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Antioxidant potency of Iranian newly cultivated wild mushrooms of *Agaricus* and *Pleurotus* species.

Sharare Rezaeian[1], Sara Saadatmand[1], Taher Nejad Sattari[1], Amin Mirshamsi[2]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Biotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

Although *Agaricus bisporus* and *Pleurotus ostreatus* are two major cultivated mushrooms in Iran, a range of different other species of *Agaricus* and *Pleurotus* mushrooms grow wild in various habitats of the country. However, limited study has been undertaken to investigate antioxidant potency of the Iranian cultivated or wild mushrooms. This study aimed to evaluate antioxidant capacity of methanol-dichloromethane (1:1) extracts of commercially cultivated and newly cultivated wild species of *Agaricus* and *Pleurotus*, using chemical and biochemical techniques. In general, the results revealed that all the tested mushroom demonstrated substantial antioxidant activities against various oxidant systems. Specifically, the newly cultivated wild *A. devoniensis* proved to be a superior antioxidant among the tested mushrooms, due to having high levels of phenols, efficient scavenging activities on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, relatively high chelating abilities, and an apparent high reducing power. In view of total flavonoids and prevention of lipid peroxidation, however, it was a commercial strain of *P. ostreatus* that ranked the first. The findings of this research might have implications in preventing oxidative stress-related diseases through the diet; thus they may justify efforts to extract natural antioxidant ingredients from the wild mushrooms tested in this study, or to use them directly in the diet.

Keywords: Antioxidant activity, Iranian wild mushrooms, *Agaricus devoniensis*, *Agaricus gennadii*, *Pleurotus spp.*

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Introduction

Imbalance between formation and removal of highly reactive micro-molecules including reactive oxygen species (ROS) and reactive nitrogen species (RNS) may cause oxidative stress and cellular damages [1]. Consequently, these cellular damages may lead to cancer, cardiovascular diseases, diabetes, aging, and a vast number of other illnesses and disorders [2]. In living organisms, however, there are several cellular defence systems against damages caused by ROS or RNS, including antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione [3]. In addition to the endogenous defence system, ingestion of exogenous antioxidants through diet and supplements can help prevent oxidative damage [4,5]. Thus far, the most extensively used antioxidant supplements have been propylgallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone. However, these compounds are suspected of causing liver damage and carcinogenesis [6,7]. Thus, there has been an increasing interest in research on natural antioxidants, namely vegetables, fruits, and mushrooms.

Mushrooms are well known to possess considerable nutritional values due to their high fiber, minerals and protein content, as well as low fat. In addition, they contain useful bioactive metabolites with a broad spectrum of pharmacological benefits [4], including antioxidant activity. In addition to cultivated mushrooms, different wild mushroom species have been demonstrated to have antioxidant activity, many of which have been previously reviewed by Ferreira *et al.* [8]. The antioxidants found in mushrooms are mainly phenolic compounds (phenolic acids and flavonoids), followed by tocopherols, ascorbic acid and carotenoids, which could be used as antioxidant ingredients. In addition, mushrooms can be used directly into diet and benefit from the synergistic effects of all the bioactive compounds [8].

Mushrooms have a long history of use as a traditional food and medicine in different parts of the world, especially among ethnic groups [4]. However, there is no a clear record on medicinal values of wild mushrooms in Iran, as opposite to medicinal plants that have long been used in the folk medicine of the country. Even today, there is shortage of the public awareness regarding special medicinal values of mushrooms, although a sharp increase in the consumption of mushrooms can be seen. In a previous study, we utilized internal transcribed spacer (ITS) analysis to authenticate several wild isolates from *Agaricus* and *Pleurotus* species collected from north-eastern Iran. In this study, thus, several commercial strains and ITS-identified wild mushrooms of two genera *Agaricus* and *Pleurotus* were subjected to cultivation, followed by assessment of their antioxidant activity through various chemical and biochemical techniques. In particular, it is the first time in Iran that *A. devoniensis* and *A. gennadii* have been cultivated and their antioxidant potencies have been studied during this research. In addition, all the commercially cultivated strains utilized in this study are regularly used by the Iranian mushroom market.

Therefore, the findings of this research might be applicable directly to both the mushroom industry and public health.

Materials and Methods

Samples

Totally eight mushroom samples of two genera *Agaricus* and *Pleurotus* were analyzed (Table 1). The commercial strains were obtained from our strain collection. The wild strains have previously been collected and authenticated using ITS analysis. Pure cultures of the mushrooms were prepared using a compost extract agar medium. Then, the cultures were subjected to spawn production and mushroom growing based on procedures adapted in our institute. Wild mushrooms *A. devoniensis* and *A. gennadii* were grown in the same spawn and compost as those for *A. bisporus*; however, these mushrooms required a higher temperature (30° C) to grow properly, as compared to the white button mushrooms.

Table 1. *Collection information of the mushroom strains used in this study*

No	Species	Origin	Strain name
1	*Agaricus bisporus*	Commercially cultivated	A15
2	*Agaricus bisporus*	Bred commercially cultivated[a]	IM008
3	*Agaricus bisporus*	Iranian wild[b]	As007
4	*Agaricus devoniensis*	Iranian wild[b]	Ad005
5	*Agaricus gennadii*	Iranian wild[b]	Ag001
6	*Pleurotus osteratus var florida*	Commercially cultivated	725
7	*Pleurotus ostreatus*	Bred commercially cultivated[a]	19
8	*Pleurotus cf. eryngii*	Iranian wild[c]	Kerman

[a]*Developed through breeding programs by our group*
[b] *Collected from the North-eastern Iran and assigned to a species using ITS sequence analysis*
[c]*Collected from Kerman (the south of Iran) nd morphologicallay confirmed to be Pleurotus cf. eryngii*

Chemicals

All the reagents used for this study were freshly prepared before use. Butylated hydroxyanisole (BHA), trichloroacetic acid (TCA), and thiobarbituric acid (TBA), methanol (grade 99.9%), dichloromethane (grade 99.9%), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), hydrochloric acid (grade 37%), acetic acid (grade 99.8%), and 1-butanol (grade 99%), were all purchased from Merck (Darmstadt, Germany). Other reagents included gallic acid (Fluka, Spain), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), folinciocaltue (Panreac, Spain), catechin (Fluka, USA), ferrous chloride (Chem-Lab NV, Belgium), ferrozin (Sigma-Aldrich, Germany), and ascorbic acid (Scharlab S.L. Sentmenat, Spain).

Production of mushroom extract

Fresh fruiting bodies were collected, air dried, and powdered. Each 30 g of the mushroom powder was macerated with 300 ml of dichloromethane-methanol (1:1, v/v) mixture at room temperature at 150 rpm for 48 hours. The residue was also re-extracted with additional 100 ml of each solvent. The broths were filtered, combined and concentrated in a rotating vacuum evaporator (RE 300, Heidolph, Germany) at 40° C, and incubated until dryness. The dry extracts were aliquoted (9 g in 10-cm gamma sterile petri dishes) and stored at dark and 4° C until use. The extracts were re-constituted in 100% methanol at 40 mg/ml.

Analysis of total phenolics

Total phenol content (TPC) was quantified based on a modified method of Mayur *et al.* [9] in 96-well plates. From a stock solution of gallic acid (1.6 g/ml), 10 µL of various dilutions (3.1 up to 1600 µg/ml) was loaded into each well followed by addition of 100 µL of Folin-Ciocalteau 10% (0.2 N). After five minutes, 80 µL of

sodium carbonate solution (7.5%, v/w%) was added and the plate was kept in the dark for 30 minutes. The absorbance was then read at 750 nm by a Nanodrop apparatus (Epoch-Biotek, USA). The absorbance for gallic acid generated a standard curve with the following equation: $y = 1.3721x + 0.3535$ ($R^2 = 0.9884$). The same procedure was carried out with the mushroom extracts at 10 mg/ml. TPC was calculated using the curve equation and expressed as mg of gallic acid equivalents (GAEs) per g of dry weight (dw).

Flavonoid determination

Flavonoids were determined based on modifications of Barros *et al.* [3]. Shortly, a standard curve was generated using 10 dilutions of catechin (4.9 up to 2500 µg/ml). Then, 62.5 µL of each dilution was mixed with distilled water (312.5 µL) and $NaNO_2$ (5%, 18.7 µL). After five minutes, an $AlCl_3$.H2O solution (10%, 37.5 µL) was added. After six minutes, NaOH (1 M, 125 µL) and distilled water (68.7 µL) were added. Into each well, 125 µL of the mixture was dispensed and the spectrophotometry was done at 510 nm. A standard curve was plotted based on the absorbance of catechin, with the following equation: $y = 9\times10^{-5}x + 0.0672$ ($R^2 = 0.9966$). The same procedure was carried out with each mushroom extract at 10 mg/ml. Flavonoid content of each mushroom sample was measured using the curve equation and expressed as mg of catechin equivalents (CEs) per g of dw.

Scavenging ability on DPPH radicals

Scavenging ability of the mushroom extracts on DPPH radicals was assessed using a micro-titer plate method established by Arbaayah and Kalsom [10] with modifications. Shortly, 50 µL of the mushroom extracts (Twelve 1.5-fold dilutions, starting from 110 µg/ml) and 50 µL of the DPPH solution (0.5 mg/ml in methanol) were added into each well. While methanol replaced the mushroom extracts to make negative controls, BHA served as a positive control. The plates were left to stand in the dark for 30 minutes with shaking. The radical scavenging activity was measured at 517 nm using the following equation: % RSA = (Ac–As/Ac) ×100; where RSA: radical scavenging activity; Ac: the absorbance of the negative control; As: the absorbance of the mushroom samples. The extract concentration providing 50% of radicals scavenging activity (DPPH-EC_{50}) was measured by interpolation using regression analysis.

Reducing power determination

A 96-well plate-based reducing power method was established, modified from Oyaizu [11]. Various concentrations of mushroom methanolic extracts (3.75, 7.5, 15, and 30 mg/ml) at 500 µL were mixed with 500 µL of sodium phosphate buffer (pH 6.6, 200 mM) and 500 µL of potassium ferricyanide, K3Fe(CN)6, (1% w/v). The mixture was incubated at 50º C for 20 min. TCA (10%, 500 µL) was added following by centrifugation at 2500 g for 10 min. The upper layer (500 µL) was mixed with deionised water followed by the addition of 100 µL of ferric chloride (0.1% w/v). The absorbance was measured spectrophotometrically at 700 nm. The extract concentration providing 50% of absorbance (EC_{50}) was calculated from the graph of absorbance. BHA was used as standard.

Chelating abilities against ferrous ions

Chelating ability assay was adapted from a method of Yeh *et al.* [6]. To 400 µL of each mushroom dilution (the same as those utilized for the DPPH assay), 50 µL of ferrous chloride (2 mM) was added and shaken vigorously. The reaction was initiated by the addition of 50 µL of ferrozine (5 mM). Then, 100 µL/well of the mixture was dispensed and allowed to stand at room temperature for 10 minutes, after which the absorbance was measured at 562 nm. Methanol served as the negative control, while EDTA (at the same doses as the mushrooms) was taken as the reference standard. The chelating ability percentage was determined using the following equation: % CA = (Ac–As/Ac) ×100; where CA: chelating ability; Ac: the absorbance of the negative control; As: the absorbance of the mushroom samples.

Inhibition of lipid peroxidation

The inhibition effect of the mushrooms on lipid peroxidation was evaluated based on a modified TBARS (Thiobarbituric acid reactive substances) assay [12], using egg yolk homogenate as lipid rich media. Briefly, fresh egg homogenate (10%, in ice-cold 1.15% KCl, w/v) was centrifuged at 2500 g for 15 minutes. Then, 500 µL of the supernatant was mixed with 100 µL of the mushroom samples (at 40 and 20 mg/ml), prepared immediately before use, and made up to 1.0 mL with sterile distilled water. FeSO4 (50 µL, 10 µM) and ascorbic acid (50 µL, 100 µM) were added to induce lipid peroxidation, followed by incubation at 37° C for 30 minutes. The following controls were also taken: BHA (as the reference control) and sterile distilled water (as the fully oxidized control). The reaction was stopped by addition of 50 µL of TCA (20%, pH 3.5), 1.5 ml of TBA (0.8%, w/v, in 1.1% SDS, w/v) and 1.5 ml of acetic acid (20%, w/v, pH 3.5), followed by heating at 100° C for 60 minutes. After cooling, 5.0 ml of 1-butanol was added to each tube to stabilize the colour, followed by centrifugation at 2500g for 15 minutes. The absorbance of the organic upper layer was measured at 532 nm. Inhibition rate was determined through the following formula: Inhibition ratio (%) = (A-Al)/A × 100; where A was the absorbance value of the fully oxidized and A1 was the absorbance value of the sample.

Statistical analysis

All the treatments were applied in triplicate, and each experiment was independently repeated at least three times. GraphPad Prism version 6 was utilized to conduct statistical analyses and ANOVA tests. Means were compared using Tukey method with a significance level (*p* value) of 0.05.

Results and Discussion

DPPH radical scavenging activity

DPPH radical scavenging activity was performed through measuring decrease in DPPH radical absorption after exposure to the mushroom extracts. It is assumed that putative antioxidants present in the mushrooms react with DPPH radicals, resulting in discolouration of DPPH radicals from purple to yellow. Overall, all the mushrooms exhibited levels of DPPH inhibition in a dose-dependent manner. Except *A. bisporus*-A15, dose-dependent DPPH scavenging active to the *Pleurotus* ones ($p<0.05$) (Fig. 1). *A.bisporus*-IM008, *A. devoniensis*-Ad005, and *A. gennadii*-Ag001 showed the most remarkable DPPH scavenging activities (higher than 90%) at 10 mg/ml, as compared to the rest ($p<0.05$). The maximal DPPH inhibition at 10 mg/ml in *Pleurotus* mushrooms were 87% and 84% for *P. cf. eryngii*-Kerman and *P. ostreatus*-19, respectively ($p<0.05$). Compatible with these findings, the DPPH-EC_{50} values of the afore-mentioned *Agaricus* and *Pleurotus* mushrooms were significantly lower than the rest ($p<0.05$) (Table 2).

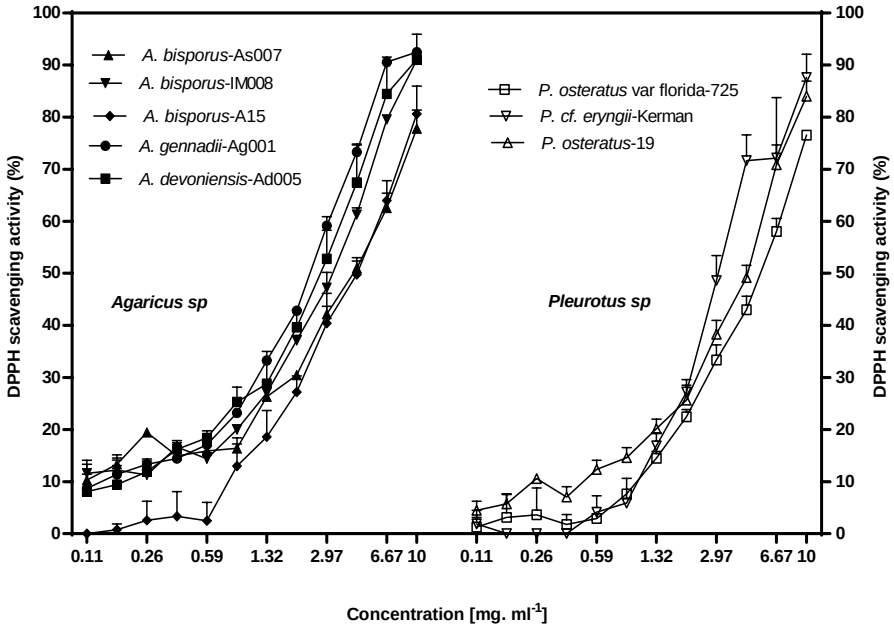

Figure 1. *DPPH scavenging activity of the tested mushrooms,determined by measuring the absorption at 517 nm.*

Table 2. *Biomolecules and antioxidant activity EC_{50} values obtained for Agaricus and Pleurotus mushroom*

Mushroom	**DPPH inhibition** (mg/ml)	**Reducing power** (mg/ml)	**Chelating ability** (mg/ ml)	**TBARS** (mg/ml)	**TPC** (mg GAE/g dw)*	**TFC** (mg CE/g dw)**
A. bisporus-A15	5.3 ± 0.12^{ab}	5.27 ± 0.16^{a}	> 10	> 40	2.5 ± 0.25^{a}	6.46 ± 0.13^{a}
A. bisporus-IM008	4.1 ± 0.22^{cd}	4.43 ± 0.0007^{ab}	> 10	> 40	3.6 ± 0.63^{b}	1.11 ± 0.24^{b}
A. bisporus-As007	5.1 ± 0.19^{ace}	9.1 ± 0.28^{c}	1.65 ± 0.07^{a}	~ 40	4.2 ± 0.12^{bc}	4.48 ± 0.05^{c}
A. devoniensis-Ad005	3.8 ± 0.38^{df}	1.44 ± 0.17^{d}	> 10	> 40	5.8 ± 0.88^{d}	3.48 ± 0.09^{d}
A. gennadii-Ag001	3.5 ± 0.11^{d}	4.1 ± 0.05^{be}	8.45 ± 0.35^{b}	> 40	4.9 ± 0.13^{e}	4.48 ± 0.25^{c}
P. osteratus var florida-725	5.9 ± 0.09^{be}	7.6 ± 0.01^{f}	1.725 ± 0.03^{a}	> 40	1.7 ± 0.38^{f}	9.05 ± 0.3^{e}
P. ostreatus-19	5.0 ± 0.63^{abc}	8.8 ± 0.15^{cg}	> 10	~ 40	1.9 ± 0.37^{f}	9.05 ± 0.61^{e}
Pleurotus cf. eryngii-Kerman	4.7 ± 0.44^{acf}	7.1 ± 0.11^{f}	> 10	> 40	2.5 ± 0.25^{a}	3.08 ± 0.11^{df}

Values are expressed as mean ± standard deviation, generated through at least three independent experiments. Means that do not share a letter are significantly different ($p<0.05$).
**TPC; total phenolic contents are expressed as mg gallic acid equivalents (GAEs) per one g of dry weight (dw)*
***TFC; total flavonoid contents are expressed as mg catechin equivalents (CEs) per one g of dry weight (dw)*

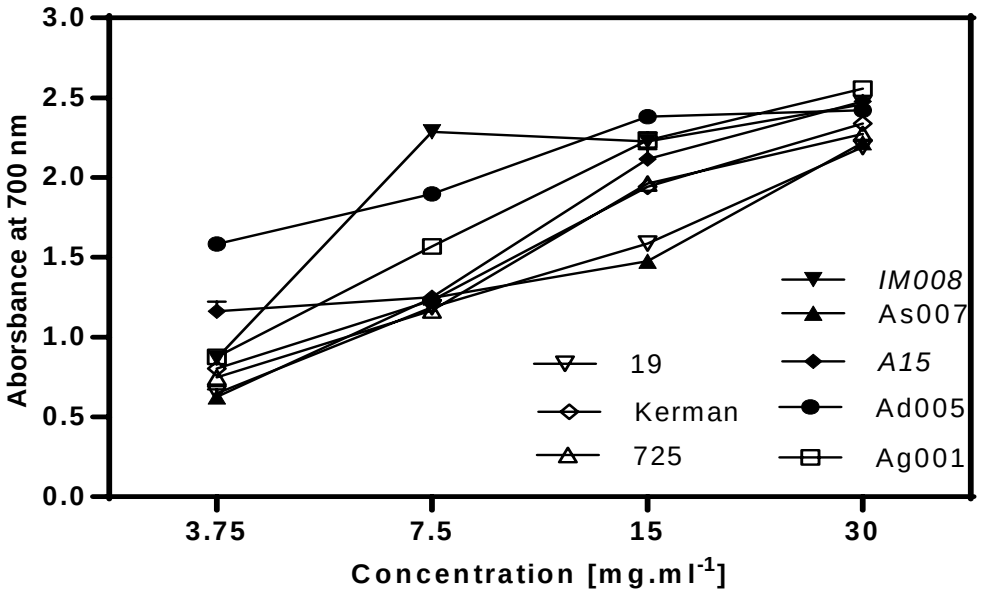

Figure 2. *Reducing power of the mushrooms by measuring the intensity of Perl's Prussian blue at 700 nm*

According to our findings, two mushrooms *A. devoniensis*-Ad005 and *A. gennadii*-Ag001 could, thus, be considered the most powerful DPPH scavengers amongst the tested mushrooms. To the best of our knowledge, this is the first report on assessment of antioxidant potential of wild *A. gennadii* and *A. devoniensis.* Regarding the *A. bisporus* strains, the DPPH-EC_{50} values represent higher free radical inhibition than methanolic extracts of Turkish [13] and Portuguese [14] wild *A. bisporus* mushrooms, where they showed EC_{50} values of 19.51 and 9.61 mg/ml, respectively. In contrast, another study revealed that a Turkish isolate of *A. bisporus* showed a considerable DPPH inhibition of 77.5% at only 180 µg/ml [15]. Also, an ethanolic extract of Chinese *A. bisporus* [16] showed over 90% radical inhibition with an EC_{50} of only 0.38 mg/ml.

The maximal DPPH inhibition obtained with *P. ostreatus*-19 at 10 mg/ml (84%) was much higher than the methanolic extract of an Indian *P. ostreatus* isolate (44%) [17] and higher than ethanolic extract of a Romanian *P. ostreatus* isolate (78%) [18]. In line with these findings, the DPPH-EC_{50} of *P. ostreatus*-19 (5 mg/ml) was much lower than that of Turkish [13] and Malaysian [10] isolates of *P. ostreatus*. In contrast, another Turkish isolate of *P. ostreatus* showed a considerable DPPH inhibition of 77.5% (at only 180 µg/ml) [15]. In our study, *P.cf. eryngii*-Kerman showed a great DPPH inhibition (87%, EC_{50} of 4.7 mg/ml), which might be comparable to a Korean *P. eryngii* (15%) [19].

Reducing power

Reducing capacity of the mushroom samples was assessed using their ability to convert Fe^{3+} to Fe^{2+}. Intensity of Perl's Prussian blue caused by this reduction was measured at 700 nm; a higher absorbance indicated higher reducing power. Thus, reducing ability of a compound can be contributed to its antioxidant properties. As depicted in Fig. 2, the amounts of reducing power of the mushroom extracts were functions of their concentrations. At 30 mg/ml, all the mushroom ext racts exhibited an outstanding reducing power of over 2. The amount of reducing ability remained over 2 at the concentration 15 mg/ml in the *Agaricus* spp, except *A. bisporus*-As007. In particular, *A. devoniensis*-Ad005 and *A. bisporus*-A15 exhibited a reducing power of over 1 even at the concentration 3.75 mg/ml. The data showed that *Agaricus* mushrooms had lower EC_{50} as compared to *Pleurotus* strains ($p<0.05$), except *A. bisporus*-As007 with an EC_{50} of 9.1 mg/ml (Table 2). The lowest EC_{50} (1.44 mg/ml) was found with *A. devoniensis*-Ad005 that significantly differed from the rest ($p<0.01$). In addition, Pearson's correlation test showed that the reducing power-EC_{50} values considerably correlated with those of the DPPH-EC_{50} (r= + 0.72).

Similar studies from Portugal reported strains of *A. bisporus* having had EC_{50}-reducing power values of 3.63 (mg/ml) [3], and 1.80 (mg/ml) [20], which were lower than *Agaricus* mushrooms (except *A. devoniensis*) tested in the present study. In addition, other studies from China [16] and France [21] reported substantial reducing power values for an ethanolic extract (EC_{50} = 0.37 mg/ml) and a methanolic extract (EC_{50} = 1.26 mg/ml) of *A. bisporus*, respectively. However, no data was found in the literature as to reducing power of *A. devoniensis,* which showed a distinguished reducing power among the mushrooms tested in our study.

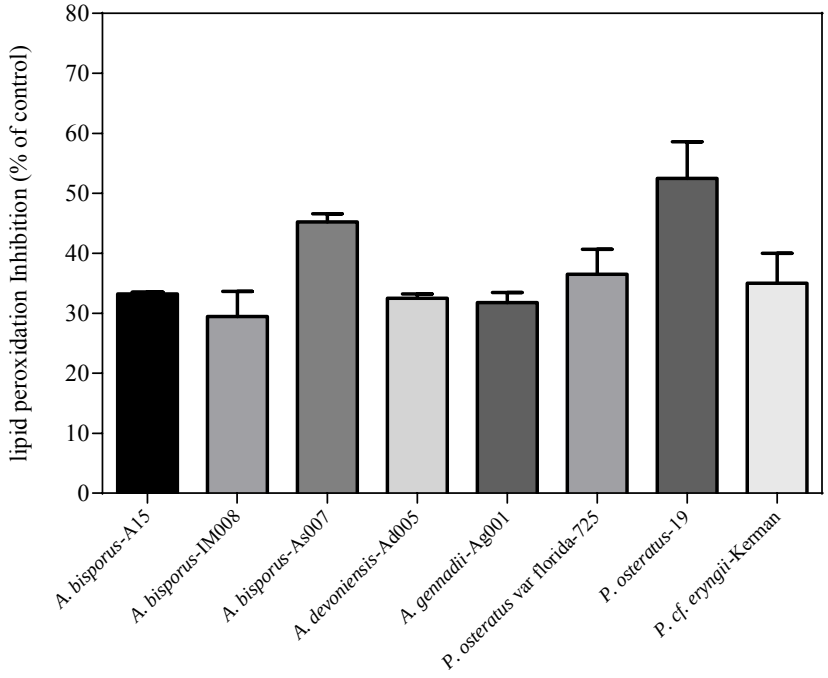

Figure 3. *Inhibition of lipid peroxidation by the mushroom extracts at 40 mg/ml; The decrease in the pinkish-red color of TBA-MDA complex was measured at 532 nm*

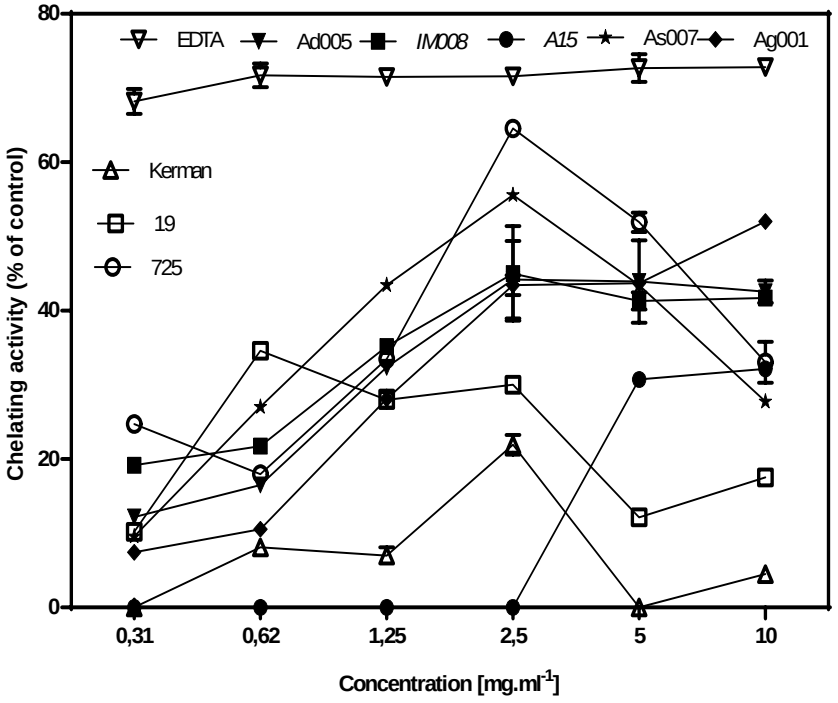

Figure 4*. Chelating ability of the mushroom extracts. Inhibition of the formation of ferrozine-Fe^{2+} complex was detected at 562 nm.*

With regard to oyster mushrooms, the ethanolic extract of a Romanian *P. osreatus* at 10 mg/ml showed a reducing power similar to our findings [18]. However, *P. osreatus* strains reported from India [17] and Malaysia [10] at 10 mg/ml had reducing power values of 1 and 0.397, respectively; which were weaker than those of our findings. In contrast, the methanolic extract of a Turkish isolate of *P. ostreatus* showed significant amounts of reducing power at very low concentrations (600 µg/ml) [15].

Inhibition of lipid peroxidation

Effect of the mushroom extracts on lipid peroxidation was measured by a modified TBARS method using egg yolk homogenates as lipid rich media. Amounts of decrease in the pinkish-red color developed by TBA-MDA complex were attributed to inhibitory effects of the mushrooms on lipid peroxidation. The results showed that no considerable color change was occurred at concentrations below 40 mg/ml (data not shown). Therefore, the lipid peroxidation assay was performed using the mushroom extracts at 40 mg/ml. Amongst the mushroom extracts, only *P. ostreatus*-19 exhibited over 50% inhibitory potency, followed by *A. bisporus*-As007 with about 45% inhibition (Fig. 3) While, there was no significant difference in lipid peroxidation inhibition between *P. ostreatus*-19 and *A. bisporus*-As007 ($p>0.05$), these two mushrooms significantly differed from the rest ($p<0.05$). Consistent with our findings, the methanolic extract of a Romanian *P. ostreatus* had significant lipid peroxidation prevention; 70% (at 10 mg/ml) [18].

As opposed to the data obtained with DPPH and reducing power, the tested mushrooms were found to exert moderate or slight inhibition of lipid peroxidation. These findings are in agreement with those of Barros *et al.* [3], where they reported that the antioxidant activities generated by chemical assays were greater than those of biochemical assays such as lipid peroxidation. Accordingly, TBAR-EC_{50} for *A. bisporus* was found to be higher than 50 mg/ml.

Chelating ability

Chelating effects of the test mushrooms on Fe^{2+} were determined by the formation of ferrozine-Fe^{2+} complexes. The ability of an agent to chelate Fe^{2+} is based on the assumption that it can capture Fe^{2+} before ferrozine, thus preventing the formation of ferrozine-Fe^{2+} complex. This reaction can be spectrophotometrically detected through absorbance at 562 nm; less absorbance indicates higher chelating ability. Firstly, the chelating ability of EDTA was assessed as a reference. EDTA at all the tested concentrations (0.31-10 mg/ml) showed a plateau of about 70% chelating ability (Fig. 4). This amount of chelating ability remained stable down to the concentration 0.11 mg/ml, after which it fell to 40% (data not shown).

Generally, the chelating effects of all the mushroom extracts increased as their concentrations increased from 0.31 mg/ml to 2.5 mg/ml. Among the mushroom extracts, *A. devoniensis*-Ad005 and *A. gennadii*-Ag001 showed an obvious dose-dependent increase in chelating ability as the concentrations increased up to 10 mg/ml, where they exhibited 42% and 52% chelating activity, respectively. However, the other mushrooms showed fluctuations in the chelating activity at the concentrations above 2.5 mg/ml. At 2.5 mg/ml, *P. osteratus* var florida-725, *A. bisporus*-As007, and *A. bisporus*-IM008 showed notable chelating effects of 64%, 55%, and 45%, respectively, which were significantly different from each other and the rest of mushrooms as well ($p<0.05$). *A. bisporus*-A15, *P. osteratus*-19, and *P. cf. eryngii*-Kerman did not show considerable chelating activities at any tested concentrations (Fig. 4). Therefore, the order of chelating effects of the mushrooms at 2.5 mg/ml on ferrous ion was *P. osteratus* var florida-725 (64%)> *A. bisporus*-As007 (55%)> *A. bisporus*-IM008 (45%)> *A. devoniensis*-Ad005 (44%)>*A. gennadii*-Ag001 (43%)> *P. osteratus*-19 (30%)> *P. cf. eryngii*-Kerman (22%)> *A. bisporus*-A15 (0%).

Other studies showed higher levels of iron chelating for *Agaricus* and *Pleurotus* mushrooms as compared to our findings; the methanolic extract of a Turkish *A. bisporus* [15] with 58% inhibition at only 100 µg/ml, *P. osteratus* reported from India [17], and Romania [18] with 90% inhibition, and *P. osteratus* reported from Turkey with 62.5% inhibition (at 10 mg/ml) [15]. The present study revealed that *P. osteratus* var florida-725, wild *A. bisporus*-As007, *A. devoniensis*-Ad005 and *A. gennadii*-Ag001, could be considered good ferrous chelators. Basically, the metal chelating ability is considered important, as it may lower the level of transition metals that involve in generating the first few radicals to initiate the radical-mediated oxidative chain reactions in both biological and food systems [6]. Therefore, the aforementioned mushrooms may serve as chelating agents to protect against oxidative stress.

Estimation of total phenolic contents (TPC)

Phenolic content of the mushrooms was quantitatively measured using Folin–Ciocalteu assay method adapted in 96-well plate. Thus, TPC of each sample was measured and expressed as mg gallic acid equivalent per one g dry weight (Table 2). Overall, *Agaricus sp* mushrooms were found to possess higher amounts of TPC in comparison to *Pleurotus sp* mushrooms ($p<0.05$), except *A. bisporus*-A15 that exhibited the same TPC as *P. cf. eryngii*-Kerman (2.5 mg GAEs/g dw). While *A. devoniensis*-Ad005 showed the highest amount of TPC with 5.8 mg GAEs/g dw, the lowest amount was seen in *P. osteratus* var florida-725 with only 1.7 GAEs/g dw. The TPC values of both *A. devoniensis*-Ad005 and *A. gennadii*-Ag001 were significantly greater than the rest of mushrooms ($p<0.05$); however there was no significant difference between *A. devoniensis*-Ad005 and *A. gennadii*-Ag001 ($p>0.05$).

The maximal TPC observed in wild *A. devoniensis* is lower than that of wild *A. bisporus* isolated reported from Turkey (13 $GAEs.g^{-1}$) [15] and China (6.8 $GAEs.g^{-1}$) [16]. This amount of TPC, on the other hand, is higher than extracts of wild *A. bisporus* from another Turkish study (4.02 $GAEs.g^{-1}$) [13] and from Portugal (4.49 $GAEs.g^{-1}$) [14], respectively. Total phenols observed in the *Pleurotus* strains tested in our study were almost twice as high as those of *Pleurotus sp* reported from India [17]. Malay-

sian wild and commercial strains of *P. ostreatus* showed around 6 and 5 GAEs.g^{-1}, respectively [22].

Some authors have expressed TPC as mg GAEs per extract or fresh weight of mushroom. Thus, in addition to dry weight, we also calculated the TPC values to be expressed as mg GAEs per extract (data not shown) in order to make them more comparable with similar reports. Accordingly, TPC values in *P. osteratus* var florida-725, *P. cf. eryngii*-Kerman and *P. osteratus*-19 were 9.5, 14, and 10.5 GAEs.g^{-1} extract, respectively. These amounts are much less than those reported with *P. ostreatus* reported from Romania [18] and Malaysia [10].

Phenols are major antioxidant component in mushrooms and their measurement has been well known to be associated to antioxidant activity, including free radical scavenging and chelating ability. Accordingly, a strong negative Pearson's correlation (r = - 0.80) was observed between DPPH-EC_{50} values and total phenols. Similarly, total phenols significantly correlated with reducing power-EC_{50}s (r= - 0.69).

Estimation of total flavonoid contents (TFC)

Flavonoid contents were quantitatively measured through an $AlCl_3$ method adapted in 96-well plate, expressed as mg catechin equivalent per one g dry weight. Contrary to the findings obtained with phenolic contents, two *Pleurotus* strains *P. osteratus*-19 and *P. osteratus* var florida-725 exhibited considerable amount of flavonoids (9.05 CEs/g dw) that was significantly greater ($p<0.01$) than those of *Agaricus* mushrooms (Table 2). Significant amounts of flavonoid in *Pleurotus* mushrooms tested in the present study are in agreement with other studies, displaying a significant amount of flavonoids[18]. Despite having a low TPC, *A. bisporus*-A15 showed a noticeable amount of flavonoids (6.46 CEs/g dw) that was significantly higher than the rest of *Agaricus* mushrooms. The lowest TFC was seen in *A. bisporus*-IM008 with just over 1 mg CEs/g dw (Table 2).

The TFC values of *A. bisporus* were higher than ones reported for cultivated strains of *A. bisporus* from Portugal [3] and Malaysia [5], while flavonoids reported from methanolic extracts of white and brown button mushrooms in Tuskegee, Alabama (USA) were significantly higher than ours [23].

TFC values in *Pleurotus* strains tested in present study were much greater than those reported from India [17], and Malaysia [10, 22]. Expressed as mg CEs/g extract, TFC values for *P. osteratus* var florida-725, *P. cf. eryngii*-Kerman and *P. osteratus*-19 were 88.78, 134.44, and 48.85, respectively. A *P. ostreatus* isolate from Romania was shown to possess TFC as much as 212 and 118 (mg quercetin equivalent per g extract) for ethanolic and methanolic extracts, respectively [18].

Conclusion

This is the first report on antioxidant potency of well-authenticated wild mushrooms in Iran. Our findings revealed that the wild and commercial stains of *Pleurotus* and *Agaricus* had considerable *in vitro* antioxidant activities against various oxidant systems. Specifically, wild *A. devoniensis* might be regarded as a superior natural antioxidant, as compared to other tested mushrooms, due to having high levels of phenols, efficient DPPH scavenging and chelating abilities, and an outstanding reducing power. To the best of our knowledge, this is the first report on antioxidant activity of this mushroom in the literature. In addition, its cultivation process has been standardized in this study, which may make it more applicable and reliable source of natural antioxidants. However, in light of total flavonoids and prevention of lipid peroxidation, commercial strain of *P. ostreatus*-19 ranked the first. At the moment, the importance of protein content of mushrooms has been taken into its place in Iran; however, other medicinal properties, including antioxidant activity, of mushrooms is overlooked. Thus, the findings presented here might have implications in the population diet and disease prevention through diet. Fractionation and profiling of main antioxidant components present in these mushrooms would merit further studies.

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Correspondence to:

Sara Saadatmand
Department of Biology
Science and Research Branch
Islamic Azad University
Tehran, Iran