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Evaluation of Antibacterial Effect on *Propionibacterium acnes* and Antioxidant Activity of Various Mushrooms, Chinese Herbs, and Plant Essential Oils

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The inhibitory effects of 30 kinds of mushrooms, Chinese Herbs, and plant essential oils on *Propionibacterium acnes* (*P. acnes*) were compared to screen high activity antibacterial materials. Furthermore, their minimum inhibitory concentration (MIC), antioxidant activity, total flavonoids, and total phenol content were determined.
Methodology: The optimal antibacterial samples were screened using the paper diffusion method. The MIC was determined using the microdilution method. The antioxidant activity was evaluated by the 1,1-Diphenyl-2-Trinitrophenylhydrazine (DPPH), Ferric Ion Reducing Antioxidant Power (FRAP), and 2, 2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) methods, and the total flavonoids and total phenol contents were determined using Rutin Method and Folin-Ciocalteu Method.
Results: The MIC of mushroom *Tricholoma quercicola* and *Boletus edulis*'s extracts were 0.391mg/mL and 3.125mg/mL, showing better antibacterial effects on *P. acnes*. The Chinese herbs *Pericarpium granat*i and *Folium artemisia argyi*'s extracts (MIC 3.13mg/mL), plant essential oils of *Eugenia caryophyllus* flower, and *Lavandula angustifolia* (MIC 61.76mg/mL and 112.7mg/mL) exhibited satisfactory antibacterial effects on *P. acnes*. Antioxidant evaluation of the above six

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samples with high antibacterial activity revealed that *Folium artemisiae argyi* and *Pericarpium granati*'s extract had good antioxidant activity. In addition, the determination results of total flavonoids and total phenols were consistent with the antioxidant activity.
Conclusion: The above results showed that *Boletus edulis*, *Tricholoma quercicola*, *Pericarpium granati,* and *Folium artemisiae argyi* could be used as candidate materials for the antibacterial, antioxidant materials, providing a feasible idea for the development of subsequent skincare products for the treatment of *P. acnes*.

Keywords: Propionibacterium acnes; Antibacterial; Antioxidant activity; Total flavonoids; Total phenols.

1. INTRODUCTION

Acne is a common chronic inflammatory skin disease, with a high incidence in adolescents. Its key pathogenic factors include abnormal keratinization of the pilosebaceous ducts, increased sebum secretion, colonization of *Propionibacterium acnes* (*P. acnes*), inflammation, and sebaceous glands, immune response [1]. Among them, androgens produced during puberty are the main reason for the high incidence of acne in adolescents, and *P. acnes* is involved in the whole process of acne occurrence and development.

P. acnes is a Gram-positive bacteria that mainly grows in human skin. Puberty is accompanied by increased androgens and sebum secretion, increasing *P. acnes* [2]. The proliferation of the bacteria will induce the occurrence of an inflammatory response and at the same time promote sebum secretion and keratinization, resulting in blockage of sebaceous gland ducts and accumulation of sebum, which will continue to increase the proliferation of the bacteria and form a vicious circle. Therefore, the inhibition or killing of *P. acne* is one of the key ways to prevent and treat acne. At present, evaluating the inhibitory effect of *P. acnes in vitro* is a critical evaluation method for related cosmetics [3]. The existing drugs for treating *P. acnes* are mainly chemically synthesized drugs, such as nitroimidazoles, sulfonamides, and commonly used antibiotic drugs (like tetracyclines, macrolides, and lincomycins) [4]. These drugs generally have specific adverse reactions (skin allergies, pigmentation, and scarring) and quickly induce *P. acnes* drug resistance and reduce efficacy.

Recent studies have shown that traditional Chinese medicinal materials [5] and natural mushroom products have apparent microbial inhibition and bactericidal effects. *Hericium erinaceus* [6] and M*orchella esculenta* [7] 's extracts have satisfactory bactericidal and therapeutic effects on various common pathogenic bacteria. These discoveries have made mushrooms already hot for new resource development. In addition, plant essential oils are also potential sources of antibacterial drugs. Zu *et al.* [8] and Veerasophon *et al.* [9] found that *Lavandula angustifolia* and cinnamon oil have an inhibitory effect on *P. acnes*. In addition, plant essential oils have other biological functions, such as antioxidant and anti-inflammatory. At the same time, the unique fragrance of plant essential oils also has substantial commercial potential and application market.

To sum up, since the research on the anti- *P. acnes* from natural sources in China is still relatively small, the cross-category comparison is not comprehensive enough. In this experiment, *P. acnes* was used to screen excellent antibacterial candidate materials from mushrooms, Chinese herbs extracts, and plant essential oils to evaluate the antibacterial effect. Furthermore, the antioxidant activity, flavonoids, and phenolic content were also measured. The development of natural materials of *P. acnes* provides a specific theoretical basis, which is of great significance to solving adolescent acne's troubles.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

P. acnes was purchased from the Guangdong Provincial Microbial Culture Collection Center, and the strain ATCC number is 11827. The origin of mushroom fruiting bodies, Chinese herbs, and plant essential oils are shown in Table 1. The mushroom fruiting bodies were identified by He Xinsheng, a professor of microbiology at the School of Life Science and Engineering, Southwest University of Science and Technology, and the samples were stored in the School of Life Science and Engineering,

Southwest University of Science and Technology. The mushroom fruiting bodies and Chinese Herbs were dried at 60℃, crushed through a 40-mesh sieve, and dried at 4℃ in the dark for future use.

DPPH (1,1-diphenyl-2-trinitrophenylhydrazine), ABTS (2,2'-diazo-bis-3-ethylbenzothiazoline-6-sulfonic acid), TPTZ (tripyridine triazine), BHA (butylated hydroxyanisole), Vc (ascorbic acid or 2,3,5,6-tetrahydroxy-2-hexeno-4-lactone), ampicillin, agar, cysteine hydrochloride, potassium persulfate, and gallic acid were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.. Folin phenol reagent, sodium carbonate, dimethyl sulfoxide, and sodium hydroxide were purchased from Chengdu Kelong Chemical Co., Ltd.. The micro biochemical identification tube and BHI medium were purchased from Qingdao Haibo Biotechnology Co., Ltd., Ultrapure water was made by the laboratory (Resistivity was 18.3M).

2.2 Activation and Culture of *P. acnes*

P. acnes was a Gram-positive anaerobic bacterium that must be cultured in anaerobic bags (Qingdao Hi-Tech Industrial Park Haibo Biotechnology Co., Ltd.) In this experiment, the protocol of Shu *et al.* [10] was adopted, agar and a trace amount of cysteine hydrochloride were added to the BHI medium, inoculated after sterilization, and placed in an anaerobic culture bag. At the same time, add 1 g of gallic acid and 10 mL of 10% sodium hydroxide to the culture bag, seal the bag, and put it into a biochemical incubator at 37℃ for 48 h.

2.3 Sample Extraction and Preparation

The extraction of mushrooms and Chinese Herbs referred to Jiang *et al.* [11] with slight modifications. 20 g of material were weighed and added 85% ethanol according to the material-to-liquid ratio of 1:10, soaked for 20 minutes, and then ultrasonically extracted at 40 ℃ for 30 minutes. After filtering with filter paper and 0.45μm filter membrane, the filtrate was spin-dried at 65 ℃. The obtained extract was added dimethyl sulfoxide (DMSO) to prepare a 500 mg/mL sample stock solution**,** stored at 4 ℃ for later use.

Preparation of plant essential oil samples: add DMSO into the oil to prepare a 500 mg/mL sample solution, and store it at 4 ℃ for future use.

Table 1. Material name and origin

Mushrooms	Origin	Chinese Herbs	Origin	Plant essential oils	Origin
Xylaria striata	Mianyang City, Sichuan Province	*Pericarpium granati*	Sichuan Mianyang Kelun Pharmacy	*Lavandula angustifolia*	YakYeTi Aromatic Pharmaceutical Technology (Qingdao) Co.,Ltd.
Lentinus edodes	Qingchuan County, Sichuan Province	*Arctium lappa*		*Ocimum basilicum*	
Sclerodema verrucosum	Mianyang City, Sichuan Province	*Galla chinensis*		*Rosmarinus officinalis*	
Tricholoma quercicola	Qingchuan County, Sichuan Province	*Cortex pseudolaricis*		*Eugenia caryophyllus*	
Agrocybe aegirit	Qingchuan County, Sichuan Province	*Nepeta cataria*		*Melaleca alternifolia*	
Boletus edulis	Qingchuan County, Sichuan Province	*Rheum officinale*		*Citrus auranyium dulcis*	
Morchella esculenta	Jiangyou City, Sichuan Province	*Lonicera japonica*		*Citrus medica L*	
Xylaria nigripes	Mianyang City, Sichuan Province	*Folium artemisiae argyi*		*Illicium verum*	
Auricularia auricular	Qingchuan County, Sichuan Province	*Angelica dahurica*		*Mentha canadensis Linnaeus*	
Armillaria luteovirens	Ganzi Prefecture, Sichuan Province	*Salvia miltiorrhiza*		*Simmondsia chinensis*	

2.4 Determination of Antibacterial Activity

2.4.1 Disc diffusion method

Referring to the method of Diao *et al.* [12] with a slight modification, the filter paper was punched into a 6mm disc, sterilized, and soaked in the sample for 30 minutes. Then, it was placed on the culture medium of the coated strain, and at the same time, the paper was soaked in 25 mg/mL ampicillin/DMSO was used as a positive/negative control. The above dishes were incubated at 37℃ for 48 hours in a biochemical incubator according to the protocol in 2.2. Moreover, the inhibition zone diameter was measured with a vernier caliper.

2.4.2 Minimum inhibitory concentration (MIC)

After comparing the antibacterial activity of 30 kinds of materials using the Disc diffusion method**,** the MIC of the six samples with the highest antibacterial activity was determined by the micro double broth dilution method. Referring to Kim *et al.*[13] with a slight modification, after anaerobic cultivation of *P. acnes* in BHI liquid medium for 48 hours, the concentration was adjusted to 0.5 OD value with blank BHI liquid medium at a wavelength of 600 nm. In a 96-well plate, the first column was an antibiotic control; the second column was bacterial medium without sample; the third column was sterile medium; every three columns in columns 4 to 12 was a sample. After adding a 50 μL sample with an initial 25 mg/mL concentration to the first well, it was double-diluted from well A to well E. Columns 4, 7, and 9 didn't add any bacterial medium. 50 μL of the sample was added for gradient dilution, and then 100 μL of blank BHI liquid medium was added. Add 50 μL bacterial solution and 50 μL blank BHI liquid medium to the remaining columns. The above 96-well plate was placed in an anaerobic culture bag incubated at 37 ℃ for 48 hours according to the protocol in 2.2. Furthermore, the absorbance value was measured at 600 nm with a microplate reader. The smallest value in the entire row of well plates was taken as the MIC of the sample.

2.5 Determination of Antioxidant Activity

1,1-Diphenyl-2-Trinitrophenylhydrazine(DPPH) determination: Refer to Stratil *et al.* [14] with slight modification, the blank control group (A0) was added with 160 μL DPPH solution and 40 μL absolute ethanol. The sample test group (Ai) was added with 160 μL DPPH solution and 40 μL sample solution; the control group (Aj) was added with 160 μL absolute ethanol and 40 μL sample solution. Each column A ~E well was followed by eight concentration gradients of one type of sample. After mixing, the samples were put in a dark place for 30 minutes. After the reaction was completed, the absorbance was measured at 517 nm with a microplate reader. From the measurement results, the DPPH radical scavenging rate of the sample is calculated according to formula (1).

DPPH free radical scavenging rate (%)
= (1- (Ai-Aj)/A0) × 100%

(1)

The measurement method of 2, 2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) ABTS refers to Stratil *et al.*[14] with a slight modification. The concentration of ABTS was 7.00 mmol/L, and the concentration of potassium persulfate was 2.45 mmol/L. The two solutions of the same volume were mixed, and the reaction was kept at room temperature for 12 h in the dark. After 12 hours, the ABTS solution was diluted with phosphate buffer, and the absorbance value of the solution was diluted to 0.7 (±0.02) with a UV spectrophotometer, and the measurement wavelength was 734 nm. The blank control group (A0) was added with 200 μL ABTS solution and 10 μL absolute ethanol. The sample test group (Ai) was added with 200 μL ABTS solution and 10 μL sample solutions of various concentrations; the control group (Aj) was added with 200 μL absolute ethanol and 10 μL sample solutions of various concentrations, and each row of wells A to E was followed by 8 concentration gradients of one type of sample, mixed well and protected from light for 15 min. After the reaction was completed, the absorbance was measured at 517 nm with a microplate reader. For the measurement results, formula (2) was used to calculate the ABTS clearance rate of the sample.

ABTS clearance rate (%) = (1-(Ai-Aj)/A0) × 100%

(2)

Ferric Ion Reducing Antioxidant Power (FRAP) determination: refer to Tepe *et al.*[15] with slight modifications, the blank control group (A0) was added with 150 μL FRAP working solution and 5 μL absolute ethanol; the sample determination group (Ai) was added with 150 μL FRAP working solution and 5 μL sample solutions of various concentrations; the control assay group (Aj), 150 μL of absolute ethanol and 5 μL of sample

solutions of various concentrations were added. Each column of wells A to E was followed by 8 concentration gradients of one sample type. After mixing, the color was developed for 30 min in the dark. The absorbance value was measured with a microplate reader at a wavelength of 593 nm. The concentration was taken as the abscissa, and the absorbance value was drawn on the ordinate.

Natural antioxidant Vc and synthetic antioxidant (BHA) were used in these tests as positive controls. By determining the clearance rate of different concentrations, the concentration for 50% of maximal effect (EC_{50}) was calculated, respectively.

2.6 Determination of Total Flavonoids and Total Phenols

The content of flavonoids was determined by the Rutin control method [16], and the content of total phenols was determined by the Folin-Ciocalteu method [17].

2.7 Statistical Analyses

All experiments were repeated three times. The experimental data were analyzed using Excel 2010, and the data were presented as mean ± standard error (n=3).

3 RESULTS AND DISCUSSION

3.1 *In Vitro* Antibacterial Test

The disk diffusion method (Table 2) showed that *Boletus edulis* (15.1mm) and *Tricholoma quercicola* (18.3mm) had the best bacteriostatic effect among ten kinds of mushroom materials. *Folium artemisiae argyi* (16mm) and *Pericarpium granati* (12mm) had higher antibacterial activity in traditional Chinese herbs. *Eugenia caryophyllus* (21.43mm) and *Lavender angustifolia* essential oil (17.32mm) had the best antibacterial effect among the plant essential oils.

Table 2. The diameter of the inhibition zone of the samples against *P. acnes*

Mushrooms (50mg/mL)	Diameter of inhibition zone (mm)	Chinese herbs （50mg/mL)	Diameter of inhibition zone(mm)	Plant essential oils （50mg/mL）	Diameter of inhibition zone (mm)
Morchella esculenta	11.2±0.8	*Nepeta cataria*	22±0.11	*Eugenia caryophyllus*	21.43±0.06
Boletus edulis	15.1±0.9	*Pericarpium granati*	30±0.31	*Melaleca alternifolia*	16.52±0.07
Agrocybe aegirit	6.5±0.5	*Angelica dahurica*	22±0.24	*Mentha canadensis Linnaeus*	14.43±0.05
Lentinus edodes	13.4±0.7	*Arctium lappa*	14±0.15	*Citrus medica L*	6.23±0.04
Tricholoma quercicola	18.3±0.8	*Lonicera japonica*	18±0.54	*Citrus auranyium dulcis*	9.13±0.05
Xylaria nigripes	10.2±0.4	*Cortex pseudolaricis*	9±0.21	*Lavandula angustifolia*	17.32±0.07
Armillaria luteovirens	13.2±0.6	*Rheum officinale*	19±0.43	*Ocimum basilicum*	16.11±0.08
Sclerodema verrucosum	11.8±0.5	*Folium artemisiae argyi*	30±0.23	*Rosmarinus officinalis*	13.83±0.10
Xylaria striata	6.0±0.3	*Galla chinensis*	14±0.64	*Simmondsia chinensis*	6.13±0.02
Auricularia auricula	6.3±0.4	*Salvia miltiorrhiza*	28±0.34	*Illicium verum*	8.67±0.09
Positive control		Ampicillin	28.1±1.0		
Negative control		DMSO	8±0.24		

Note: Data are mean ± standard error (SE)

Table 3 revealed the MIC of the above six best antibacterial materials. The MIC of *Boletus edulis* was 3.125 mg/mL, the MIC of T*richoloma quercicola* was 0.391 mg/mL, and the MIC of both *Pericarpium granati* and *Folium artemisiae argyi* extract were 3.13 mg/mL. The MIC results were consistent with the disc diffusion. Especially the *Tricholoma quercicola* showed strong antibacterial application potential as literature [18]'s report.

The MIC of *Lavandula angustifolia*_essential oil with the most potent antibacterial ability among plant essential oils was 112.7 mg/mL. The MIC of *Eugenia caryophyllus* essential oil was 61.76 mg/mL. The results were weaker than mushroom and Chinese Herbs extracts and were inconsistent with the inhibition zone data. It was possibly related to the lower solubility of essential oils in this test.

3.2 Antioxidant Test Results

DPPH, ABTS, and FRAP are commonly used antioxidant assay methods. Among the six samples, the antioxidant activities of *Boletus edulis*, *Pericarpium granati,* and *Folium artemisiae argyi* were all strong, and the EC_{50} values of *Pericarpium granati, Boletus edulis,* and *Folium artemisiae argyi* were close to the two positive controls (Table 4). Compared with other researchers' results [19-20], our experiment showed that the extract from *Boletus edulis* and *Folium artemisiae argyi* showed superior antioxidant activity. Hence, They were excellent targets for antioxidant development. In addition, the performance of essential oils was relatively weak, with only *Lavandula angustifolia* showing little antioxidant capacity. This result is also consistent with the results of other researchers [21].

Table 3. MIC values of 6 samples

Samples	MIC（mg/mL）
Boletus edulis	3.125±0.07
Tricholoma quercicola	0.391±0.04
Pericarpium granati	3.13±0.2
Folium artemisiae argyi	3.13±0.2
Lavandula angustifolia	112.7±0.7
Eugenia caryophyllus	61.76±0.4

Note: Data are mean ± standard error (SE)

Table 4. Antioxidant activity of samples

Samples	EC_{50} (mg/mL)		
	DPPH	ABTS	FRAP
Positive control：V_C	0.03±0.01	0.08±0.02	0.02±0.01
Positive control：BHA	0.02±0.01	0.02±0.01	0.03±0.01
Boletus edulis	2.79±0.05	1.37±0.02	0.32±0.04
Tricholoma quercicola	4.52±0.08	6.34±0.06	9.92±0.1
Pericarpium granati	0.095±0.01	0.12±0.01	1.46±0.01
Folium artemisiae argyi	0.49±0.01	1.22±0.01	0.72±0.03
Lavandula angustifolia	18.4±0.2	10.2±0.2	24.4±0.7
Eugenia caryophyllus	5.95±0.1	8.9±0.1	1.50±0.03

Note: Data are mean ± standard error (SE)

Table 5, Total flavonoids and total phenol content（%）

Element	*Tricholoma quercicola*	*Boletus edulis*	*Folium artemisiae argyi*	*Pericarpium granati*	*Eugenia caryophyllus*	*Lavandula angustifolia*
Total flavonoids	0.11±0.02	2.10 ± 0.06	16.54±0.6	9.54±0.35	2.487±0.05	1.675 ± 0.02
Total phenols	8.56±0.23	13.06 ± 0.4	6.36±0.3	12.54±0.5	3.674±0.2	0.655 ± 0.01

Note: Data are mean ± standard error (SE)

3.3 Content of Total Flavonoids and Total Phenols

It is known that flavonoids and phenolic compounds are related to antioxidant activity, and the antioxidant activity is further revealed by measuring the content of total flavonoids and total phenolics. From the results in Table 5, the flavonoid content of *Folium artemisiae argyi* and *Pericarpium granati* was high (16.54% and 9.54%), which is consistent with the literature [22]. The total phenolic content of *Tricholoma quercicola*, *Boletus edulis,* and *Pericarpium granati* is high (8.56%, 13.06%, and 12.54%), which was higher than the result measured in the literature [23]. The above results were consistent with the antioxidant results.

Chrysargyris *et al.* [24] reported that plant essential oils were mainly terpenoids, and the content of total flavonoids and total phenols was low, which was also proved by our result.

4. CONCLUSIONS

The abuse of antibiotics in acne treatment has made *P. acnes* resistant, and the treatment effect has declined [25]. Natural materials such as Chinese herbal medicines and mushrooms have attracted more and more attention because of their pharmacological activities in antibacterial, antiviral, antitumor, and other aspects. This study found that *Pericarpium granati*, *Folium artemisiae argyi*, and *Salvia miltiorrhiza* had higher *in vitro* antibacterial activities. This result was consistent with most of the research results of Chinese herbal antibacterial experiments in relevant literature [26]. *Pericarpium granati* and *Folium artemisiae argyi* also had particular antioxidant activity. The antibacterial activities of *Boletus edulis* and *Tricholoma quercicola* were relatively high, especially the latter, which was worthy of follow-up development and research.

Plant essential oils, known as "liquid gold," are the volatile aromatic substances of plants and the essence of plant immune and self-healing systems. This experiment found that the MIC of E*ugenia caryophyllus* essential oil with the most potent inhibitory ability was 61.76 mg/mL, significantly lower than that of Chinese herbal medicines and mushroom extracts, which may be related to the poor water solubility of essential oils. At present, E*ugenia caryophyllus* essential oil has been used in cosmetics based on its excellent antibacterial activity, consistent with our research results. In addition, the antioxidant activity of *Lavandula angustifolia* essential oil was vigorous, showing a particular application prospect.

This study compared the antibacterial effects, tested the antioxidant activity and total phenols and flavonoids contents of various Chinese herbs, mushrooms, and plant essential oils. In the future, we will conduct research on the formulation of these materials to develop antibacterial and antioxidative formula materials used in skincare products for acne treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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