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Antioxidant Activity of Different Solvents Extracts from Fruiting Bodies of *Trametes gibbosa* (Pers.) Fr.

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ABSTRACT

In this study, the content of total phenols, total flavonoids, triterpenoid saponins and polysaccharides as well as the antioxidant activity of different solvents (water, 70% ethanol, methanol, acetone and ethyl acetate) crude extracts from fruiting bodies of Trametes gibbosa were investigated. The results showed that the water extract had the highest total phenols content (28.6 mg/g) and the methanol extract had the highest polysaccharides content (561.6 mg/g). The water extract and 70% ethanol extract demonstrated significant DPPH radical scavenging effect higher than methanol, acetone and ethyl acetate extracts at all tested concentration while the same effect for ABTS radical scavenging was only found at 0.6 mg/mL and above. For ferric-reducing power assay, the water extract was found with the highest antioxidant activity among five extracts at the concentration from 0.8 to 1.6 mg/mL. Correlation analysis showed that there was a higher correlation between total phenols, polysaccharides and antioxidant activity.

Keywords: *Trametes gibbosa*, total phenols, triterpenoid saponins, polysaccharides, antioxidant activity

INTRODUCTION

The body in the process of metabolic reactions will produce reactive oxygen species (ROS), such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) [1]. The overproduction of ROS results in oxidative stress defined as an imbalance between the production of free radicals and reactive metabolites and the oxidative stress has been implicated in various pathological conditions such as cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion and aging [2,3]. Studies have found that antioxidants can slow down the harmful effects caused by oxidative stress [4]. However, the synthetic antioxidants have some potential side effects such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) for their carcinogenicity [5]. Therefore, it caused tremendous concern in finding natural antioxidants in recent years.

Trametes gibbosa, commonly known as the lumpy bracket, is a polypore mushroom that causes white rot woods. Studies have reported the polysaccharide fractions from the fruiting bodies of *T. gibbosa* having pharmacological properties, such as anti-inflammatory, vascular protective [6], hypoglycemia [7], antiviral [8] and cytotoxic activity [9]. The preliminary investigation on antioxidant and antibacterial activity of *T. gibbosa* has been done by Johnsy G et al. [10] and the phenolic composition, antioxidant and enzyme inhibitory activities of methanolic and water extracts of *T. gibbosa* have been reported by Zengin G et al. [11]. Moreover, antigenotoxic effect of *Trametes* spp. extracts against DNA damage on human peripheral white blood cells has also been studied [12].

However, the correlation between different chemical components and the antioxidant activity of *T. gibbosa* was ignored in previous studies. We didn't know which component plays a better role in antioxidant activity. Therefore, the present study seeks to evaluate and compare with antioxidant properties of different solvents extracts from the fruiting bodies of *T. gibbosa*, using 2,2,-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-2-ethylbenz-othiazoline -6-sulfonate (ABTS) radical- scavenging methods and ferric-reducing antioxidant power (FRAP) assay. In addition, the total content of phenols and flavonoids, triterpenoid saponins and polysaccharides from crude extracts were also measured. And the correlation among these components was analysed.

MATERIALS AND METHODS

Plant material

Fresh fruiting bodies of *T.gibbosa* was collected from Santai County, Sichuan Province of China. The fully mature samples were authenticated by Prof. He (School of Life Science and Engineering, Southwest University of Science and Technology, China) based on their microscopic and macroscopic characteristics. The fruiting bodies of the sample were cut and hot air-dried in an oven at 55˚C for 48 h.

Extracts preparation

The dried samples were crushed to a fine powder (20 mesh). A sub-sample (10 g) was extracted with different solvents (water, 70% ethanol, methanol, acetone and ethyl acetate) at room temperature (25˚C) at 150 rpm for 24 h. The extract was filtered and the residue was extracted again with two additional 100 mL portions of extraction solvent as described above. The filtrates were combined and concentrated using a rotary evaporator (RE-52AA, Shanghai YaRong Biochemistry Instrument Factory, Shanghai, China) to dryness at 45˚C. The crude extracts were weighed and dissolved respectively with the extracted solvents into a concentration of 10 mg/mL. All prepared extracts were stored at 4˚C.

Determination of total phenols

The total phenols content of different solvent extracts was determined by the Folin-Ciocalteu colorimetric assay with slight modifications [13]. Briefly, 1 mL sample was mixed with 1 mL Folin-Ciocalteu's phenol reagent. After 3 min, 1 mL of 7.5% Na_2CO_3 was added to the mixture and it was made up to 10 mL by adding distilled water. After the reaction was kept in the dark for 90 min the absorbance was read at 725 nm. Total phenols content was expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Determination of total triterpenoid saponins

The total triterpenoid saponins of different solvent extracts was measured with the method described by Chen Y, et al. [14]. The different samples were transferred into a 10 mL test tube respectively. After the solvent was heated to evaporation in a water-bath at 75˚C, 0.2 mL vanillin-acetic acid solution (5%, w/v) and 1.2 mL hydrochloric acid were added, mixed and incubated at 70˚C for 15 min. The tubes were taken out and cooled in running water to room temperature. Immediately, ethyl acetate was added to bring the final volume to 5 mL. Absorbance was then measured at 550 nm. Total triterpenoid saponins content was expressed as mg of Oleanolic acid equivalents (OCEs) per g of extract.

Determination of polysaccharides

The polysaccharides content of each extract was determined according to the method of Dubois M et al. with some modifications [15]. One milliliter of samples mixed with 0.5 mL 6% phenol solution, and quickly added 2.5 mL concentrated sulfuric acid. And then the mixture was thoroughly vortex-mixed and kept for 20 min at room temperature. The absorbance of the solution was measured at 490 nm. Total sugar content was expressed as glucose equivalents.

DPPH assay

The scavenging effect of different solvents extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was detected by the method of Zheng Y, et al. [16] An aliquot of 1.5 mL of 0.1 mM DPPH in 95% (v/v) ethanol was added to 1.5 mL of different solvents extracts. The absorbance was measured at 517 nm after 30 min incubation at room temperature in the dark. Results were expressed as a percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation (1):

$$Scavenging\ rate\ (\%) = \frac{A0 - Ai - Aj}{A0} \qquad (1)$$

Where A_0 is the absorbance of DPPH solution without a sample, A_i is the absorbance of the test sample mixed with DPPH solution, and A_j is the absorbance of the sample without DPPH solution.

ABTS assay

The ABTS radical scavenging activity was measured by using ABTS radical cation decolorization assay with some modifications [17]. Briefly, the different solvents extracts (0.4 mL) were mixed with ABTS+ solution (4 mL). The absorbance was measured at 734 nm after reacting for 20 min at room temperature. The ABTS scavenging activity was calculated according to the equation (1), where A_0 is the absorbance of $ABTS^+$ solution mixed with PBS, A_i is the absorbance of the test sample mixed with the $ABTS^+$ solution, and A_j is the absorbance of the sample mixed with PBS.

FRAP assay

FRAP assay was performed refer to the method of Alothman M, et al. [18]. Briefly, a calibration curve was prepared using an aqueous solution of ferrous sulfate (Fe_2SO_4. $7H_2O$). FRAP values were expressed as Fe (II) equivalents (µmol/L).

Statistical analysis

All the analyses were carried out in triplicate. Data were analyzed using SPSS 22.0 software. Values were expressed as mean ± standard deviation. Statistical differences between extracts were evaluated by using one-way variance (ANOVA) followed by RSD. P values <0.05 were regarded as significant and P values <0.01 as very significant.

RESULTS AND DISCUSSION

Effect of solvents on extraction yield

The highest extraction yield from different solvents extracts of T. gibbosa fruiting bodies was 11.82% in water extract and the lowest extraction yield was only 0.92% in ethyl acetate extract. While 70% ethanol, methanol, and acetone were used as the solvent, the yields of crude extract were 6.47%, 4.67%, and 1.09% respectively. Our results showed that the extraction yield increased with the polarity of the solvent increasing, in agreement with the extraction yields of Ren G, et al. [9], who reported the petrol ether, ethyl acetate and methanol extracts of eight medicinal polypore fungi from China including *T. gibbosa*. However, Zengin G, et al. [11] found that the water extraction yield was lower than methanol extracts.

Effect of solvents on total phenols, total flavonoids, total triterpenoid saponins and polysaccharides content

The total phenols, total flavonoids, total triterpenoid saponins and polysaccharides contents of different solvents from the fruiting bodies of *T. gibbosa* are presented in Table 1.

Table 1: Total phenols, total flavonoids, total triterpenoid saponins and polysaccharides contents of different extracts from fruiting bodies of *T gibbosa*; Data from three repetitions, with mean ± standard deviation; Values with different letters in each column are significantly different (p<0.05); GAEs: gallic acid equivalents; QEs: quercetin equivalents; TSEs: triterpenoid saponins equivalents; GEs: glucose equivalents; ND: content below detection limit

Extracts	Total phenols	Total flavonoids	Total triterpenoid saponins	Polysaccharides
	(mg GAEs per g extract)	(mg QEs per g extract)	(mg TSEs per g extract)	(mg GEs per g extract)
Water extract	28.6±3.4^a	ND	50.4±3.4^d	251.4±22.1^b
70%Ethanol extract	23.2±0.8^b	0.5±0.1^b	46.8±2.0^d	348.4±8.6^a
Methanol extract	20.2±0.3^c	0.7±0.2^b	85.4±1.6^c	561.6±27.0^a
Acetone extract	14.1±0.7^d	2.7±0.7^a	209.8±14.0^a	43.9±3.3^c
Ethyl acetate	7.5±1.5^e	ND	188.6±3.0^b	44.6±3.4^c

Among five crude extracts investigated, the content of polysaccharides and total triterpenoid saponins were higher than total phenols and total flavonoids. Total flavonoids content was found very low (from 0.5 to 2.7 mg QEs/g extract) and was not detected in water and ethyl acetate extracts. Similar results also showed the low content of total flavonoids of methanol and water extract from *T. gibbosa* [10,11] and no flavonoids were detected in 16 species of Portuguese wild mushrooms [19].

The total phenols content was significantly different among five solvents extracts ($p<0.05$) ranged from 7.5 to 28.6 mg GAEs/g extract, being the highest in water extract and the lowest in ethyl acetate extract. What's more, it was apparent that higher extraction yields of phenolic compounds were obtained with an increase in polarity of the solvent. In a previous study, Zengin G, et al. [11] found many kinds of phenolic compounds, such as protocatechuic acid, (+)-catechin and chlorogenic acid etc. The total phenols content of methanol extracts from *Trametes* spp. ranging from 10.54 to 23.28 mg/g extract [20]. The content of total phenols of *T. versicolor* extract was 52.59 mg/mL of distilled water extract, 14.44 mg/mL of ethanol extract and 4.07 mg/mL of methanol extracts [21].

The polysaccharides content of methanol (561.6 mg GEs/g extract), 70% ethanol (348.4 mg GEs/g extract) and water extract (251.4 mg GEs/g extract) was significantly higher than other extracts. In the cases of Song W, et al. [22], the polysaccharide content of *T. hirsuta* aqueous extract was evaluated as 19.1 ± 0.63 glucose equivalent µg/100 µg extract, much less than our study evaluated.

High total triterpenoid saponins content was determined in acetone extract (209.08 mg TSEs/g extract) and ethyl acetate extract (188.6 mg TSEs/g extract), while the content of other extracts was in the range from 46.8 to 85.4 mg TSEs/g extract.

Effect of solvents on DPPH assay

The DPPH scavenging activity of five different solvent extracts from fruiting bodies of *T. gibbosa* as a function of their concentration was shown in Figure 1. We discovered that the scavenging effects of these extracts on DPPH radical increased with concentration. When the concentration reached to 0.8 mg/mL, the excellent scavenging activity was apparently observed by 92.36% for water extract and 94.04% for 70% ethanol extract. However, the DPPH scavenging effect of the ethyl acetate extract was only 30.35% at the concentration of 1.4 mg/mL. But we can infer that the inhibition rate will increase with the concentration increasing. The acetone extract exhibited the highest antioxidant activity (54.9%), followed by the methanol extract (40%) at 0.5 mg/mL from *T. versicolor* [23], compared with our results that the methanol extract with 48.04 % at 0.6 mg/mL and 37.41 % at 0.4 mg/mL.

On the other hand, the EC_{50} values were further obtained by nonlinear regression analysis and presented in Table 2. The lower the EC_{50} value of a sample demonstrated the stronger antioxidant activity. According to Table 2, both water and 70% ethanol extracts exhibited higher scavenging potential and their EC_{50} values were close to 0.23 mg/mL. This result may be explained by that when the concentration of these two higher antioxidant extracts reached a certain value, the antioxidant activity would be very close so that we can't recognize the difference between them. The EC_{50} of methanol, acetone and ethyl acetate extract were 0.59 ± 0.011 mg/mL, 0.59 ± 0.04 mg/mL, 0.89 ± 0.04 mg/mL and the ethyl acetate extract over 1.4 mg/mL, respectively. The EC_{50} of the ethanol extract of *Agaricus bisporus* at 0.38 mg/mL from China [24] and 0.52 mg/mL from Spain [25] were higher than our data and the EC_{50} of Malaysian Trametes extracts ranging from 7.296 to 27.324 mg/mL has also been reported [20].

Whether in Figure 1 or Table 2, we can conclude that crude extracts of scavenging capacities of DPPH followed the order: 70% ethanol/water extract>methanol extract>acetone extract>ethyl acetate extract and the results noted that the scavenging activity of ascorbic acid was higher than all solvent extracts.

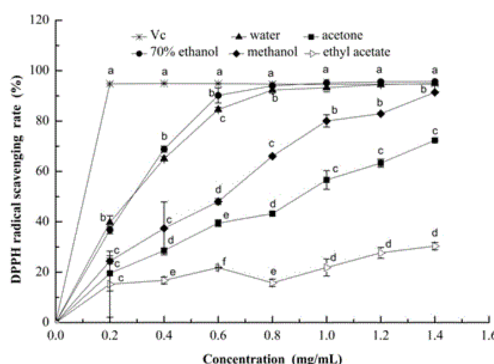

Figure 1: The DPPH radical scavenging activity of different solvent extracts from fruiting bodies of *T gibbosa*. Vertical bars show standard deviation of means from three replicates. Values with different letters at the same concentration show significant difference as determined by LSD Test at $p<0.05$

Effect of solvents on ABTS assay

The scavenging effects of samples on ABTS radicals were measured and shown in Figure 2. When the concentration was less than 1.0 mg/mL, the scavenging activities were also dependent on concentration as well as DPPH assay. At 0.8 mg/mL, the scavenging activity of ABTS in order of water extract >70% ethanol extract > methanol extract > acetone extract > ethyl acetate extract were found as completely 100%, 92.26%, 77.42%, 61.15% and 21.86%, respectively, in accordance with the EC_{50} demonstrated in Table 2.

From Table 2, the EC_{50} of scavenging capacities for ABTS of five different solvents from fruiting bodies of *T. gibbosa* were statistically a significant difference ($p<0.05$). In comparison with the DPPH assay, the order of water and 70% ethanol extracts for antioxidant activity was a little different. In this assay, we can clearly discover that the antioxidant activity of water extract was stronger than 70% ethanol extract, and their EC_{50} was 0.30 and 0.42 mg/mL, respectively. However, the scavenging ability of extracts was less than ascorbic acid in the range from 0.2 to 0.8 mg/mL, and the difference can't be detected exceeding 1.0 mg/mL. The ABTS scavenging activity of polysaccharides extraction from other mushrooms, such as *Trametes robiniophila* with 83.6 ± 1.2% [17] and *Dendrobium denneanum* with 15.3% at 1.0 mg/mL [26], were less than the 70% ethanol (93.22%) and water extract (99.94%) in our study. The EC_{50} values of *T. gibbosa* in our study also indicated much higher antioxidant activity than other mushrooms like *Agaricus blazei* and *Lentinus edodes* [27].

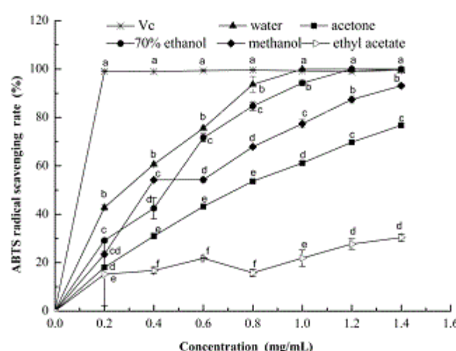

Figure 2: The ABTS radical scavenging activity of different solvent extracts from fruiting bodies of *T gibbosa*. Vertical bars show standard deviation of means from three replicates. Values with different letters at the same concentration show significant difference as determined by LSD Test at $p<0.05$.

Effect of solvents on FRAP assay

The total antioxidant activity was determined in an evaluation of the FRAP assay (Figure 3). The FRAP values of water, 70% ethanol and methanol extracts were higher than acetone and ethyl acetate extracts at all concentration. Therefore, water, 70% ethanol, and methanol extracts indicated higher total antioxidant activity than other two extracts. At 1 mg/mL, the order of the FRAP value was water extract >70% ethanol extract> methanol extract > acetone extract > ethyl acetate extract, for 347.83 ± 7.80, 304.09 ± 15.00, 292.84 ± 17.18, 142.86 ± 3.75, 101.61 ± 17.18, respectively. Compared with another study that the 95% ethanol extract from *Trametes hirsute* showed higher FRAP value which was 512.24 ± 0.15 µmol/L at 0.5 mg/mL [28]. The FRAP values of positive control ascorbic acid reached vertex under 0.8 mg/mL revealed that the FRAP values of all the extracts from the fruiting bodies of *T. gibbosa* were less than it.

We use SPSS 22.0 software to estimate EC_{200} which the FRAP value was equal to 200 as shown in Table 2. Both the EC_{200} of the acetone and acetate extracts were higher relatively, whereas the water extract was the lowest only with 0.50 ± 0.04 mg/mL. From our results, we can get the same conclusion as ABTS assay about the order of extracts for antioxidant activity. Another studies also found that the water extract had higher antioxidant activity [29]. What's more, Sławińska found that the water extract from *Flammulina velutipes* possessed the high equivalent capacity for all applied extraction methods and was characterized by high level of total phenols [30].

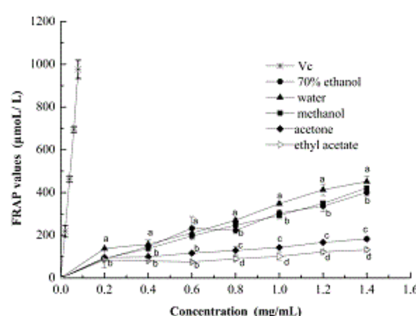

Figure 3: The ferric reducing antioxidant power (FRAP) of different solvent extracts from fruiting bodies of *T gibbosa*. Vertical bars show standard deviation of means from three replicates. Values with different letters at the same concentration show significant difference as determined by LSD Test at $p<0.05$

Table 2: EC_{50} of radical scavenging capacities and EC_{200} of ferric reducing power (FRAP) of different solvent extracts from fruiting bodies of *T gibbosa*: Data from three repetitions, with mean ± standard deviation; Values with different letters in each column are significantly different ($p<0.05$). EC_{200} indicates the concentration of extracts at FRAP value 200

Extracts	EC_{50} of radical scavenging (mg/mL)		EC_{200} of FRAP
	DPPH	ABTS	(mg/mL)
Water extract	0.23 ± 0.02d	0.30 ± 0.00e	0.50 ± 0.04c
70% Ethanol extract	0.23 ± 0.01d	0.42 ± 0.01d	0.60 ± 0.03b
Methanol extract	0.59 ± 0.04c	0.51 ± 0.01c	0.62 ± 0.03b
Acetone extract	0.89 ± 0.04b	0.79 ± 0.02b	>1.40a
Ethyl acetate extract	>1.40a	>1.40a	>1.40a

Correlation between bioactive compounds and antioxidant activity

Correlation analysis was performed on the three chemical compounds content (polysaccharides, total phenols, and triterpenoid saponins) for three antioxidant assays at 1 mg/mL (Table 3). The total phenols content highly correlated with the DPPH, ABTS scavenging activities and FRAP values, their correlation coefficient was 0.956 ($p<0.05$), 0.973 ($p<0.01$) and 0.966 ($p<0.01$), respectively. The correlation between polysaccharides content was less than total phenols content, while the correlation coefficient was also more than 0.774. On the contrary, the triterpenoid saponins showed the negative correlation but also had a higher correlation coefficient, which was 0.873, 0.842 and 0.958 respectively. The results revealed that both the polysaccharides and total phenols are important groups in representing the antioxidant activity and these two bioactive constituents can be an indicator of the evaluation of antioxidant activity. The result is in accord with Song W, et al. [31], who discovered that the relationship between the content of phenols, polysaccharide and DPPH Trolox equivalent antioxidant activity was well correlated in commercial mushroom extracts including *T versicolor*, which their correlation coefficient were 0.93 and 0.72, respectively. And the positive correlation between the scavenging activity of the methanol extract with total phenols content in *L. edodes* (r^2 = 0.99) and *Volvariella volvacea* (r^2 =0.93) have also found by Cheung LM, et al. [32]. Similar results also showed that good correlation (r^2 >0.94) between total phenols content, DPPH, and FRAP data in edible mushrooms [33].

Polyphenols as primary antioxidants can inactivate free radicals by the hydrogen atom transfer, the single electron transfer, and the metals chelation [34]. The possible antioxidant mechanisms of polysaccharides were summarized by Wang et al. [35], in which the polysaccharide conjugates can donate protons to electron deficient radicals and polysaccharide mixture can also chelate metal and inhibit the generation of free radicals.

Table 3: The correlations between the content of chemical components and antioxidant activity at the extract concentration 1 mg/mL ;*means a significant correlation at the 0.05 level and ** means a significant correlation at the 0.01 level; RSC: radical scavenging activity; FRAP: ferric reducing antioxidant power

Chemical components	DPPH RSC	ABTS RSC	FRAP
Total phenols	0.956*	0.973**	0.966**
Total triterpenoid saponins	-0.873	-0.842	-0.958*
Polysaccharides	0.842	0.774	0.889*

CONCLUSION

To summarize, the results indicated that water and 70% ethanol extracts showed higher antioxidant activity than other extracts and the antioxidant activities were well correlated with the content of phenolic compounds and polysaccharides. Therefore, further analysis of the composition and antioxidant capacity of the phenolic and polysaccharide compounds of the fruiting bodies of *T. gibbosa* are needed. Our result suggested that the chemical components of the fruiting bodies of *T. gibbosa* possess the good antioxidant capacity and might have potential applications in the food and medical industries.

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