

Preliminary Investigation on the Chemical Constituents and Antioxidant Activities of Two *Phellinus* Mushrooms Collected in Foshan

Feng Zhu^{1*}, Weihong Lu², Weijie Feng¹, Zhaofeng Song¹, Chunyan Wang¹, Xin Chen¹

¹Department of Chemical Engineering, Foshan University, Foshan, China

²School of Life Science and Engineering, Foshan University, Foshan, China

Email: *zhufeng@fosu.edu.cn

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Abstract

The *Phellinus* mushrooms have been known for its immunomodulatory, hypolipidemic, and anticancer activities. In the current work, the chemical constituents of two *Phellinus* mushrooms, *Phellinus pini* 141016# and *Phellinus* sp. 150802#, which were collected in Foshan city, Guangdong province, were investigated preliminarily by pre-test tube method and by comparison with the known natural compounds from *Phellinus pini*, and their antioxidant activities were evaluated by the biochemical assay of hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity *in vitro*. The results show that both the two *Phellinus* mushrooms contain phenolics, alkaloids, lactones, steroids and terpenoids, and have potent antioxidant activities. The IC₅₀ values of DPPH radicals and hydroxyl radicals for *Phellinus pini* 141016# were 74.37 µg/mL and 59.69 µg/mL, respectively, and the IC₅₀ values of DPPH radicals and hydroxyl radicals for *Phellinus* sp. 150802# were 98.95 µg/mL and 165.47 µg/mL, respectively. They are comparable to the antioxidant activity of the standard antioxidant ascorbic acid.

Keywords

Mushroom, *Phellinus* sp., *Phellinus pini*, Chemical Constituent, Antioxidant Activity

1. Introduction

Edible and medicinal mushrooms are an important source of natural antioxidants [1]. They are ubiquitous in nature and produce various classes of biologically active primary and secondary metabolites. Mushrooms in the genus *Phellinus* have attracted much attention due to its long history and high medicinal

value [2]. Among them, *Phellinus pini*, also known as Huang Zhi, pine white rot fungus, which has anti-cancer, can enhance immunity, lower blood sugar and other activities, is a good medicine for cancer treatment [3] [4] [5]. During an ongoing study to discover natural products with antioxidant activity from mushrooms, we collected two mushrooms in Foshan city, Guangdong province, and identified them as *Phellinus* mushrooms. It was known that the chemical constituents isolated from the fungi of the genus *Phellinus* are steroids, terpenoids, polysaccharides and fatty acids. In addition, flavonoids and flavanone derivatives, enzymes, pyrone pigments, and alkaloids were also found in the genus of *Phellinus* [4] [6]. But to our best knowledge, the *Phellinus* mushrooms in Foshan had not yet been studied. Herein we report the preliminary investigation on the chemical constituents and antioxidant activities of the two *Phellinus* mushrooms. The aim of the study was to provide theoretical basis for the development and efficient utilization of them.

2. Materials and Methods

2.1. Chemistry

Analytical grade chemicals and reagents were directly used to perform various experiments.

2.2. Analytical

UV-2501 PC absorption spectrometer was used to measure the absorbance values.

2.3. Mushrooms

The No. 141016# wild fruiting bodies (**Figure 1**) identified as *Phellinus pini* were collected in October in 2014 from Leigang Mountain in Foshan city, Guangdong Province, China. The No. 150802# wild fruiting bodies (**Figure 2**) were collected in August in 2015 from Leigang Mountain in Foshan City, Guangdong Province, China, and were identified as *Phellinus* sp., and it maybe is a novel specie of the genus of *Phellinus*.



Figure 1. The mushroom of *Phellinus pini* 141016#.



Figure 2. The mushroom of *Phellinus* sp. 150802#.

2.4. Preparation of the Crude Extracts

The fresh fruiting bodies of the two *Phellinus* mushrooms were dried by air, and grinded into powder. The crude powders were extracted continuously with methanol for 24 hrs. The methanol extracts were combined and the methanol was recovered by vacuum rotary evaporation to obtain the crude extracts. A 1.23 g of crude extracts was obtained from 73 g of crude powders of the fruiting bodies of *Phellinus pini* 141016#, yielding 1.68%, and a 1.00 g of crude extracts was obtained from 56 g of crude powders of the fruiting bodies of *Phellinus* sp. 150802#, yielding 1.79%. All the crude extracts were solved in methanol and prepared into sample solutions in methanol with concentration of 5 mg/mL.

2.5. Preliminary Test on the Chemical Constituents in the Methanol Crude Extracts

The chemical constituents of the methanol crude extracts were determined by preliminary-test tube method [7] [8]. The ferric chloride reagent was used to check the phenolic components. Alkaloids were detected by Dragendorff reagent. The hydrochloric acid-zinc powder test was used to check the flavonoids. The anthraquinones were examined by Bornträger reaction and magnesium acetate test. Cardiac glycosides were examined by Kedde reagent. The lactone and coumarin components were examined by the ferric hydroxamate reaction. The sterols and triterpenoids were examined by Liebermann-Burchard reaction and Salkowski reaction.

2.6. Antioxidant Activity Assay

Scavenging Activity on Hydroxyl Radical

The hydroxyl radicals scavenging activity of the crude extracts were evaluated by salicylic acid-FeSO₄-H₂O₂ method [8]. Spectrophotometric method was used to determine the hydroxyl radicals scavenging ability of different concentrations of sample solution *in vitro*. A 0 - 1.2 mL of sample solution in methanol was added to a clean plug tube, then methanol was added to make up the volume to 5 mL, then 1 mL of 6 mol/L salicylic acid solution in methanol was added. After shaking well, 1 mL of 2 mol/L aqueous ferrous sulfate solution was added, then 1 mL of 1.5% aqueous hydrogen peroxide solution was added. Distilled water instead of 1.5% aqueous hydrogen peroxide solution is as a blank control. After shaking well, methanol was added until reaching the constant volume of 10 mL. Finally, the tubes were placed in 37°C bath for 60 min. The absorbance values were measured at λ_{\max} 510 nm, and calculated as a percentage of radical scavenging activity (%RSA) according to "Equation (1)". The experiment was repeated three times. The %RSA values were converted to activity of inhibition concentration at 50 percent (IC₅₀) using a graph plotted between %RSA and the concentration. The standard antioxidant ascorbic acid was used in this experiment.

$$\%RSA = \left[(A_0 - A_s) \div A_0 \right] \times 100 \quad (1)$$

A_0 is the absorbance value of salicylic acid-FeSO₄-H₂O₂ system, and A_s is the absorbance value of sample-salicylic acid-FeSO₄-H₂O₂ system.

Scavenging Activity on DPPH Radical

The DPPH radical scavenging activity of the crude extracts was measured using the method of Seephonkai [2] and Wu [8]. Spectrophotometric method was used to determine the DPPH radicals scavenging ability of different concentrations of sample solution *in vitro*. A 1 mL of different concentrations of the sample solution in methanol was mixed with 2 mL of 0.2 mmol/L DPPH solution in methanol. After 30 min at room temperature, the absorbance values of the sample were measured at λ_{\max} 517 nm and calculated as a percentage of radical scavenging activity (%RSA) according "Equation (2)". The %RSA values were converted to activity of inhibition concentration at 50 percent (IC₅₀) using a graph plotted between %RSA and the concentration. Standard antioxidant ascorbic acid was used in this experiment.

$$\%RSA = \left[(A_{DPPH} - A_s) \div A_{DPPH} \right] \times 100 \quad (2)$$

A_{DPPH} is the absorbance value of 1 mL of methanol mixed with 2 mL of 0.2 mmol/L DPPH solution in methanol, and A_s is the absorbance value of 1 mL of sample solution in methanol mixed with 2 mL of 0.2 mmol/L DPPH solution in methanol.

3. Results and Discussion

3.1. Chemical Constituents Preliminary Analysis

To date, at least fourteen natural compounds 1-14 (Figure 3) had been found in

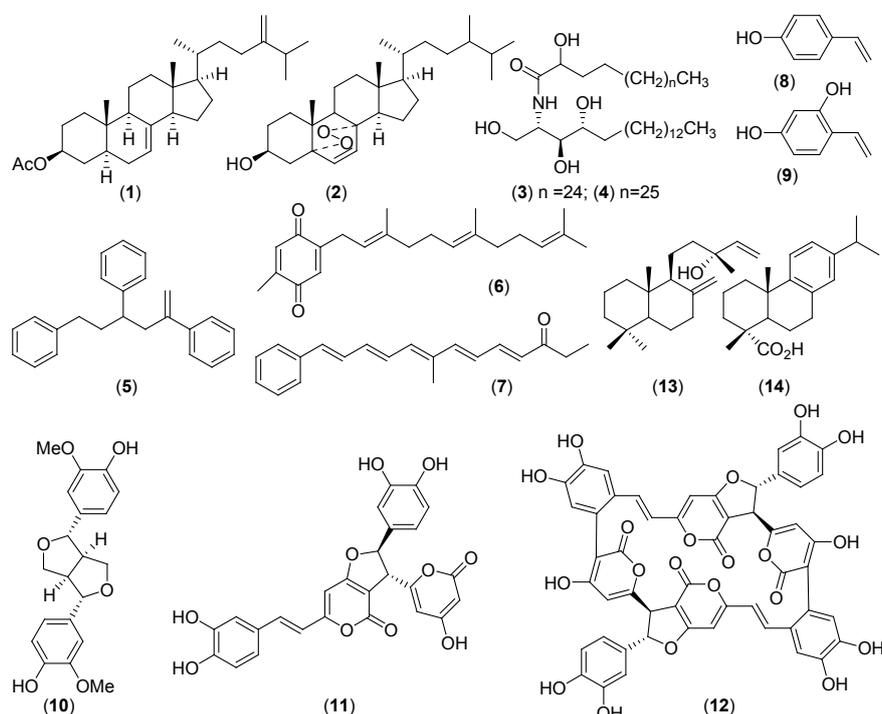


Figure 3. The known natural compounds from *Phellinus pini*.

Phellinus pini. In 1996, Lourenco *et al.* [9] reported that the steroids episteryl acetate (1) and ergosterol peroxide (2) and the lignan (+)-pinoresinol (10) had been isolated from the wild fruiting bodies of the fungus *Phellinus pini*. In addition, two new ceramides, N-(2'-hydroxynonacosanoyl)-D-erythro-1, 3, 4-trihydroxy-2-amino-octadecane (3) and N-(2'-hydroxytriacontanoyl)-D-erythro-1, 3, 4-trihydroxy-2-amino-octadecane (4), also had been obtained from the same source. At the same year, Ayer *et al.* [10] reported that five compounds, 2, 4, 6-triphenylhex-1-ene (5), 2-farnesyl-5-methylbenzoquinone (6), 8-methyl-13-phenyltrideca-4, 6, 8, 10, 12-pentaene-3-one (7), 4-vinylphenol (8) and 4-vinyresorcinol (9) had been isolated from the cultured mycelium of the fungus *Phellinus pini*, and all the five compounds showed activity against most fungi examined. In 2007, two phenylpropanoid-derived polyketides, hypholomine B (11) and pinillidine (12), with potent xanthine oxidase inhibitory activities were isolated from the cultured mycelium of *Phellinus pini* by bioassay-guided isolation by Wangun *et al.* [11]. Recently, two diterpinoids [12], 8, 14-labdadien-13-ol (13) and dehydroabietic acid (14), were isolated from the fruit body of *Phellinus pini*, they could inhibit NO production in LPS-activated RAW 264.7 cells. In addition, many scholars [13] [14] also studied the polysaccharides of *Phellinus pini* and its biological activities.

In this work, the sample solution of the methanol crude extracts from *Phellinus pini* 141016 # showed a blue-violet color reaction with ferric chloride, suggesting the presence of phenolic components. The sample solution evaporated in water bath to get the residue, the residue was dissolved in 5% sulfuric acid, then filtration, the filtrate reacted with Dragendorff reagents to produce red precipi-

tate, indicating that the sample contains alkaloids. The sample solution in the hydrochloric acid-zinc powder test did not occur color reaction, indicating that the sample does not contain flavonoids. In the Bornträger test, the sample solution produced a red reaction with 10% sodium hydroxide, but the red color faded when heating after adding a small amount of 30% hydrogen peroxide solution. Additionally, the sample solution did not occur color reaction with 1% magnesium acetate. All these phenomena suggest that there is no anthraquinone ingredient in the sample. The sample solution did not occur color reaction with Kedde reagent, indicating that no cardiac glycosides in the sample. In the ferric hydroxamate test, the sample solution showed red reaction, indicating the presence of lactones. The sample solution produced a remarkable color reaction in the Liebermann-Burchard reaction and the Salkowski reaction, indicating the presence of sterols and triterpenes.

The experimental results show that *Phellinus pini* 141016 # contain phenolics, alkaloids, lactones, steroids and terpenoids, which were consistent with the types of the known natural compounds of *Phellinus pini* (Figure 3). Since the sample solution of the methanol crude extracts from *Phellinus* sp. 150802# showed the same reaction phenomena as the methanol crude extracts from *Phellinus pini* 141016# in the chemical constituents preliminary test, it was concluded that *Phellinus pini* 141016# also contain phenolics, alkaloids, lactones, steroids and terpenoids.

3.2. Antioxidant Activity

The *Phellinus* mushrooms are an important group of medicinal fungi. Cao *et al.* [15] found all the extracts from fruiting bodies of *Phellinus mori*, *P. baumii* and *P. vaninii* had *in vitro* antioxidant activities. Seephonkai *et al.* [2] investigated the antioxidant activity and total phenolic content from the crude extracts and crude fractions of ten species of *Phellinus* mushrooms collected from northeast Thailand, and the experimental results showed that some of the investigated extracts exhibited potent radical scavenging activity, while the samples with potent antioxidant activity were also determined to have high amount of total phenolics. Wang *et al.* [16] studied on preparation and detection antioxidant capacities of three polysaccharides, water-extracted polysaccharides (PSI), alkali-extracted polysaccharides (PSII) and sulfate polysaccharides (PSIII), from the fruiting bodies of *Phellinus pini*, and found PSII and PSIII showed the better result of antioxidant activities with concentration dependence. They [17] also studied the effects of the three polysaccharides on antioxidant capacity of normal mice, and the results showed that the three polysaccharides significantly enhanced antioxidant capacity in mice, but also increased oxidative stress *in vivo*. Yang *et al.* [18] obtained a polysaccharide designated as PP60-S1 from the fruiting bodies of *Phellinus pini*, and found PP60-S1 showed a potent antioxidant activity *in vitro*. Two heteropolysaccharides, PPM and PPE, were isolated from mycelium and culture medium of *Phellinus pini*, respectively, and Jiang *et al.* [19] found PPM possessed better antioxidant activity *in vitro* than PPE.

In this work, the antioxidant activities of the methanol extracts from the wild fruiting bodies of *Phellinus pini* 141016 # and *Phellinus* sp. 150802# were evaluated by free radical scavenging test. The results are shown in **Figure 4** and **Figure 5**. The results showed that the two species had significant antioxidant activities, and the free radicals scavenging activity increased with the increase of the concentration of the samples. The IC_{50} values of DPPH radicals and hydroxyl radicals for *Phellinus pini* 141016# were 74.37 $\mu\text{g/mL}$ and 59.69 $\mu\text{g/mL}$, respectively. And the IC_{50} values of DPPH radicals and hydroxyl radicals for *Phellinus* sp. 150802# were 98.95 $\mu\text{g/mL}$ and 165.47 $\mu\text{g/mL}$, respectively. They are comparable to the antioxidant activity of the standard antioxidant ascorbic acid, which IC_{50} values of DPPH radicals and hydroxyl radicals were 66.01 $\mu\text{g/mL}$ and 103.34 $\mu\text{g/mL}$, respectively.

4. Conclusions

The chemical constituents preliminary test results show that both the methanol extracts from the wild fruiting bodies of *Phellinus pini* 141016# and *Phellinus* sp. 150802# contain phenolics, alkaloids, lactones, steroids and terpenoids which were consistent with the types of the known natural compounds of *Phellinus pini*.

Free radicals scavenging activity experimental results show that both the methanol extracts from the wild fruiting bodies of *Phellinus pini* 141016# and *Phellinus* sp. 150802# have potent antioxidant activities, and the free radicals scavenging activity has increased with the increase of the concentration of the samples. They are comparable to the antioxidant activity of the standard antioxidant ascorbic acid.

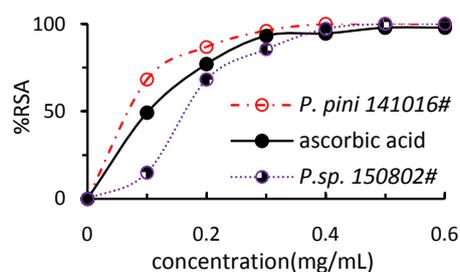


Figure 4. Hydroxyl radicals scavenging activity of *Phellinus pini* 141016# and *Phellinus* sp. 150802#.

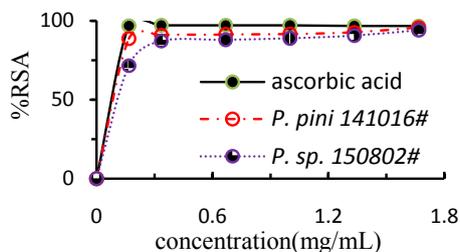


Figure 5. DPPH radicals scavenging activity of *Phellinus pini* 141016# and *Phellinus* sp. 150802#.

In conclusion, the results of the present study indicate that the mushrooms *Phellinus pini* 141016# and *Phellinus* sp. 150802# collected in Foshan have a great potential to be a good source of natural radical scavenger to be used in the food and pharmaceutical industries. Its antioxidant activities could be related to other biological activities for its use in traditional medicine. The next work is to isolate and purify natural compounds from these two species, and to evaluate their antioxidant activities *in vitro* and *in vivo*, finally to explore the two *Phellinus* mushrooms as novel antioxidants in functional foods and medicine.

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