

# Low-Polarity Components Analysis and Antioxidant Activity Evaluation of Two Polyporaceae Mushrooms

## Abstract

**Aims:** To evaluate the antioxidant and antibacterial activity of two kinds of Polyporaceae mushrooms (*Coriolus versicolor* and *Trametes robiniophila*), and investigate chemical constituents of their low polarity part.

**Methodology:** Extracting the powder of two mushrooms by soxler extraction method. The above extract were sequentially extracted with petroleum ether, ethyl acetate, n-butanol and distilled water. The antioxidant activity of different solvent extracts was investigated by the DPPH free radical clearance method. The antibacterial activity of four different solvent extracts was determined by drilling method. Finally, the petroleum ether extracts of the two mushrooms were methylation and analyzed by GC-MS.

**Results:** The antioxidant activity of each solvent extraction of the two mushrooms showed clear dose-effect relationship and the ethyl acetate and n-butanol extract from *Coriolus versicolor* had stronger antioxidant activity. However, there was no obvious antibacterial effect of these two mushrooms. The results of GC-MS analysis showed that there were 27 compounds in petroleum ether extraction part of the *Coriolus versicolor*, and 39 compounds in *Trametes robiniophila*. Methyl linoleate, 1,2-Benzenedicarboxylic acid, Methyl 2-hydroxy-tetracosanoate, Palmitic acid and Ergosta-14,22-dien-3-ol, (3 $\beta$ , 5 $\alpha$ , 22E) had a high content in two mushrooms.

**Conclusion:** This study had clarified some of the chemical constituents of these two mushrooms and their antibacterial and antioxidation activity had been studied, it would provide some theoretical basis for their further development and utilization in anti-aging drugs and health food.

**Keywords:** *Coriolus versicolor*; *Trametes robiniophila*; Antioxidant activity; antibacterial activity; GC-MS.

## 1. INTRODUCTION

The polyhydric fungi is a kind of fungi belonging to the hymenomycetes that has important economic value<sup>[1]</sup>. The fungi metabolites are diverse in structure. polysaccharides<sup>[2]</sup>, terpenoids<sup>[3]</sup>, steroid, alkaloids, benzoquinones and organic acids<sup>[4]</sup> are all included. These metabolites often play a role in anti-tumor, anti-inflammatory, antiviral and other biological activities<sup>[5-6]</sup>. Therefore, the medicinal and edible fungi that can be used in medical care and health care which have been widely concerned, such as *Ganoderma lucidum*, *Wolfiporia cocos* and *Polyporus umbellatus*.

In recent years, *Coriolus versicolor* and *Trametes robiniophila* have gradually entered the public view, because of its application in medical and health care products. It has been proved that these two fungi have various effects on anti-tumor<sup>[7-8]</sup> and regulating immunity<sup>[9-10]</sup>. At present, there are many relational products in the market, such as locust granule and yunzhi hetai granule, which can effectively inhibit the growth and reproduction of tumor cells. The later has an ability to treat chronic hepatitis as an immunomodulator.

At present, the research on the composition of *Coriolus versicolor* and *Trametes robiniophila* is mainly concentrated on the polar compounds of polysaccharides and glycopeptides<sup>[11]</sup>. The activity studies were mainly focused on anti-tumor and immune regulation, and there were few reports on antioxidant and antibacterial activity. Therefore, we explored the antibacterial and antioxidant activity of two species of fungi in the same family but different genus, namely, *Coriolus versicolor* and *Trametes robiniophila*, and analysed its compound ingredients of low polarity part by GC-MS, to expand the development of the efficacy of these two kinds of fungi for medical reference.

## 2. MATERIALS

### 2.1 Mushrooms and test bacteria

The fruiting bodies of the wild *Coriolus versicolor* and *Trametes robiniophila* were collected from Beichuan county, Sichuan Province of China in September 2017. They were identified by one author of this article, professor He Xinsheng. And the voucher specimens were kept in the Microbiology Laboratory of Southwest University of Science and Technology, Mianyang, Sichuan Province. After 50°C drying in infrared-ray oven, these mushrooms were crushed and separated through 100 mesh sieves. The prepared samples were keeping at 4°C until later use. Two test bacteria: *Escherichia coli* and *Staphylococcus aureus*, provided by Guangdong Institute of Microbiology were obtained from stock cultures and grown in nutrient broth and incubated at 37°C for 18 h.

### 2.2 Reagents

DPPH (1,1-diphenyl-2-picrylhydrazyl free radical) was purchased from Tokyo Chemical Industry (Shanghai) Co., Ltd. *n*-alkanes ( $C_1 \sim C_{31}$ ) standard solution ( $10 \text{ mg} \cdot \text{mL}^{-1}$ ) was purchased from the Sigma-Supelco company. Vitamin C (ascorbic acid) and Neomycin were purchased from Aladdin Industrial Corporation. Ethanol, petroleum ether, ethyl acetate and other reagents were purchased from ChengDu Chron Chemicals Co., Ltd. All above reagents were of analytical grade.

### 2.3 Equipments

Electronic Precision Balance (BS223S, Germany Sartorius), Ultrasonic cleaner (KQ-200KDE, Kunshan Ultrasonic Instrument Co., Ltd.), Rotary evaporator (RE-52AA, Shanghai Ya Rong Biochemistry Instrument Factory), Spectrophotometer (UV7200, Unico(Shanghai) Instrument Co., Ltd.), Light incubator (MGC-250, Shanghai bluepard instruments Co., Ltd.), High-speed refrigerated centrifuge (CR22G III, Hitachi).

## 3 METHOUD

### 3.1 Preparation of extract from *Coriolus versicolor* and *Trametes robiniophila*

Referring to Madeja, Katarzyna<sup>[12]</sup> and other schemes, Soxhlet extraction method was used to extract the dry powder of the two mushrooms by refluxing for two hours with 85% ethanol at 1:5 (mass to volume ratio). The supernatant was filtered, and the residue was extracted with the same amount of solvent. After 3 times of repetition, the supernatant was combined and dried.

The ethanol extract was reextracted by petroleum ether, ethyl acetate, n-butanol and distilled water according to the order of polarity from small to large. Ultrasonic extraction was carried out in accordance with the volume ratio of extract and extraction solvent at 1:5 for 20 min each time. The supernatant was filtered and combined. The above operation was repeated three times. The combined solution was dried in vacuo evaporator and weighed respectively.

### 3.2 Analysis of volatile constituents of petroleum ether extracts

Before the GC-MS analysis, the petroleum extract from two mushrooms (50 g of each) were methylated with mixed solution composed of H<sub>2</sub>SO<sub>4</sub> (1%): Methanol = 1:9 (v/v) for 80 min at 80°C<sup>[13]</sup>. When the reaction fluid was cooled to room temperature, it was centrifuged at 10000 r/min, before which 2 mL *n*-hexane and 5 mL distilled water were added. The above operation was repeated three times. All the supernatant was combined for GC-MS separation.

Volatile composition analysis of the extract obtained above was run on an Agilent 7890A/5975C gas chromatography - mass spectrometry, equipped with a split-splitless injector and Agilent 19091S-433 column (30m×250µm ID, 0.25 µm film thickness). The sample was injected, and the split ratio was 10:1. The injector and detector temperatures were 290°C and 220°C, respectively. The oven temperature programmed as follows: firstly, hold 5 min at 40°C, then 40~150°C at 10°C/min, held for 5 min at 180°C, and then 150~225°C at 5°C/min, held for 5 min, lately, 225~250 at 5°C/min, held for 10min, Finally, 250~300°C at 10°C/min, held for 20min. Helium (99.9%) was used as carrier gas at a flow rate of 1.0 mL/min. The mass detector was set to scan ions between 33-700 m/z using full scan mode and electron impact (EI, 70 eV). A hydrocarbon mixture of n-alkanes (C<sub>1</sub>-C<sub>31</sub>) was applied separately on GC-MS using the same chromatographic conditions as above. Identification of compounds was achieved from their GC retention indices (RI) relative to n-alkanes and by computer search using libraries of NIST08.

KI(Kovats index) determination: Take n-alkane standards analyzed in accordance at the same conditions as sample analysis, record each n-alkane standard peak retention time, and take the data into the linear temperature formula<sup>[14]</sup> to calculate the KI value of each component

$$KI = 100n + 100 (t_x - t_n) / (t_{n+1} - t_n)$$

Where  $t_x$ ,  $t_n$  and  $t_{n+1}$  are the outflow time (min) of the measured components and n-alkanes with n and n + 1 as carbon number, respectively ( $t_n < t_x < t_{n+1}$ ).

### 3.3 Antioxidant activity

The antioxidant activity was determined by the DPPH free radical scavenging assay. The scavenging effect on DPPH• radical by the sample was carried out according to the method of Elbadrawy<sup>[15]</sup> with a slight modification. Sample of 0.2 ml with 5 different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 g·L<sup>-1</sup>) was mixed with 4 ml of DPPH• in ethanol prepared daily (25 mg/L). After 5 min incubation in darkness, the absorbance at 517 nm was measured against absolute

ethanol blank. The inhibition percentage of DPPH• radical was calculated according to the formular:

$$SR\% = (A_0 - A_i) / A_0 \times 100\%.$$

where A<sub>0</sub> and A<sub>i</sub> are the absorbance values of the control and tested samples, respectively. Ascorbic acid (0.2 mg/mL) was used for comparison. The determinations were performed in triplicate.

### 3.4 Antibacterial activities

The antibacterial activities of the four extract from two mushrooms were carried out according to the puncture method, which had been utilized by De Andrade EF<sup>[16]</sup>, using two bacteria (*Escherichia coli* and *Staphylococcus aureus*). The specific operation is as follows: The prepared plate is divided into two groups. A set of plates was punctured with six evenly sized wells on each plate with a punch, positive control (neomycin), negative control (10% DMSO) and four samples 50μL of each solution, all of which has the same concentration of 0.5 mg/mL 10% DMSO as solvent, were added into and marked. The plates inoculated with the two bacteria were inoculated at 37°C for 18 h. The determinations were performed in triplicate for each sample and the values were averaged.

### 3.5 Statistical Analysis

The data collected were triplicate. The significant means were separated and compared using Duncan multiple range tests.

## 4 RESULT AND DISCUSSION

### 4.1 Sample Extraction

1.982g and 1.857g extract were obtained from *Coriolus versicolor* and *Trametes robiniophila* (50g of each), respectively, using a Soxhlet apparatus for 2 h with ethanol (85%) as the solvent. Four solvent different in polarity, Petroleum ether, ethyl acetate, n-butanol and distilled water, were used to reextract the above extracts. Various solvent extraction yield was shown in Table 1, representing that the petroleum ether part of both two mushrooms had the highest yield, which suggests that the two mushrooms contain more low-polar substances.

Table 1 Various solvent extraction yield

Solvent	<i>Coriolus versicolor</i>		<i>Trametes robiniophila</i>	
	Extracting amount (g)	Yield (%)	Extracting amount (g)	Yield (%)
petroleum ether	0.664	34.44	0.675	36.35
ethyl acetate	0.322	16.70	0.227	12.22
n-butanol	0.292	15.15	0.116	6.25
distilled water	0.040	2.07	0.072	3.88

### 4.2 Volatile components of the petroleum ether extracts

After the determination by GC - MS analysis of petroleum ether extraction part of two kinds of mushrooms, the ion flow spectra (Fig. 1, 2) and mass spectrum data were got, illustrating that 27 compounds were identified in *Coriolus versicolor* and 39 compounds in *Trametes*



Compound name	Molecular formula	Molecular weight	Coriolus versicolor			Trametes robiniophila			KI*
			retention time	% area	KI	Murr			
						retention time	% area	KI	
Phenol, 3,5-di-tert-butyl	C <sub>14</sub> H <sub>22</sub> O	206	21.617	6.37	1551				1555
9,12-Octadecadienoic acid, Methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	36.462	23.92	2092	36.462	4.29	2092	2093
13-Octadecenoic acid, Methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	36.614	5.06	2083				2085
1,2-Benzenedicarboxylic acid, Methyl ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	47.838	18.68	2160	47.852	9.60	2161	2162
Methyl 2-hydroxy-tetracosanoate	C <sub>25</sub> H <sub>50</sub> O <sub>3</sub>	398	53.561	15.78	2835	53.580	15.54	2837	2836
Palmitic acid, Methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	32.882	3.17	1874	32.875	3.78	1876	1878
Ergosta-14,22-dien-3-ol (3β, 5α, 22E)	C <sub>28</sub> H <sub>46</sub> O	398	58.076	3.79	2641	58.102	10.38	2640	2640
Ergosta-7, 22-dien-3-ol (3β, 5α, 22E)	C <sub>28</sub> H <sub>46</sub> O	398				59.125	2.01	2643	2640
trans-Dehydroandrosterone, heptafluorobutyrate	C <sub>23</sub> H <sub>27</sub> F <sub>7</sub> O <sub>3</sub>	484				58.318	5.86	2035	2034
9-Octadecenoic acid, Methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296				36.639	22.43	2083	2085
Methyl 2-hydroxy-pentacosanoate	C <sub>26</sub> H <sub>52</sub> O <sub>3</sub>	412				54.933	5.63	2934	2935
Stigmasta-5,22-dien-3-ol,(3β,22E)	C <sub>29</sub> H <sub>48</sub> O	412	58.305	2.18	2739				2739
Cholest-5-en-3-ol,(3β)	C <sub>27</sub> H <sub>46</sub> O	386	59.221	2.16	2598				2596
Tetrapentacontane, 1,54-dibromo	C <sub>54</sub> H <sub>108</sub> Br <sub>2</sub>	914	54.926	2.45	5986				5981
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl]	C <sub>23</sub> H <sub>32</sub> O <sub>2</sub>	340				44.756	1.78	2789	2788

Note: KI, The calculated value; KI\*, the reference value

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153 As it was shown from table 2, there was little difference in the composition of petroleum ether  
154 part between two mushrooms, which were rich in regard to both fatty acids and sterol. Methyl  
155 linoleate (23.92%), the highest content in the *Coriolus versicolor*, has been reported at an early  
156 time that it can directly affect the respiratory epithelial cells and regulate the gene expression,  
157 production, and secretion of mucus [17]. Moreover, its derivatives, linoleic acid, and conjugated  
158 linoleic acid have anti-cancer, inhibit atherosclerosis, reduce body fat, and improve lean body  
159 weight, regulate immune and inflammatory response, etc. [18, 19]. The highest content in  
160 *Trametes robiniophila* turned out to be methyl oleate (22.43%), which has a function of  
161 increasing the weight of androgen-sensitive tissue and plasma testosterone level [20]. Methyl  
162 linoleate, monoethyl hexyl ester of phthalate, methyl palmitate, 2-hydroxy-tetracyclide and  
163 ergostane 14,22 diene-3-alcohol are included as the common substance. Among them, methyl  
164 palmitate has protective effects on the heart, liver, and lungs [21, 22] as well as anti-inflammatory  
165 activity [23].

166

### 167 4.3 Antioxidant activities

168 Antioxidant activity of the samples was characterized using the DPPH method, which  
169 assesses the scavenging capacity of hydrogen donating antioxidants toward the stable free  
170 radical. Antioxidant activities of four solvent extracts were summarized in Table 2.

171 **Table 3 DPPH free radical scavenging rate of four extraction fractions at different**  
172 **concentrations**

concentration (mg·mL <sup>-1</sup> )	clearance rate (%)								
	<i>Coriolus versicolor</i>				<i>Trametes robiniophila</i>				Vc
	NB	PE	EA	DW	NB	PE	EA	DW	
0.2	72.46 <sup>a</sup>	48.27 <sup>c</sup>	48.27 <sup>c</sup>	56.86 <sup>b</sup>	56.38 <sup>b</sup>	52.08 <sup>d</sup>	52.08 <sup>d</sup>	40.22 <sup>e</sup>	98.42
0.4	80.20 <sup>a</sup>	55.39 <sup>cd</sup>	55.39 <sup>cd</sup>	58.95 <sup>c</sup>	74.17 <sup>b</sup>	52.25 <sup>d</sup>	52.25 <sup>d</sup>	42.53 <sup>e</sup>	NA
0.6	87.01 <sup>a</sup>	57.70 <sup>c</sup>	57.70 <sup>c</sup>	66.60 <sup>b</sup>	83.26 <sup>a</sup>	55.93 <sup>c</sup>	55.93 <sup>c</sup>	49.00 <sup>d</sup>	NA
0.8	88.58 <sup>a</sup>	70.16 <sup>c</sup>	70.16 <sup>c</sup>	71.31 <sup>c</sup>	86.77 <sup>a</sup>	58.23 <sup>d</sup>	58.23 <sup>d</sup>	51.62 <sup>e</sup>	NA
1	90.26 <sup>b</sup>	74.45 <sup>c</sup>	74.45 <sup>c</sup>	75.08 <sup>c</sup>	87.09 <sup>b</sup>	63.95 <sup>d</sup>	63.95 <sup>d</sup>	56.86 <sup>e</sup>	NA

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\*PE: petroleum ether; EA: ethyl acetate; NB: n-butanol; DW: distilled water; NA: not application

175 It showed in table 3 that the DPPH free radical clearance rate of different components of the  
176 two mushrooms could increase with the increasing of sample concentration, showing a good  
177 correlation between quantity and effect. All samples exhibited DPPH inhibition activity ranging  
178 from 40.22% to 91.51%, relative to ascorbic acid with 98.42%. Scavenging effect on the DPPH  
179 radical in *Coriolus versicolor* decreased in the following order: n-butyl alcohol > ethyl acetate >  
180 distilled water > petroleum ether; while, ethyl acetate > n-butanol > petroleum ether > distilled  
181 water in *Trametes robiniophila*. Antioxidant activity of all samples were less than that of  
182 positive control. The present study also suggested that extracts from ethyl acetate and  
183 n-butanol parts revealed better DPPH scavenging activity than petroleum ether and distilled  
184 water parts. The development of antioxidant active substances in ethyl acetate and n-butanol

185 parts can be highlighted.

186

#### 187 4.4 Antibacterial activity

188 Table 4 Antibacterial activity of different components of two mushrooms

Samples	<i>Coriolus versicolor</i>		<i>Trametes robiniophila</i>	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Neomycin	14.26±1.04	14.08±1.49	14.07±0.89	14.16±1.57
DMSO	0	0	0	0
petroleum ether	3.18±0.58	1.14±0.21	0.02±0.01	0.03±0.01
ethyl acetate	1.2±0.56	0	0.04±0.01	0.05±0.02
n-butanol	0	0	0	0
distilled water	0	0	0	0

189 \*ND: not detected

190 The antibacterial results as shown in table 4, Neomycin (0.4 g/ml) showed maximum zone of  
191 inhibition on both bacteria, while DMSO showed no antibacterial effect. In addition to a weak  
192 antibacterial effect of the *Coriolus versicolor* petroleum ether components, all the other  
193 components had no obvious antibacterial effect. Both mushrooms were poor in antibacterial  
194 activity. There is no significance for a deep research in antibacterial activity.

195

#### 196 5 CONCLUSION

197 In recent years, with people's attention to health, advocating nature. Antioxidants, especially  
198 antioxidants qualitative screening of natural products become a hot job, many natural extract  
199 or monomer compounds of drug sources can inhibit the generation of free radicals and has the  
200 oxidation resistance, and effect of anti-aging, even cure some diseases. Such as Resveratrol  
201 in grape, Polygonum and Mulberry<sup>[24]</sup>. From the above screening results, the antioxidant  
202 activity of the various sites of *Coriolus versicolor* was higher than that of *Trametes robiniophila*  
203 Murr, especially the antioxidant activity of ethyl acetate and n-butanol. It has been reported in  
204 the literature that polysaccharides of *Coriolus versicolor* have anti-oxidant activity<sup>[25]</sup>. However,  
205 the polysaccharides are very water-soluble and scarcely exist in the above two parts. We can  
206 actively look for new antioxidants from the two parts of *Coriolus versicolor*.

207 The results of GC-MS showed that the chemical compositions of the two polar fungi at low  
208 polarity were similar, with polyunsaturated fat as the main component. Peng et al.<sup>[26]</sup> reported  
209 that human polyunsaturated fatty acids play a number of key roles in host defense and  
210 immunity, including anticancer, anti-inflammatory, antioxidant activity, and competition for  
211 enteric pathogens. Therefore, the low polarity parts of the two fungi can also be used as raw  
212 materials for the production of anticancer and anti-inflammatory drugs. To sum up, this study  
213 provides some theoretical basis for further comprehensive utilization of *Coriolus versicolor* and  
214 *Trametes robiniophila*.

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