Microbiology Research Journal International

31(1): 26-32, 2021; Article no.MRJI.65905
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Screening of Antioxidant Activity of 20 Kinds of Mushrooms from Southwest of China

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Authors' contributions

This work was carried out in collaboration among all authors. Author YH designed the study, author DHH performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZHY and DHH managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2021/v31i130289
Editor(s):
(1) Dr. Laleh Naraghi, Iranian Research Institute of Plant Protection, Iran.
Reviewers:
(1) Hanan Farouk Aly, National Research Centre, Egypt.
(2) Oom Komala, Pakuan University, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65905

Original Research Article

Received 03 December 2020
Accepted 07 February 2021
Published 10 February 2021

ABSTRACT

The ethanol and water extracts of 20 kinds of mushrooms from Sichuan Province, Southwest of China, were investigated for their antioxidant activity using DPPH assay. The results showed the ethanol extract of *Serpula lacrymans, Daldinia concentrica,* and *Scleroderma verrucosum* had well DPPH free radical scavenging activities 91.33%, 79.76% and 62.82%, respectively. Besides, the water extracts of *Serpula lacrymans*, *Armillaria luteo-virens,* and *Lentinus edodes* also possessed pretty high DPPH free radical scavenging activity were 95.59%, 88.76% and 86.93%, respectively. Based on the above comparison, the EC_{50} and total phenolic content of the ethanol extracts of *Serpula lacrymans*, *Daldinia concentrica,* and *Scleroderma verrucosum* were also measured. Their EC_{50} and total phenolic content valued of 17.87mg·mL^{-1}, 11.19mg·mL^{-1} and 35.01mg·mL^{-1}, as well as 0.0769μg·L^{-1}, 0.0673μg·L^{-1} and 0.0545μg·L^{-1}, respectively. The results showed there was a correlation between antioxidant activity and total phenolic content. Besides, the reaction time of the DPPH test affected the free radical scavenging, which reflected the difference of the extract component would impact the test method.

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Keywords: Antioxidant; mushroom; EC_{50}; DPPH; total phenolic.

1. INTRODUCTION

Free radical is an extremely reactive compound with unpaired electrons. It obtained a pair of electrons by oxidizing surrounding molecules to achieve atomic stability. This phenomenon can produce oxidative stress on the body, contributing to atherosclerosis or cancer, and so forth [1-4]. Recent reports have confirmed that bioactive compounds' antioxidant activity can efficiently maintain cell structure and functions and inhibit free radicals reactions and prevent other oxidative damage [4-6].

Numerous studies found that many natural compounds like flavonoids and total phenolic compounds had exhibited antioxidant properties. Searching natural antioxidant components had become a hot topic in the scientific field in recent years, and many of them, in plants, had been found [7-9]. However, there was little scientific information on the antioxidant properties of mushrooms [10]. Therefore, the assessment of antioxidant properties from mushrooms is an essential and exciting task. It provides a theoretical basis for finding new sources like natural antioxidants, cosmetics, and functional foods [11], nutraceuticals and even drugs.

Herein, to exploit enormous mushroom resources, the DPPH free radical scavenging method was used to evaluate and compare the antioxidant activity of 20 kinds of mushrooms collected in Mianyang, Sichuan Province of China. The EC_{50} and total phenolic content of three kinds of mushrooms with the most potent antioxidant activity were determined. These results provided a theoretical basis for their further research as a bioactive antioxidant.

2. MATERIALS AND METHODS

2.1 Material and Chemicals

Twenty kinds of mushrooms' bodies were collected in the southwest of China and authenticated by He Xinsheng , a professor of mycology of Southwest University of Science and Technology(Table 1.), using morphology [12] and ITS analysis [13]. All voucher specimens were stored in the Microbiology laboratory of Southwest University of Science and Technology. The fruiting bodies of mushrooms were dried at 60 □ to constant weight, smashed through a 50 mesh sieve, stored at 4 □ and protected from light. The whole experiment was completed within one month.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) was purchased from TCI (Shanghai) Chemical Industry Development Co., Ltd. Folin-Ciocalteu was obtained from Nanjing Oddfoni Biological Technology Co., Ltd. Gallic acid and L-Ascorbic acid (Vitamin C) was purchased from Chinese Medicines Group Chemical Reagent Co., Ltd. BHA (Butyl hydroxyanisole) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Anhydrous sodium carbonate and other reagents were purchased from Chengdu Kelon Chemical Reagent Factory. All chemicals were analytical grade. Pure water was self-made (electrical resistivity was 18 MΩ•cm).

2.2 Sample Extraction

500 mg of the dry sample was mixed with 10 mL of absolute ethanol or 10 mL distilled water in a 50 mL conical flask and vortexed for 10 min followed by ultrasonic treatment for 5 min. These steps were done with three replications for each sample. Last, the obtained supernatant by filtration was conducted in the analysis process.

2.3 DPPH Radical Scavenging Assay

7.5 mg DPPH was added to a 250 mL volumetric flask accurately and dissolved by anhydrous ethanol. The solution was prepared into a concentration of 0.03mg·mL-1 and stored in the dark for later use. The absorbance value of this solution at 517 nm was around 0.8.

The antioxidant activity of the extract was measured using Pyrzynska's method [14] with slight modification. 0.2 mL supernatant of various extracts were added into 4mL of DPPH solution separately. The mixture was vigorously shaken and incubated for 5 and 30 min in the dark at room temperature. Then the supernatant was transferred to the cuvette and the absorbance was measured at 517nm. The decrease in the absorbance indicated radical-scavenging activity. The sample's antioxidant capacity could be expressed by the scavenging rate (SR(%)) and calculated using the following formula:

$$\mathrm{SR}(\%) = \mathrm{f}(\mathrm{x}) = \left(1 - \frac{\mathrm{A_i} - \mathrm{A_j}}{\mathrm{A_0}}\right) \times 100\%$$

$\mathrm{A_i}$: The absorbance of 0.2 mL test solution

mixed with 4 mL DPPH solution;

A_j: The absorbance of 0.2 mL test solution mixed with 4 mL anhydrous ethanol solvent;

A_0: The absorbance of 0.2 mL solvent(used in preparing the test solution) mixed with 4 mL of DPPH solution.

After preliminary screening of 20 kinds of mushrooms, EC_{50} (concentration of the extract for 50% scavenging rate of DPPH) of the four most potent antioxidant activity samples were determined for better evaluation. L-Ascorbic acid was used as the reference compound.

Table 1. The identification results of 20 kinds of mushrooms

			
Serpula lacrymans Division: Basidiomycota Family: Serpulaceae Genus: Serpula	*Daldinia concentrica* Division: Ascomycota Family: Hypoxylaceae Genus: Daldinia	*Scleroderma verrucosum* Division: Basidiomycetes Family: Sclerodermataceae Genus: Scleroderma	*Tremella fuciformis* Division: Basidiomycota Family: Tremellaceae Genus: Tremella
			
Clitocybe gibba Division: Basidiomycota Family: Tricholomataceae Genus: Infundibulicybe	*Inonotus obliquus* Division: Basidiomycota Family: Hymenochaetaceae Genus: Inonotus	*Boletus edulis* Division: Basidiomycota Family: Boletaceae Genus: Boletus	*Tricholoma matsutake* Division: Basidiomycota Family: Tricholomataceae Genus: Tricholoma
			
Pycnoporus cinnabarinus Division: Basidiomycota Family: Polyporaceae Genus: Pycnoporus	*Xylaria polymorpha* Division: Ascomycota Family: Xylariaceae Genus: Xylaria	*Coriolus versicolor* Division: Basidiomycota Family: Polyporaceae Genus: Trametes	*Morchella deliciosa* Division: Ascomycota Family: Morchellaceae Genus: Morchella
			
Xylaria longipes Division: Ascomycota Family: Xylariaceae Genus: Xylarias	*Agrocybe aegirit* Division: Basidiomycota Family: Strophariaceae Genus: Cyclocybe	*Xylaria nigripes* Division: Ascomycota Family: Xylariaceae Genus: Xylarias	*Trametes robiniophila* Division: Basidiomycota Family: Polyporaceae Genus: Trametes

Armillaria luteovirens
Division: Basidiomycota
Family: Physalacriaceae
Genus: Armillaria

Lentinus edodes
Division: Basidiomycota
Family: Omphalotaceae
Genus: Lentinula

Xylaria striata
Division: Ascomycota
Family: Xylariaceae
Genus: Xylarias

Auricularia auricula
Division: Basidiomycota
Family: Auriculariaceae
Genus: Auricularia

2.4 Total Phenolic Content

Using the Folin-Ciocalteu method to determine these mushrooms' total phenolic content, as described by Tumbarski et al. [15]. Sample solutions were made by diluting the stock solution (1.5 mg gallic acid was dissolved in 10 mL absolute ethanol) to five different concentrations, including 50, 75, 100, 125 and 150μg·mL-1. 0.2 mL of sample solution was mixed with 0.5 mL Folin-Ciocalteau reagent and 4.0 mL of pure water in a 10 mL volumetric flask. Then 200 μL of 20% sodium carbonate solution was added in and the absorbance was measured at 760 nm after incubation for 30 minutes at room temperature. The total phenolic content was expressed as micrograms of gallic acid equivalent per milligram of crude extract.

3. RESULTS AND DISCUSSION

3.1 Comparison of DPPH Antioxidant activity

Table 2. and Fig. 1. summarized the DPPH free radical scavenging rate of 20 kinds of mushrooms. When using anhydrous ethanol as the extraction solvent, *Serpula lacrymans* had the highest clearance (91.33% of 5 min, 96.05% of 30 min), followed by *Daldinia concentrica* (79.76%, 80.99%), *Scleroderma verrucosum* (62.82%, 74.25%). While extracted by water, three mushrooms with the highest clearance rate were *Serpula lacrymans* (95.59%, 93.40%), *Armillaria luteovirens* (88.76%, 89.48%), and *Lentinus edodes* (86.93%, 87.21%), respectively. In general, these results showed that most of the extracts had an excellent scavenging effect on DPPH. Especially, utilizing water as an extraction solvent had a slightly better effect on the extraction of antioxidant substances than anhydrous ethanol. However, test reaction time had less influence on most of the mushrooms.

3.2 Determination of EC_{50}

Compared with water, ethanol could extract more active constituents and reduce the complexity of subsequent separation [16]. Therefore, the EC_{50} of ethanol extract from the three most potent antioxidant activity mushrooms (*Serpula lacrymans*, *Daldinia concentrica*, and *Scleroderma verrucosum*) were determined for further evaluation using Vitamin C and gallic acid as standard. Scavenging activity was expressed as EC_{50} (effective concentration in mg/mL of samples or positive control that reduces the absorbance of DPPH by 50% compared with negative control). As provided in Table 3, the extracts of *Daldinia concentrica*, *Serpula lacrymans*, and *Scleroderma verrucosum* showed EC_{50} values of 11.19mg·mL^{-1}, 11.87mg·mL^{-1} and 35.01mg·mL^{-1}, respectively, where the EC_{50} values of Vitamin C and gallic acid were 0.087mg·mL^{-1} and 0.019μg·mL^{-1}. The results show that *Daldinia concentrica* and *Serpula lacrymans* had low EC_{50} values, which meant they had higher antioxidant activity [17].

3.3 Determination of Total Phenolic Content

Phenolic is a significant group of compounds acting as free radical scavenging or primary antioxidants [18]. Determining phenolic content in mushrooms' extracts could estimate all flavonoids and non-flavone phenolic compounds [19]. Based on EC_{50} values, the total phenol content was determined by the method of Folin-Ciocalteau. Table 4 revealed that the ethanol extract of *Serpula lacrymans* had the highest content, followed by *Daldinia concentrica* and *Scleroderma verrucosum*. This result had a significant correlation with the DPPH value. It indicated a viewpoint that total phenol's presence mainly contributed to the antiradical activity, verified by Li FH et al [20].

However, the correlation was less remarkable for *Daldinia concentrica* between EC_{50} values and total phenolic content. This phenomenon possibly indicated some other components(Not phenols) in *Daldinia concentrica* extract exhibited antioxidant activity.

Table 2. The scavenging rate of 20 kinds of mushrooms

Mushroom	DPPH scavenging rate (%)			
	EtOH extract		Distilled water extract	
	5 min	30 min	5 min	30 min
Serpula lacrymans	91.33±3.28	96.05±2.97	95.59±1.15	93.40±0.95
Daldinia concentrica	79.76±3.92	80.99±3.53	61.02±2.80	65.14±5.08
Scleroderma verrucosum	62.82±3.26	74.25±1.47	77.81±1.03	78.12±1.15
Clitocybe gibba	41.77±1.21	60.73±1.47	23.71±2.34	38.35±3.05
Inonotus obliquus	40.24±1.73	44.11±1.58	68.30±6.65	68.32±7.76
Boletus edulis	28.05±2.21	38.41±1.29	55.87±2.75	54.58±2.85
Pycnoporus cinnabarinus	14.22±3.11	17.68±3.14	33.03±3.61	47.70±5.79
Xylaria polymorpha	12.56±3.72	21.33±6.12	56.12±2.63	70.80±2.50
Coriolus versicolor	6.69±1.41	11.32±2.09	62.87±1.53	85.61±4.51
Xylaria longipes	6.53±1.15	10.60±2.43	49.08±3.67	69.66±4.98
Agrocybe aegirit	6.00±2.01	8.93±2.25	86.17±1.13	89.17±1.43
Xylaria nigripes	5.91±0.50	10.00±1.83	64.99±0.73	80.42±1.21
Armillaria luteovirens	5.42±0.84	7.92±1.82	88.76±7.02	89.48±6.39
Lentinus edodes	5.25±1.94	8.42±2.26	86.93±4.27	87.21±5.86
Xylaria striata	4.96±1.43	6.85±1.96	76.52±9.20	80.80±10.66
Trametes robiniophila	4.80±1.30	6.86±1.11	63.88±4.21	79.59±2.70
Morchella deliciosa	4.44±0.38	8.39±1.83	85.69±6.27	88.68±8.97
Auricularia auricula	4.31±1.75	9.46±0.73	61.58±3.94	82.92±2.42
Tremella fuciformis	-0.15±0.55	0.27±0.55	9.75±0.71	15.05±0.75
Tricholoma matsutake	-0.38±1.45	2.45±1.15	73.79±0.66	73.60±1.79

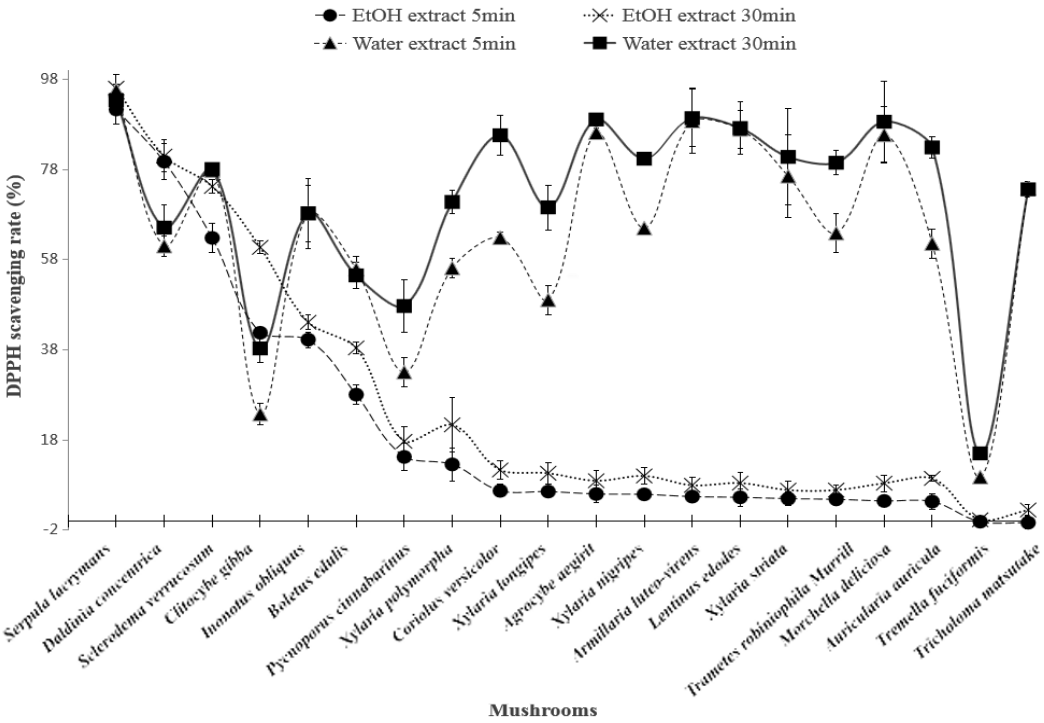

Fig. 1. Antioxidant activity of 20 kinds of mushrooms

Table 3. The EC_{50} of ethanol extracts from 3 kinds of mushrooms

Name	EC_{50}
Daldinia concentrica	11.19mg·mL^{-1}
Serpula lacrymans	17.87mg·mL^{-1}
Scleroderma verrucosum	35.01mg·mL^{-1}
Gallic acid	0.019μg·mL^{-1}
Vitamin C	0.087mg·mL^{-1}

Table 4. Determination of polyphenol content in extracts of three mushrooms

Mushrooms	Average OP	Corresponding concentration of gallic acid (ug/mL)
Serpula lacrymans	0.843	0.077
Daldinia concentrica	0.740	0.067
Scleroderma verrucosum	0.604	0.054

4. CONCLUSION

This study compared the antioxidant activities of 20 kinds of mushrooms and determined the EC_{50} and phenolic content of three highly active mushrooms. The result showed that the extract of *Serpula lacrymans* in ethanol and water had the strongest radical scavenger in the DPPH assay. The ethanol extracts of *Daldinia concentrica* and *Scleroderma verrucosum* also possessed high radical scavenging abilities. On the other hand, the water extract of *Armillaria luteovirens* and *Lentinus edodes* performed relatively weaker antioxidant than *Serpula lacrymans*. For most of the mushrooms, the water extract had a slightly higher effect than the ethanol extract. This conclusion might deduce that these active antioxidant substances were more likely soluble in water, similar to flavones or polyphenols, with strong water solubility. Besides, different test time had less impact on results.

The above results provided a reference for the further research of these mushrooms, which were few literature reports on the antioxidant activity. Along with the demonstrated antioxidant properties of *Serpula lacrymans, Daldinia concentrica* and *Scleroderma verrucosum* indicated that these mushrooms would become a healthy antioxidant source using in cosmetics or the food field.

ACKNOWLEDGEMENTS

The work was supported by the Doctor Foundation of Southwest University of Science and Technology(16zx7161), Innovative Training Program for National College students (201710619023). Undergraduate Students Innovation Training Program of Sichuan Province (20xcy078). Student Innovation Fund Program of Southwest University of Science and Technology (jz19-062).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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