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Research Article

Distribution of Antioxidant Activities and Total Phenolic Contents in Acetone, Ethanol, Water and Hot Water Extracts from 20 Edible Mushrooms via Sequential Extraction

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Abstract

Distribution of antioxidative properties and total phenolic content (TPC) of 20 commonly consumed edible mushrooms in four extraction solvents were investigated. The results showed that aqueous extracts (water extracts and hot water extracts) possessed much higher antioxidant properties than organic solvent extracts (acetone extracts and ethanol extracts). Among all extracts, the water extract of *Agaricus subrufescens* presented the best antioxidant activity. Similar as antioxidants, the TPC values of the samples extracted with water and hot water were much higher than those extracted with acetone and ethanol. In general, water extract of *Ophiocordyceps sinensis* exhibited the highest TPC value among all extracts.

Keywords: Antioxidant properties; Edible mushrooms; Extraction solvent; Phenolics.

Introduction

Mushrooms have been a part of human diet in many regions of the world for centuries due to their organoleptic characteristics as well as the nutritional values [1,2]. The consumption of mushrooms has even increased remarkably over the past few decades [3]. Generally, mushrooms provide low energy while usually contain large amounts of dietary fibers, proteins, vitamins and minerals [4]. Some mushrooms are even consumed for medicinal purpose as they contain valuable bioactive components, for example, *Ganoderma lucidum* [5]. Polysaccharides and polysaccharide-protein complexes extracted from some edible mushrooms were found to have stimulation effects on non-specific immune system and have anti-tumor properties by stimulating the defense system of the host in animal experiments [6].

It has been proven that the polysaccharides contained in cell wall of mushroom possess free radical scavenging properties [7]. In general, wild mushrooms contain significant amount of antioxidants and phenols [8]. It has been widely accepted that mushrooms are a good source of nutrients including high level of antioxidants [9]. The intake of dietary antioxidants may help the prevention of free radical damage in human body [10]. Antioxidants can scavenge free radicals through the inhibition of the initiation process or interruption of the propagation process of lipid oxidation and provide preventive function by several actions [10].

The objective of this study was to determinate the antioxidative properties and the antioxidant-related composition of phenolics in 20 commonly consumed edible mushrooms in China. The study compared the antioxidant activities of different mushroom species and how different solvents affected the effectiveness of the extraction.

Materials and Methods

Mushroom samples

One species (*Ophiocordyceps sinensis*) (fruiting bodies) was produced in Shanghai Academy of Agricultural Sciences. Other mushroom samples (fruiting bodies) were purchased from several supermarkets in Zhuhai and Harbin, China. Among the samples collected, *Boletus pinophilus* was produced in Inner Mongolia, China; *Agrocybe aegerita* and *Grifola frondosa* were produced in Zhejiang Province, China; *Pleurotus citrinopileatus* and *Pleurotus eryngii* were produced in Fujian Province, China; *Boletus edulis*, *Boletus luridus* and *Boletus aereus* were produced in Wutian, Yunnan Province, China; *Auricularia auricular*, *Tremella fuciformis*, *Hypsizygus tessellates*, *Agaricus subrufescens*, *Hericium erinaceus*, *Coprinus comatus*, *Phallus indusiatus*, *Pholiota nameko*, *Armillaria mellea*, *Hohenbuehelia reniformis* and *Lentinus edodes* were all produced in Heilongjiang Province, China. All samples were dry mushroom and stored in a cool place before treatment. The information of mushrooms was listed in **Table 1**.

Chemicals and reagents

Absolute acetone and absolute ethanol were offered by Guangzhou Chemical Reagent Factory (Guangzhou, China). 2-Diohenyl-1-picryhydrazyl (DPPH) was supplied by Shanghai Yuanye Biological

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Table 1: Scientific name, common name and sources of mushroom samples.

No.	Common name	Scientific name	Mode of production	Place of origin
1	Jew's ear	*Auricularia auricula*	Cultivated	Suifenhe, Heilongjiang Province
2	Tremella	*Tremella fuciformis*	Cultivated	Suifenhe, Heilongjiang Province
3	Shimeji	*Hypsizygus tessellatus*	Cultivated	Suifenhe, Heilongjiang Province
4	Princess matsutake	*Agaricus subrufescens*	Cultivated	Suifenhe, Heilongjiang Province
5	Lion's mane mushroom	*Hericium erinaceus*	Cultivated	Suifenhe, Heilongjiang Province
6	Shaggy ink cap	*Coprinus comatus*	Cultivated	Suifenhe, Heilongjiang Province
7	Bamboo fungus	*Phallus indusiatus*	Cultivated	Suifenhe, Heilongjiang Province
8	Golden oyster mushroom	*Pleurotus citrinopileatus*	Cultivated	Gutian, Fujian Province
9	Apricot oyster mushroom	*Pleurotus eryngii*	Cultivated	Gutian, Fujian Province
10	Porcino	*Boletus edulis*	Cultivated	Wuding, Yunnan Province
11	Lurid bolete	*Boletus luridus*	Cultivated	Wuding, Yunnan Province
12	Porcino nero	*Boletus aereus*	Cultivated	Wuding, Yunnan Province
13	Caterpillar fungus	*Ophiocordyceps sinensis*	Cultivated	Shanghai
14	Nameko	*Pholiota nameko*	Cultivated	Shangzhi, Heilongjiang Province
15	Stump mushroom	*Armillaria mellea*	Cultivated	Shangzhi, Heilongjiang Province
16	Winter mushroom	*Hohenbuehelia reniformis*	Cultivated	Shangzhi, Heilongjiang Province
17	Shiitake	*Lentinus edodes*	Cultivated	Shangzhi, Heilongjiang Province
18	Pine bolete	*Boletus pinophilus*	Wild	Chifeng, Inner Mongolia
19	Poplar mushroom	*Agrocybe aegerita*	Cultivated	Lishui, Zhejiang Province
20	Maitake	*Grifola frondosa*	Cultivated	Lishui, Zhejiang Province

Technology Co., Ltd (Shanghai, China). Folin-Ciocalteu reagent was provided by Shanghai Sanjie Biotechnology Co., Ltd (Shanghai, China). Trolox was purchased from Aldrich Co. (St. Louis, MO, U.S.A.). Sodium carbonate was supplied by Tianjin Nuoke Technology Development Co., Ltd (Tianjin, China). Gallic acid was purchased from Tianjin Damao Chemical Reagent Co., Ltd (Tianjin, China). All the chemicals were of analytical grade.

Extraction of mushroom samples

Each of the twenty dry mushroom samples was pulverized; 5.0 g of each mushroom powder was accurately weighed into a set of centrifuge tubes (each of the sample prepared in triplicate); 40 mL of absolute acetone was added into each tube for the preparation of acetone extracts. The samples were extracted for 24 hours and then centrifuged by a centrifuge (Weierkang Xiangying Centrifuge Co., Ltd, Changsha, Hunan, China) at 8000 rpm for 10 min. The supernatants were transferred into new centrifuge tubes and stored at -20 degree C. The residues were dried by vaporizing and collected; 40 mL of absolute ethanol was added into the dry residues for the preparation of ethanol extracts. The same extraction procedures were repeated to prepare water extracts as acetone and ethanol extraction and finally hot water extraction was done at 99°C in water bath for 2 hours. All the extracts were stored at -20°C in the dark for further usage.

Determination of antioxidant properties

For determination of organic extracts, 1 mL of each acetone extract, ethanol extract, blank and standard solutions was added into a pre-labeled test tube and mixed with 3 mL of DPPH solution. For determination of aqueous extracts, 0.2 mL of each water extract, hot water extract, blank and standard solutions was added into a pre-labeled centrifuge tube and mixed with 3.8 mL of DPPH solution. The mixtures were mixed evenly by vortexing and stood in the dark at room temperature for 20 min. Then the mixtures were centrifuged by a centrifuge (Weierkang Xiangying Centrifuge Co., Ltd, Changsha, Hunan, China) at 3000 rpm for 10 min. The absorbance of each mixture was measured by an UV-Visible spectrophotometer (TI-1901, Beijing Purkinje General Instrument Co., Ltd, Beijing, China) at 517 nm against solvent blank. The results were expressed as Trolox equivalents (μmole of TE/g sample) in accordance to the standard curve of Trolox.

Determination of total phenolic content (TPC)

For determination of water extracts, 50 μL of each water extract, hot water extract, blank and standard solution was mixed with 3 mL of distilled water, 250 μL of Folin-Ciocalteu reagent and 750 μL of 7% Na_2CO_3 solution and the mixtures were mixed evenly by vortexing. After standing at room temperature for 8 min, 950 μL of distilled water was added into each mixture and the mixtures were allowed to stand at room temperature for 1 hour. For organic solvent extraction, 500 μL of each acetone extract, ethanol extract, blank and standard solution was mixed with 2.55 mL of distilled water, 250 μL of Folin-Ciocalteu reagent and 750 μL of 7% Na_2CO_3 solution. The absorbance of each mixture was measured by the UV-Visible spectrophotometer (TI-1901, Beijing Purkinje General Instrument Co., Ltd, Beijing, China) at 765 nm by using distilled water as the blank. The total phenolic content (TPC) was expressed as gallic acid equivalents (mg of GAE/g sample) in accordance to the standard curve of gallic acid.

Statistical analysis

The data obtained were analyzed by ANOVA with the use of SPSS Statistics (Version 17.0, SPSS Inc., Chicago, IL, U.S.A.). Duncan's multiple range test was used to test whether there was any significant difference in antioxidant activity or total phenolic content among different mushroom species.

Results and Discussion

Antioxidant properties of various extracts from mushroom

Free radical scavenging is one of the mechanisms involved in inhibiting lipid oxidation; therefore it is normally used for

Table 2: Antioxidant activities of mushroom samples extracted by different solvents.

No.	Scientific name	DPPH free radical scavenging capacities (μmole TE/g)			
		Acetone extracts	Ethanol extracts	Water extracts	Hot water extracts
1	*Auricularia auricula*	0.57 ± 0.02*g*	0.35±0.02*i*	2.42±0.09*h*	2.18±0.08*j*
2	*Tremella fuciformis*	0.28 ± 0.02*m*	0.17±0.02*k*	3.62±0.07*d*	3.66±0.15*f*
3	*Hypsizygus tessellatus*	0.37 ± 0.03*kl*	0.24±0.01*j*	1.92±0.06*j*	3.72±0.12*f*
4	*Agaricus subrufescens*	1.04 ± 0.01*e*	1.18±0.02*c*	4.94±0.09*a*	3.76±0.08*ef*
5	*Hericium erinaceus*	0.39 ± 0.01*jk*	0.39±0.03*h*	2.85±0.06*f*	2.28±0.14*ij*
6	*Coprinus comatus*	0.53 ± 0.04*gh*	0.71±0.04*e*	4.88±0.11*a*	4.07±0.08*d*
7	*Phallus indusiatus*	1.21 ± 0.06*d*	1.53±0.04*b*	2.35±0.12*h*	2.17±0.02*j*
8	*Pleurotus citrinopileatus*	0.37 ± 0.01*k*	0.47±0.02*fg*	4.11±0.10*c*	3.76±0.20*ef*
9	*Pleurotus eryngii*	0.39 ± 0.04*jk*	0.48±0.05*f*	3.72±0.07*d*	4.45±0.13*ab*
10	*Boletus edulis*	1.75 ± 0.06*b*	2.18±0.04*a*	4.58±0.04*b*	4.19±0.12*cd*
11	*Boletus luridus*	1.79 ± 0.03*b*	2.16±0.02*a*	4.82±0.23*a*	2.84±0.21*h*
12	*Boletus aereus*	1.93 ± 0.09*a*	2.20±0.02*a*	4.87±0.12*a*	4.33±0.10*bc*
13	*Ophiocordyceps sinensis*	1.29 ± 0.01*c*	0.95±0.03*d*	2.68±0.09*g*	3.40±0.15*g*
14	*Pholiota nameko*	0.50 ± 0.03*hi*	0.43±0.03*gh*	2.13±0.08*i*	3.99±0.18*de*
15	*Armillaria mellea*	0.31 ± 0.02*lm*	0.43±0.03*fgh*	2.50±0.05*h*	3.78±0.19*ef*
16	*Hohenbuehelia reniformis*	0.30 ± 0.03*m*	0.05±0.01*l*	1.32±0.06*l*	1.74±0.04*k*
17	*Lentinus edodes*	0.40 ± 0.04*jk*	0.28±0.02*j*	1.40±0.09*l*	2.93±0.05*h*
18	*Boletus pinophilus*	0.27 ± 0.01*m*	1.52±0.01*b*	3.75±0.08*d*	3.29±0.08*g*
19	*Agrocybe aegerita*	0.66 ± 0.03*f*	1.51±0.03*b*	3.32±0.06*e*	4.64±0.20*a*
20	*Grifola frondosa*	0.44 ± 0.03*ij*	0.41±0.02*h*	1.75±0.04*k*	2.47±0.10*i*

Note: Data were expressed as mean ± standard deviation (n = 3), small letters indicate significantly different within the species at 95% confident interval ($p < 0.05$).

Table 3: Total phenolic content of mushroom samples extracted by different solvents

No.	Scientific name	Total phenolic content (mg GAE/g)			
		Acetone extracts	Ethanol extracts	Water extracts	Hot water extracts
1	*Auricularia auricula*	0.12 ± 0.01*h*	0.09 ± 0.01*i*	0.82 ± 0.04*o*	0.35 ± 0.07*m*
2	*Tremella fuciformis*	0.11 ± 0.01*hi*	0.06 ± 0.01*j*	0.81 ± 0.06*o*	0.66 ± 0.03*l*
3	*Hypsizygus tessellatus*	0.09 ± 0.01*i*	0.07 ± 0.01*j*	2.16 ± 0.03*n*	2.37 ± 0.06*g*
4	*Agaricus subrufescens*	0.25 ± 0.01*d*	0.30 ± 0.01*d*	2.82 ± 0.01*i*	1.01 ± 0.03*k*
5	*Hericium erinaceus*	0.16 ± 0.01*g*	0.13 ± 0.01*h*	3.08 ± 0.09*k*	1.75 ± 0.08*i*
6	*Coprinus comatus*	0.19 ± 0.01*f*	0.18 ± 0.01*g*	2.41 ± 0.06*m*	4.12 ± 0.05*b*
7	*Phallus indusiatus*	0.28 ± 0.01*c*	0.27 ± 0.01*e*	3.21 ± 0.16*k*	2.58 ± 0.02*f*
8	*Pleurotus citrinopileatus*	0.10 ± 0.01*i*	0.10 ± 0.01*i*	9.42 ± 0.16*d*	2.96 ± 0.10*d*
9	*Pleurotus eryngii*	0.09 ± 0.01*j*	0.07 ± 0.01*j*	3.65 ± 0.11*j*	1.52 ± 0.06*j*
10	*Boletus edulis*	0.28 ± 0.01*c*	0.30 ± 0.01*d*	5.17 ± 0.09*g*	3.98 ± 0.15*bc*
11	*Boletus luridus*	0.36 ± 0.01*b*	0.38 ± 0.01*b*	10.43 ± 0.01*b*	2.72 ± 0.11*ef*
12	*Boletus aereus*	0.29 ± 0.01*c*	0.32 ± 0.01*c*	10.18 ± 0.13*c*	3.95 ± 0.07*c*
13	*Ophiocordyceps sinensis*	0.22 ± 0.01*e*	0.24 ± 0.01*f*	10.82 ± 0.19*a*	9.44 ± 0.24*a*
14	*Pholiota nameko*	0.06 ± 0.01*j*	0.06 ± 0.01*j*	7.31 ± 0.09*e*	2.15 ± 0.10*h*
15	*Armillaria mellea*	0.10 ± 0.01*hi*	0.11 ± 0.01*i*	4.81 ± 0.09*n*	2.13 ± 0.10*h*
16	*Hohenbuehelia reniformis*	0.06 ± 0.01*j*	0.03 ± 0.01*k*	2.35 ± 0.04*m*	1.37 ± 0.07*j*
17	*Lentinus edodes*	0.06 ± 0.01*j*	0.06 ± 0.01*j*	4.26 ± 0.01*i*	1.82 ± 0.01*i*
18	*Boletus pinophilus*	0.91 ± 0.06*a*	0.62 ± 0.01*a*	4.26 ± 0.07*i*	2.86 ± 0.10*de*
19	*Agrocybe aegerita*	0.13 ± 0.01*h*	0.27 ± 0.01*e*	5.71 ± 0.07*f*	2.99 ± 0.09*d*
20	*Grifola frondosa*	0.11 ± 0.01*hi*	0.11 ± 0.01*i*	3.78 ± 0.12*j*	2.34 ± 0.09*g*

Note: Data were expressed as mean ± standard deviation (n = 3) with small letters indicate significantly different within the species at 95% confident interval ($p < 0.05$).

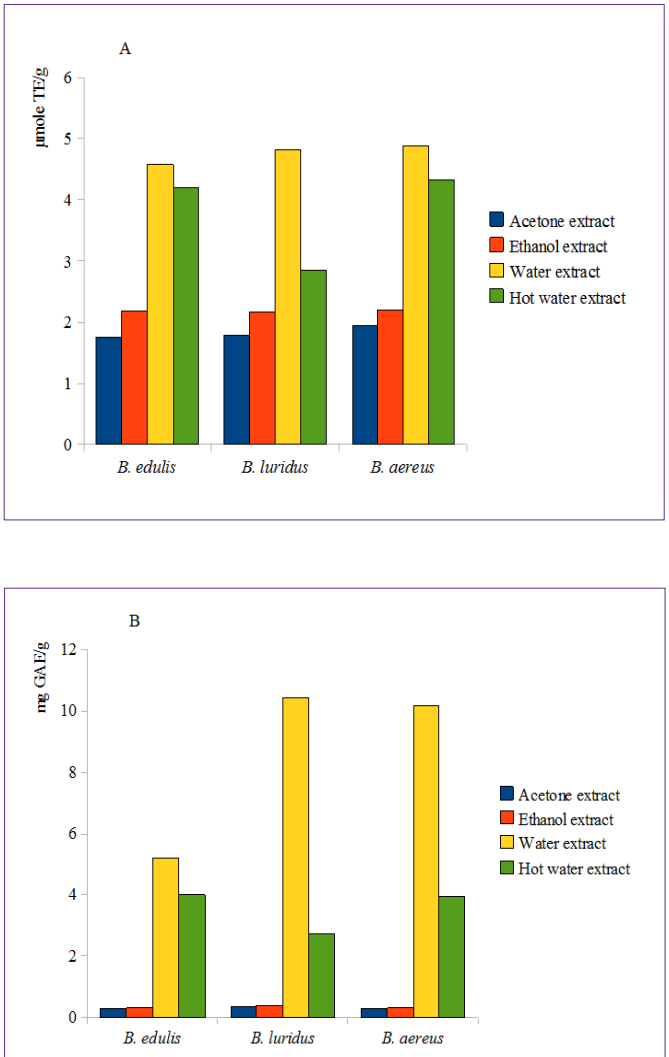

Figure 1: Antioxidant activities (A) and total phenolic contents (B) of various extracts from mushrooms *B. edulis, B. luridus and B. aereus.*

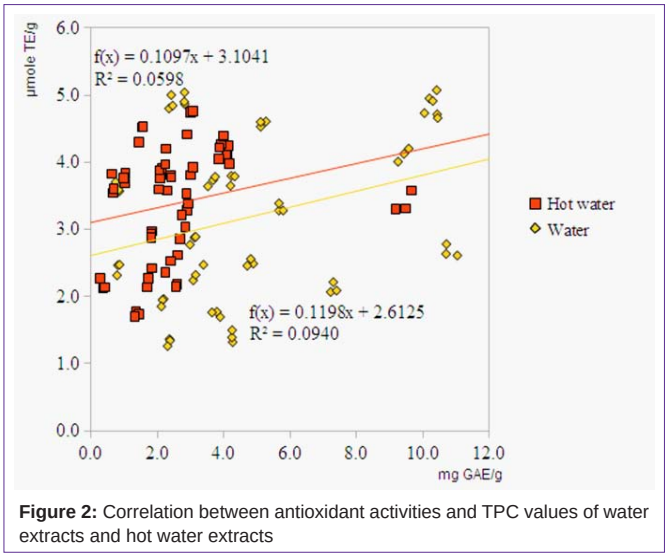

Figure 2: Correlation between antioxidant activities and TPC values of water extracts and hot water extracts

determination of antioxidant activity [11]. The DPPH free radical scavenging activity of extracts from 20 different mushrooms was tested. DPPH provides a violet color when dissolves in ethanol and the discoloration occurs when antioxidants donate protons to DPPH [11,12].

Antioxidant capacities of the 80 extracts from 20 different mushrooms with four different extraction solvents were presented in **Table 2**. Three mushroom species (*Boletus edulis*, *B. luridus* and *B. aereus*) provided a relative higher antioxidant activity for all four extraction solvents as compared to the other 17 species. **Figure 1** presented antioxidant activities of various extracts from mushrooms *B. edulis, B. luridus and B. aereus* together with their total phenolic contents. In this study, the results showed that the extracts of genus *Boletus* provided higher antioxidant activities comparing to mushrooms of other genuses.

For all mushrooms, the water extracts and hot water extracts exhibited much higher antioxidant properties than that of acetone extracts and ethanol extracts. However, the differences between water extracts and hot water extracts and the differences between acetone extracts and ethanol extracts were not significant ($p > 0.05$). Among all the extracts, the water extracts of *Agaricus subrufescens* provided the highest antioxidant activity with 4.94 μmole of TE/g sample.

Total phenolic content of mushrooms

Phenols are known as important botanical constituents due to their scavenging ability provided by the hydroxyl groups [13]. Phenolic compounds may have direct contribution to antioxidative action [14]. Phenolic compounds were reported to be associated with antioxidant activity and play important roles in the stabilizing of lipid peroxidation [15]. Natural phenolics are able to provide antioxidative function through various ways, such as intercepting singlet oxygen, decomposing primary products of oxidation, preventing continued hydrogen abstraction from substances, etc [16]. In addition, total polyphenols were considered as the major naturally occurring antioxidant compounds in the wild edible mushrooms [17].

The results of TPC values of mushrooms were shown in **Table 3**. The TPC of the samples extracted with water and hot water were much higher than those extracted with acetone and ethanol. Among the four different solvents, water extraction provided the highest TPC. There were no significant differences between acetone extracts and ethanol extracts; they presented in low amounts. Meanwhile, there were significant ($p < 0.05$) differences between different mushrooms. In organic extracts, *Boletus pinophilus* provided the highest amount of TPC with 0.91 mg GAE/g in acetone extracts and 0.62 mg GAE/g in ethanol extracts. However, comparing to other mushroom samples, it only provided medium TPC in aqueous extracts with 4.26 mg GAE/g in water extracts and 2.86 mg GAE/g in hot water extracts. In inorganic extracts, water extract and hot water extracts of *Ophiocordyceps sinensis* had the highest amount of TPC with 10.82 mg GAE/g and 9.44 mg GAE/g. The distribution of TPC in different extraction solvents depended on species of the mushrooms.

The correlations of phenolic levels and antioxidant capacities in triplicate aqueous extracts were shown in **Figure 2**. According to the results, the overall trend was that the antioxidant capacities were positively related to the phenolic levels. However, as the values of the

correlation analysis were 0.31 and 0.24 for water extracts and hot water extracts, respectively, the correlation was not highly significant.

Conclusions

The current study compared the antioxidant activities among different mushroom samples and the effects of different extraction solvents on distribution of antioxidants and phenolics. The antioxidant activity determination indicated that water extracts and hot water extracts had much higher antioxidant properties than acetone extracts and ethanol extracts. The TPC of the samples extracted with water and hot water were also much higher than those extracted with acetone and ethanol. Due to their high content of antioxidants, aqueous extracts of some mushrooms, especially *B. edulis*, *B. luridus* and *B. aereus*, may be used as materials of dietary supplements.

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