Original Research Paper

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 soxhlet 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,（3β，5α，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 antioxidant 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[1]. The fungi metabolites are diverse in structure: polysaccharides[2], terpenoids[3], steroid, alkaloids, benzoquinones and organic acids[4] are all included. These metabolites often play a role in anti-tumor, anti-inflammatory, antiviral and other biological activities [5-6]. Therefore, the medicinal and edible fungi that can be used in medical care

which have been widely studied, 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[7-8] and regulating immunity[9-10].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[11].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 analyzed 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 two mushroom were collected from Beichuan county, Sichuan Province of China in September 2017. These were identified as *Coriolus versicolor* and *Trametes robiniophila* based on their morphology, physiological-biochemical charateristies and 16S rDNA analysis 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℃ drying in infrared-ray oven, these mushrooms were crushed and separated through 100 mesh sieves. The prepared samples were keeping at 4℃ 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℃ for 18 h.

2.2 Reagents

DPPH (1,1-diphenyl-2-carnitine free radical) was purchased from Tokyo Chemical Industry (Shanghai) Co., Ltd. *n*-alkanes (C_1 ~ C_{31}) standard solution(10 mg·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 □, Hitachi).

3 METHOD

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

Referring to Madeja, Katarzyna [12] 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 vacuumm evaporator and weighed respectively. Then, the extraction yield was obtained.

$$Yield(\%) = \frac{\text{Weight of crude extract}}{\text{Weight of different solvent extracts}} \times 100\% \qquad (1)$$

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_2SO_4 (1%): Methanol =1:9 (v/v) for 80 min at 80 □[13]. 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 reparation.

Volatile composition analysis of the extract obtained above was run on an Aglient 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 □ and 220 □, respectively. The oven temperature programmed as follows: first hold 5 min at 40 □, then 40～150 □ at 10 □/min, held for 5 min at 180 □, and then 150～225 □ at 5 □/min, held for 5 min, lately, 225～250 at 5 □/min, held for 10 min, Finally, 250～300 □ at 10 □/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_1-C_{31}) was applied separately on GC-MS using the same chromatographic conditions as above. Identification of compounds was achieved by chromatography-mass spectrometry(GC-MS) combined with Kovats retention indices(KI) . The determination method of KI wais as follows:

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 [14] to calculate the KI value of each component

$$KI = 100n + \frac{100(t_x - t_n)}{t_{n+1} - t_n} \qquad (2)$$

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[15] with a slight modification. Sample of 0.2 ml with 5 different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 $g \cdot L^{-1}$) 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 formula:

$$SR(\%) = \frac{A_0 - A_i}{A_0} \times 100\% \qquad (3)$$

where A_0 and A_i are the absorbance values of the control and tested samples, respectively. Ascorbic acid (0.2 mg/mL) was used as positive control 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[16], 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 punc. 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 and expressed in the form of mean±SD. The significant means were separated and compared using Duncan multiple range tests with SPSS 20.0 software.

4 RESULT AND DISCUSSION

4.1 Sample Extraction

Coriolus versicolor extract(1.982 g) and *Trametes robiniophila* extract(1.857 g) were obtained from the dry powder(50 g of each), 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	*Coriolus versicolor*		*Trametes robiniophila*	
	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 robiniophila*. According to the comparison between KI and mass spectra, the compounds ranked in the top ten in content were presented as table 4, in which the percentage and relative retention indices of components are given.

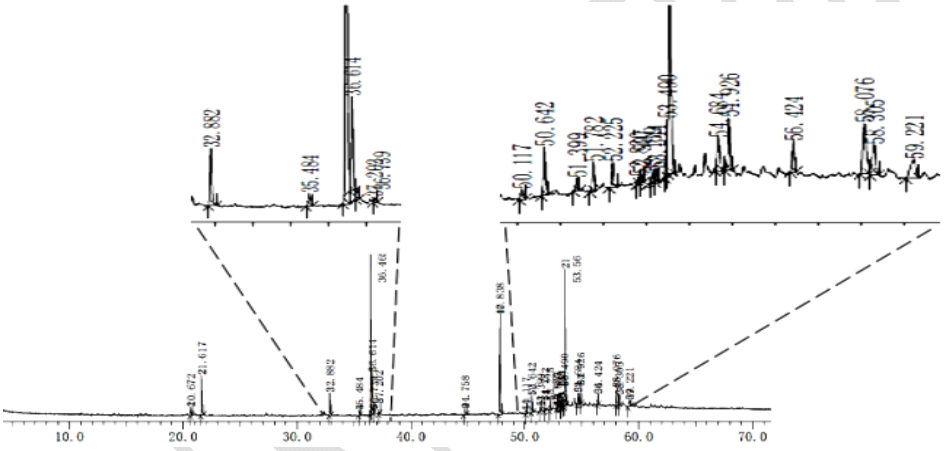

Fig 1. The GC-MS spectra of petroleum ether components from *Coriolus versicolor*

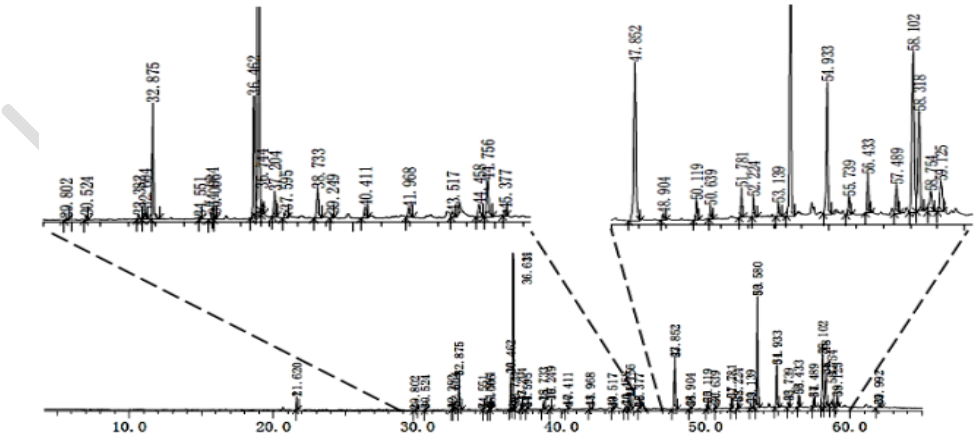

Fig 2. The GC-MS spectra of petroleum ether components from *Trametes robiniophila*

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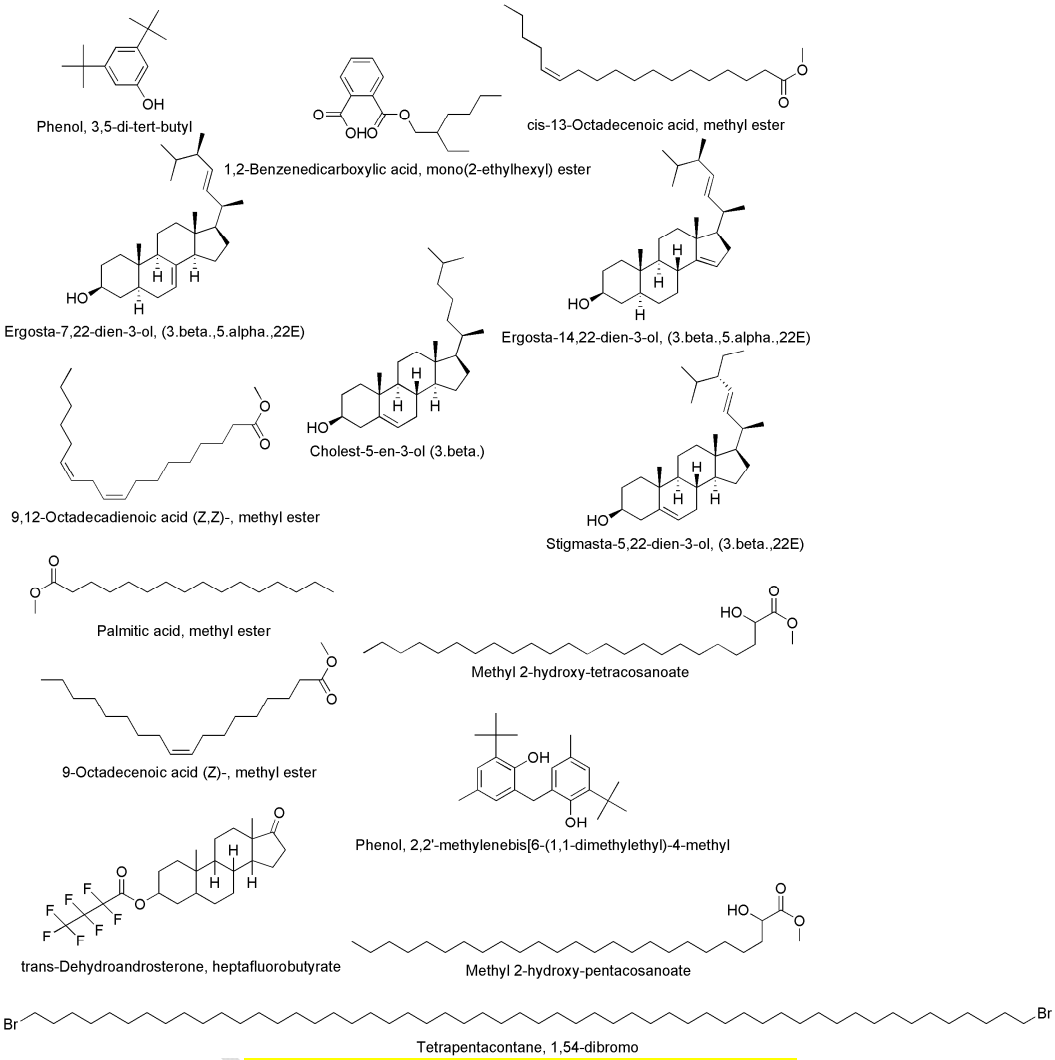
155 **Table 2 The GC-MS analysis results of petroleum ether components from two mushrooms**

Compound name	Molecular formula	Molecular weight	*Coriolus versicolor*			*Trametes robiniophila* Murr			KI*
			retention time	% area	KI	retention time	% area	KI	
Phenol, 3,5-di-tert-butyl	$C_{14}H_{22}O$	206	21.617	6.37	1551				1555
9,12-Octadecadienoic acid(Z,Z)，Methyl ester	$C_{19}H_{34}O_2$	294	36.462	23.92	2092	36.462	4.29	2092	2093
13-Octadecenoic acid，Methyl ester	$C_{19}H_{36}O_2$	296	36.614	5.06	2083				2085
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	47.838	18.68	2160	47.852	9.60	2161	2162
Methyl 2-hydroxy-tetracosanoate	$C_{25}H_{50}O_3$	398	53.561	15.78	2835	53.580	15.54	2837	2836
Palmitic acid，Methyl ester	$C_{17}H_{34}O_2$	270	32.882	3.17	1874	32.875	3.78	1876	1878
Ergosta-14,22-dien-3-ol（3β，5α，22E）	$C_{28}H_{46}O$	398	58.076	3.79	2641	58.102	10.38	2640	2640
Ergosta-7, 22-dien-3-ol（3β，5α，22E）	$C_{28}H_{46}O$	398				59.125	2.01	2643	2640
trans-Dehydroandrosterone, heptafluorobutyrate	$C_{23}H_{27}F_7O_3$	484				58.318	5.86	2035	2034
9-Octadecenoic acid，Methyl ester	$C_{19}H_{36}O_2$	296				36.639	22.43	2083	2085
Methyl 2-hydroxy-pentacosanoate	$C_{26}H_{52}O_3$	412				54.933	5.63	2934	2935
Stigmasta-5,22-dien-3-ol,(3β,22E)	$C_{29}H_{48}O$	412	58.305	2.18	2739				2739
Cholest-5-en-3-ol,(3β)	$C_{27}H_{46}O$	386	59.221	2.16	2598				2596
Tetrapentacontane, 1,54-dibromo	$C_{54}H_{108}Br_2$	914	54.926	2.45	5986				5981

Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4 -methyl]	$C_{23}H_{32}O_2$	340			44.756	1.78	2789	2788

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



157 **Fig 3. The structures of identified compounds**

All the compounds above were identified by using the NIST/NIH/EPA mass spectral library[17]. As it was shown from table 2, there was little difference in the composition of petroleum ether part between two mushrooms, which were rich in regard to both fatty acids and sterol. Methyl linoleate (23.92%), the highest content in the *Coriolus versicolor*, has been reported at an early time that it can directly affect the respiratory epithelial cells and regulate the gene expression, production, and secretion of mucus [18]. Moreover, its derivatives, linoleic acid, and conjugated linoleic acid have anti-cancer, inhibit atherosclerosis, reduce body fat, and improve lean body weight, regulate immune and inflammatory response, *etc.* [19, 20]. The highest content in *Trametes robiniophila* turned out to be methyl oleate (22.43%), which has a function of increasing the weight of androgen-sensitive tissue and plasma testosterone level [21]. Methyl linoleate, monoethyl hexyl ester of phthalate, methyl palmitate, 2-hydroxy-tetracylide and ergostane 14,22 diene-3-alcohol are included as the common substance. Among them, methyl palmitate has protective effects on the heart, liver, and lungs [22, 23] as well as anti-inflammatory activity [24].

4.3 Antioxidant activities

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

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

concentration（mg·mL-1）	clearance rate（%）								
	Coriolus versicolor				*Trametes robiniophila*				
	NB	PE	EA	DW	NB	PE	EA	DW	Vc
0.2	72.46±0.21^{a}	48.27±0.87^{c}	48.27±0.75^{c}	56.86±0.65^{b}	56.38±1.36^{b}	52.08±0.96^{d}	52.08±1.42^{d}	40.22±0.59^{e}	98.42±4.91
0.4	80.20±3.12^{a}	55.39±2.36^{cd}	55.39±1.35^{cd}	58.95±1.46^{c}	74.17±2.83^{b}	52.25±3.41^{d}	52.25±4.37^{d}	42.53±1.49^{e}	NA
0.6	87.01±1.35^{a}	57.70±0.76^{c}	57.70±1.43^{c}	66.60±2.51^{b}	83.26±0.87^{a}	55.93±1.89^{c}	55.93±0.61^{c}	49.00±2.56^{d}	NA
0.8	88.58±1.52^{a}	70.16±1.91^{c}	70.16±3.42^{c}	71.31±0.78^{c}	86.77±3.48^{a}	58.23±2.64^{d}	58.23±1.95^{d}	51.62±3.21^{e}	NA
1	90.26±4.73^{b}	74.45±1.99^{c}	74.45±2.81^{c}	75.08±2.54^{c}	87.09±3.62^{b}	63.95±1.89^{d}	63.95±3.45^{d}	56.86±0.97^{e}	NA

**PE: petroleum ether; EA: ethyl acetate; NB: n-butanol; DW: distilled water; NA: not application; The similar letters has no significative difference within a significance paraemeter*

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

acetate and n-butanol parts revealed better DPPH scavenging activity than petroleum ether and distilled water parts. The development of antioxidant active substances in ethyl acetate and n-butanol parts can be highlighted.

4.4 Antibacterial activity

Table 4 Antibacterial activity of different components of two mushrooms

Samples	*Coriolus versicolor*		*Trametes robiniophila*	
	Escherichia coli	***Staphylococcus aureus***	***Escherichia coli***	***Staphylococcus aureus***
Neomycin	14.26±1.04	14.08±1.49	14.07±0.89	14.16±1.57
DMSO	ND	ND	ND	ND
PE	3.18±0.58	1.14±0.21	0.02±0.01	0.03±0.01
EA	1.2±0.56	ND	0.04±0.01	0.05±0.02
NB	ND	ND	ND	ND
DW	ND	ND	ND	ND

**ND: not detected*

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

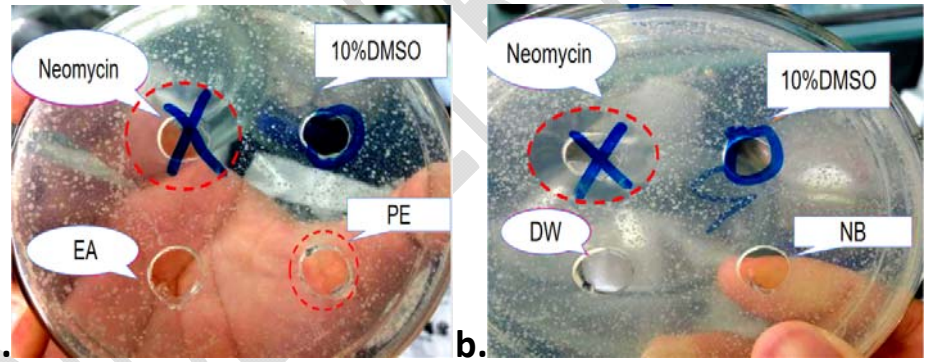


Fig 3. The antibacterial activity of different parts

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

5 CONCLUSION

In recent years, with people's attention to health, advocating nature. Antioxidants, especially antioxidants qualitative screening of natural products become a hot job, many natural extract or monomer compounds of drug sources can inhibit the generation of free radicals and has the oxidation resistance, and effect of anti-aging, even cure some diseases. Such as

Resveratrol in grape, *Polygonum* and *Mulberry*. From the above screening results, the antioxidant activity of the various sites of *Coriolus versicolor* was higher than that of *Trametes robiniophila Murr*, especially the antioxidant activity in the part of ethyl acetate and n-butanol. It has been widely known that polysaccharides of *Coriolus versicolor* have anti-oxidant activity. However, the polysaccharides are very water-soluble and scarcely exist in the above two parts. We can actively look for new antioxidants from the two parts of *Coriolus versicolor*.

The results of GC-MS showed that the chemical compositions of the two polar fungi at low polarity were similar, with polyunsaturated fat as the main component. The low polarity parts of the two mushroom can also be used as raw candidate materials for the further exploitation of anticancer and anti-inflammatory drugs, because of the anticancer, anti-inflammatory, antioxidant function of human polyunsaturated fatty acids. To sum up, this study provides some theoretical basis for further comprehensive utilization of *Coriolus versicolor* and *Trametes robiniophila.*

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