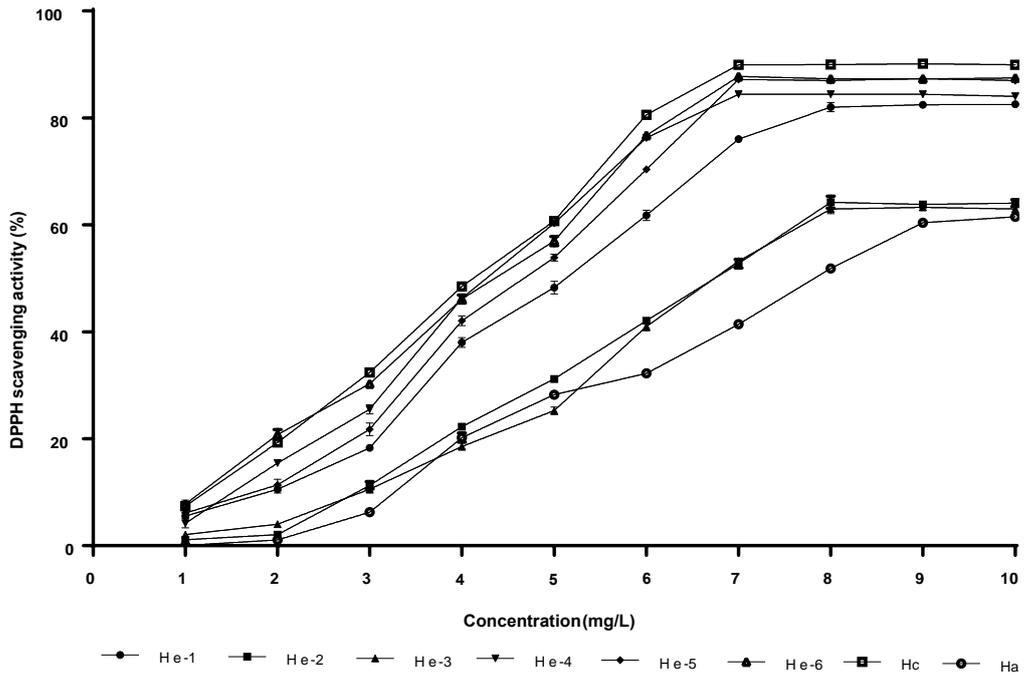
The minimum scavenging effects was found in the methanol extract of *Hericium* spp. at 1 mg/mL. The scavenging effects of the methanolic extracts from the *Hericium* spp. on DPPH<sup>•</sup> radicals increased with the increase of the concentrations and were ranged of 61.5–89.9% at 7 mg mL<sup>-1</sup>. The methanol extracts of *H. coralloides* showed a scavenging effect of 84.4% at 6.4 mg mL<sup>-1</sup> of the extract, whereas the scavenging effect of the *H. erinaceus* dry fruitbody isolate extracts ranged between 45.9 and 81.2%. Wong et al. [2009] reported that the scavenging effect of oven-dried *H. erinaceus* was measured as 63.2–67.8% at 6.4 mg mL<sup>-1</sup>. In the presented study, lower scavenging effects (35.9%) were observed with the methanol extracts of *H. americanum* at 6.4 mg mL<sup>-1</sup> of the extract.

Higher levels of TPC in turn led to higher DPPH<sup>•</sup> radical scavenging activity. *H. coralloides* revealed better antioxidant properties than other *Hericium* spp. with lower EC<sub>50</sub> values. The EC<sub>50</sub> values for the *H. americanum* extracts were nearly two times greater than the values of *H. coralloides*. The DPPH<sup>•</sup> radical scavenging EC<sub>50</sub> values of the *H. erinaceus* isolate extracts ranged from 4.12 to 6.74 mg mL<sup>-1</sup>. Among the tested *H. erinaceus* extracts, that of He-4 was the best DPPH<sup>•</sup> scavenger with the lowest EC<sub>50</sub> value, followed by the extracts of He-5, He-6 and He-1. In previous studies, the DPPH<sup>•</sup> radical scavenging EC<sub>50</sub> values of *H. erinaceus* dry fruitbody extract was reported as 5.51 [Wong et al. 2009] and 5.06 mg mL<sup>-1</sup> [Mau et al. 2002]. However, the methanolic extracts of *H. erinaceus* and *H. coralloides* were reported to have DPPH<sup>•</sup> EC<sub>50</sub> values of 24.53 mg mL<sup>-1</sup> and 22.53 mg mL<sup>-1</sup>, respectively [Heleno et al. 2015]. The values obtained in the present study for *H. erinaceus* and *H. coralloides* were lower than those presented by Heleno et al. [2015]

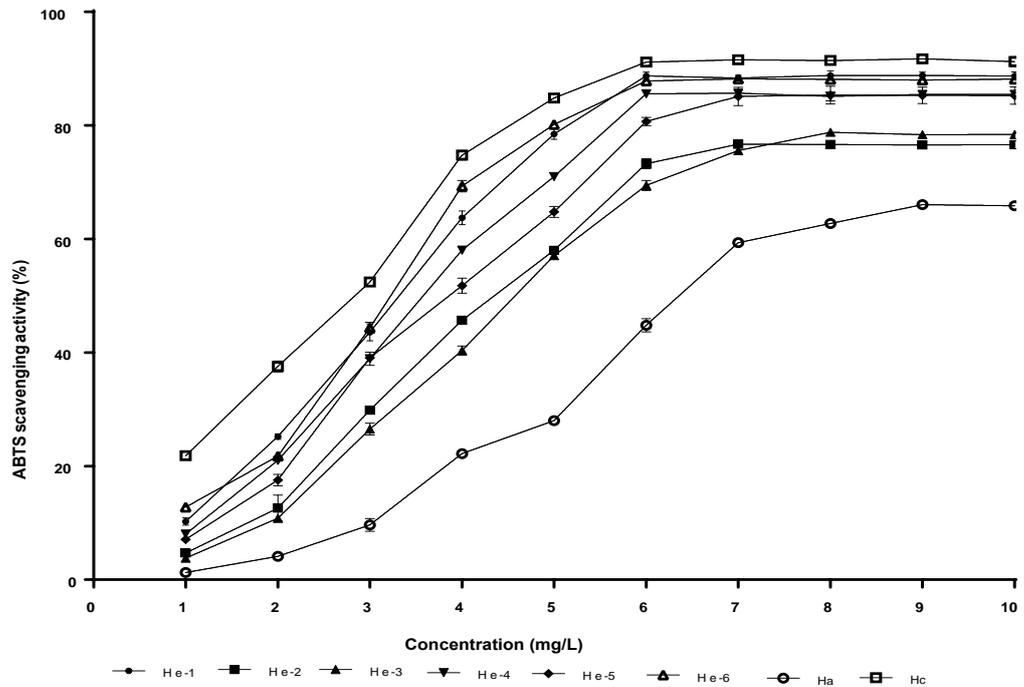
The ability of the methanolic extracts of other mushrooms to quench free radicals has been reported previously. The scavenging abilities of *Agaricus blazei* were found as 94.9% at 5 mg mL<sup>-1</sup> [Tsai et al. 2007] and *Agrocybe cylindracea* showed a scavenging ability of 93.8% at 5 mg mL<sup>-1</sup> [Tsai et al. 2006]. It seems that the free radical scavenging ability of the *Hericium* spp. was lower than that of *Agaricus blazei* and *Agrocybe cylindracea*. On the other hand, the scavenging effect of the *Hericium* spp. was higher compared to that reported for *Pleurotus ostreatus* (EC<sub>50</sub> of 11.56 mg mL<sup>-1</sup>), *Lentinula delicious* (EC<sub>50</sub> of 8.52 mg mL<sup>-1</sup>) and *Tricholoma portentosum* (IC<sub>50</sub> of 22.9 mg mL<sup>-1</sup>) by Ferreira et al [2007] and *Lentinula edodes* (EC<sub>50</sub> of 26.32 mg mL<sup>-1</sup>) by Carneiro et al. [2013]

**ABTS<sup>•+</sup> radical cation scavenging activity.** Reductions in the ABTS<sup>•+</sup> radical cation induced by antioxidants were determined by the decrease in its absorbance at 734 nm [Re et al. 1999]. The ABTS<sup>•+</sup> radical-scavenging activities of the extracts are shown in Figure 2. The ABTS<sup>•+</sup> radical-scavenging activities of the methanolic extracts of *Hericium* spp. were tested at 1–10 mg mL<sup>-1</sup>. The ABTS<sup>•+</sup> radical-scavenging activities of the *Hericium* spp. methanolic extracts rose with the increase in the concentration (Fig. 2). At the 6.0 mg mL<sup>-1</sup> concentration, the methanol extract of *H. coralloides* exhibited the highest radical scavenging activity (91.2%) when reacted with the ABTS<sup>•+</sup> radicals. The activity exhibited by *H. erinaceus* ranged from 69.4% to 88.7%, respectively, and the ABTS<sup>•+</sup> radical-scavenging activity of *H. americanum* was 44.8% at the same concentration. Reports of the ABTS<sup>•+</sup> radical-scavenging activities of the *Hericium* spp have not been found in any available literature sources.

The EC<sub>50</sub> values were shown in the sixth column of Table 2. A comparative analysis indicated that the *Hericium* spp. showed well antioxidant properties in the ABTS<sup>•+</sup> assays. With EC<sub>50</sub> values ranging between 3.23 mg mL<sup>-1</sup> and 4.58 mg mL<sup>-1</sup>, the methanolic extracts of the *H. erinaceus* isolates had lower antioxidant properties than *H. coralloides*, with an EC<sub>50</sub> value of 2.83 mg mL<sup>-1</sup>, but higher than *H. americanum*, with an EC<sub>50</sub> value of 6.29 mg mL<sup>-1</sup>. The *H. coralloides* and *H. erinaceus* isolates exhibited significant radical scavenging activity, where-



**Fig. 1.** Scavenging activity (%) on DPPH radicals of methanolic extracts from *Hericium* spp. Each value is expressed as mean  $\pm$  standard deviation (n = 3)



**Fig. 2.** ABTS radical cation scavenging activity of methanolic extracts from *Hericium* spp. Each value is expressed as mean  $\pm$  standard deviation (n = 3)

as the *H. americanum* presented moderate values. The EC<sub>50</sub> value of *H. americanum* in the ABTS<sup>+</sup> scavenging assay was more than two times higher compared to *H. coralloides*. Based on freeze-dried samples of *Agaricus bisporus*, Savoie et al. [2008] reported an EC<sub>50</sub> value of between 3.33 and 4.57 mg mL<sup>-1</sup>, which is close to the value obtained for the *Hericium* spp. On the other hand, the scavenging effect of the *Hericium* spp. was higher compared to that of *Pleurotus oeus* (IC<sub>50</sub> of 17.0 ± 0.39 mg mL<sup>-1</sup>) [Sudha et al. 2012], but lower than that of *Pleurotus ostreatus* lyophilized mycelium (IC<sub>50</sub> of 0.10–1.78 mg mL<sup>-1</sup>) [Vamanu 2014].

Although a strong correlation was obtained between the DPPH<sup>•</sup> and ABTS<sup>+</sup> radical scavenging assays, the methanolic extracts from the *Hericium* species (EC<sub>50</sub> values) had higher antioxidant activity in the ABTS<sup>+</sup> than in the DPPH<sup>•</sup>, which is in agreement with Vamanu [2014]. According to the results, it can be said that the ABTS<sup>+</sup> method is more effective for the determination of the antioxidant activities of *Hericium* spp.

#### Correlations between TPC and antioxidant activity

A correlation analysis was conducted to reveal the correlation between the TPC and the antioxidant activity of the samples. Table 3 has shown that correlations between total phenolic content and antioxidant activities of *Hericium* spp. used in the study. The TPC of the *Hericium* spp. showed a negative correlation with the corresponding EC<sub>50</sub> values of DPPH and ABTS, as evidenced by their correlation coefficients ( $r^2$ ) of -0.969 and -0.855, respectively ( $P < 0.01$ ). Furthermore, a strong positive correlation was observed between the TPC and FRAP ( $r^2 = 0.942$ ). These results were in agreement with a previously reported correlation [Koutrotsios et al. 2016] in *H. erinaceus*. This linear correlation confirmed that phenols were important antioxidant components in the *Hericium* isolates. It was also reported that the antioxidant activity of *H. erinaceus* was essentially consistent with the total polyphenol content [Mau et al. 2002]. On the other hand, Abdullah et al. [2012] found that a hot water extract of *H. erinaceus* fruitbodies showed relatively high antioxidant activity, while the polyphenolic contents did not overlap with the antioxidant activities.

#### CONCLUSION

Based on the results obtained using three standard assays, the *Hericium* spp. were effective at radical scavenging activity and possessed potent reducing power. As a result of the potential antioxidant properties of all *Hericium* spp., especially *H. coralloides*, as determined by this study, these mushrooms might be considered by the food and pharmaceutical sectors for use as natural sources of antioxidants. The potential of *H. coralloides* and *H. americanum* is constrained by the fact that these are infrequently found species in nature and are not commercially produced. The *Hericium* spp. can be cultivated on a large scale and, in view of their high antioxidant activity, have great potential to be developed as functional food and as nutraceuticals. Furthermore, to the best of our knowledge, this study is the first to report on the total phenolic content and antioxidant activity of *H. americanum*. In order for this mushroom to be utilized in the food and pharmaceutical industry, the antioxidant compounds and content of individual phenolic compounds should be investigated in more detail.

#### ACKNOWLEDGMENTS

This work was supported by the Ahi Evran University Research Council Grant No. ZRF. E2.17.013.

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