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Antimicrobial effects of extracts from 18 kinds of medicinal plants

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Abstract
Natural plants are prospective sources of the new antimicrobial drug. This study found some natural materials from plants that have antimicrobial effect. The antibacterial activity and the minimum bactericidal concentration of methanol and ethanol extracted from 18 plants against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* studied in this paper. As analyzed its terms and bacteriostatic conditions, we found that *Cyperus rotundus Linn*, *Camptotheca acuminate* Decne, *EucaLyptus* spp, *Lonicera japonica* Thunb showed good antibacterial activity. The results could provide a reference for extracting antibacterial components from these plants.

Keywords: antimicrobial, medicinal plants

1. Introduction
The continued spread of antimicrobial resistance representing one of the most serious infectious diseases threatens the health of global people [1]. The research showed that the resistance of staphylococcus aureus was recorded against ampicillin and erythromycin (88% each), while resistances against oxacillin, fosfomycin, cefoxitin, and ciprofloxacin were also worrisome [2]. It is agreed that a key component of addressing this threat is to replenish the waning pipeline of antimicrobials, with attention being paid to novel mechanisms of action [1]. Plants are prospective sources for identifying new types of antimicrobial agents in the future [3]. We can also use the plants which have high efficiency as plant pesticide [4]. Also, they're clean and harmless to the environmemt [5]. So, it is valuable to study the development of new types of plant-derived products that can effectively against recently emerging problems related to human microbial diseases. There are many kinds and wide distribution of medicinal plants in China. According to the new edition of the *Dictionary of Traditional Chinese Medicine* revised and completed in 2006, 11,146 kinds of medicinal plants have been identified [6]. In this paper, we tested the antimicrobial activity of 18 different kinds of natural plants.

The effective components of natural plant can commonly be extracted by solvents which have strong polarities, such as water, methanol, ethanol, acetone, chloroform, and petroleum ether [7-9]. The purpose of this study is to analyze the antibacterial effect in 18 varieties of medicinal plants extracted by methyl alcohol. Finally, we make a preliminary evaluation of pharmaceutical value.

2. Materials and methods
2.1 Materials
2.1.1 Collection of Plant samples
Most of the materials were collected around the campus of Southwest University of Science and Technology in Mianyang, Sichuan province on November 24, 2006. Among them, the *Cyperus rotundus* Linn and the *Echinacea purpurea* Moench were purchased and authenticated by Prof. Lin Ma in the Engineering Research Center for Biomass Resource Utilization and Modification of Sichuan Province (Mianyang, Sichuan). The names and species of the plants used in the experiment are shown in the table below.

Table 1: the name and part of the materials.

Name	Part
Cyperus rotundus Linn.	Stem
Broussonetia papyrifera(L.) Vent.	Leaves
Cinnammon camphora Linn.	Leaves
Phyllostachys nigra (Lodd.) Munro.	Leaves
Ginkgo biLoba Linn.	Leaves
Camptotheca acuminate Decne.	Leaves
Pyracantha fortuneana	Fruits
*Eriobotrya japonica(*Thumb).Lindl.	Leaves
Ricinus communis L.	Leaves
Citrus grandis (Linn.) Osbeck.	Leaves
EucaLyptus spp.	Leaves
Melia azedarch L.	Leaves
Nerium indicum.	Leaves
Lonicera japonica Thunb.	Stem and leaves
Pinus massoniana Lamb.	Leaves
Herba Paederiae.	Stem and leaves
S. japonica cv.	Leaves
Echinacea purpurea Moench.	Leaves

2.1.2 Chemicals, reagents
Beef extract (We brought it from Beijing Aobaxing Biotechnology Co. LTD Peptone (We brought it from Beijing Shuangxuan Microorganism culture and products Factory)) Agar (We brought it from Biovake Co. LTD) Sodium chloride (We brought it from CP,Sichuan Leshan Pharmaceutical Factory) Dimethyl sulfoxide (AR, We brought it from Fuchen Tianjin Chemical Reagent Co. LTD) Methyl alcohol (AR, We brought it from Chengdu Jinshan Chemical Reagent Co. LTD)

2.2 Method

2.2.1 Pretreatment of plant materials

Put the medicinal plants' stem, fruit, and leaves in the oven drying for 10-20 h (under 65 degrees). The drying time determined on the materials themselves. Generally, leaves could be dried in 10h while stem needed 15h drying to meet crushing requirements. However, a hard fruit requires 20 h. Crushed the dried materials into powder then sieved the powdor (60 mesh) [10]. Finally, put the powdor into a wild-mouth bottle with a dry environment.

2.2.2 Culture and preservation of bacterial strains

The medium used in this experiment was beef extract peptone medium, and the preparation method of the medium was as follows: the beef extract was given 10g, peptone 10g, sodium chloride 5g, agar 18g, and 1000ml distilled water. Then, adjusted the pH value to about 7.4 and the medium was sterilized for 20 min at 121 degrees [11].

The activation method of the strains was solid inclined surface inoculation. After inoculating, the inoculated strains were placed in a constant temperature incubator for 24 hours at 30 degrees. When the surface of the culture medium was generally covered with bacteria evenly, it could be used for the bacteriostasis test. Besides, the excess strains could be stored in the refrigerator.

2.2.3 Preparation of the bacterial suspension

Use the inoculating loop to take the activated strains twice, and put them into 10ml sterile water, shake them well, generally in a turbidity liquid. The whole operation is completed in the super clean table, making sure that there is no contaminated hybrid bacteria in the bacterial suspension.

2.2.4 Preparation of methanol and ethanol extract

Weight 50 g of the prepared plant powder and place it in 500 mL wide-mouth bottle respectively. Add 200 mL methyl alcohol to soak for 4 h, place it in a water bath kettle and set the temperature at 55 degrees, stirring with a constant speed agitator for 2 h. Filtrate while extractives are hot, collect the filtrate residue, and return it to the wild-mouth bottle. Then add 200 mL methanol, soaking it again for 2 h at room temperature. Put it in a 55 degree water bath kettle, and pay attention to stirring, extracting it for 2 hours. After that filter it while it is hot, discarding the filter residue. Merge the extract into the flask and concentrat the liquid with a rotary reflux evaporator in 45 degrees. Do not stop it until there is no liquid material flowing out of the rotary reflux evaporator. Record the weight of the flask at this point, subtracting the initial weight of the flask and the weight of the available concentrate. Add 25 mL sterile water into the flask to dissolve the concentrated paste, pouring the dissolved solution, while the ethanol extract dissolved by dimethylsulfoxide. Take 5 mL sterile water and rinse the flask for four times. Collect the dissolved solution and mixe it. Keep the volume to 50 mL. The rest steps of preparation of ethanol extract is in the same way.

2.2.5 Detection of antibacterial effect

In this experiment, the filter paper method was used to screen the extracts of natural plants with antibacterial effect. Preparation method of filter paper: The filter paper (12.5cm qualitative filter paper) with strong water absorption was punched into the small circular paper with a diameter of 6 mm, which was sterilized by damp heat (121 degree, 20 min) and set aside. Experimental procedure: 18 species of plants were tested and completed in three times. In addition, filter papers were put into sterile water for a control experiment, respectively marked, soaked for 5 min, and dried. The above paper was cultured in a constant temperature incubator of 30 degrees for 24 h. After 24 h, measure the diameter of the bacteriostatic circle. The diameter of the bacteriostatic circle was measured horizontally and vertically twice with the minimum scale of 1mm, and take the average value.

2.2.6 Determination of the minimum inhibitory concentration

The MIC value was determined by the double broth dilution method, and the minimum inhibitory concentration (MIC) test was conducted by which material with an initial inhibitory effect on bacteria.

The methanol extract initial concentration of 1g/mL was diluted to 1:2, 1:4, 1:8, 1:16 by multiple dilution method with sterile water for the determination of minimum inhibitory concentration (MIC), while ethanol extract initial concentration of 1g/ml was dilute to 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 multiple with the same method. Put them in 33 degrees for the next 24h, and observe the presence of colonies. The minimum mass fraction of bacterial growth is taken as the minimum inhibitory mass fraction (MIC) of the extract, and the compare group add the sterile water.

3. Results

3.1 Extraction rate of methanol and ethanol extract

From the result, we can see that when ethanol is used as a solvent, the concentration and extract content are generally higher. *Melia azedarach* Linn and *Cyperus rotundus* Linn were special, their methanol extract content is slightly higher than that of ethanol.C.L, G.L, E.L, P.L, N.I, E.S have a high yield, which maybe be just collected from the field. The strength of antibacterial activity in vitroii is closely related to the composition and concentration of the extract. When the extract is concentrated to a higher concentration, its composition is complex and often contains non-antibacterial components such as protein, starch, polysaccharide etc. Without removing impurities in the extract and increasing the concentration of antibacterial components in the extract, the antibacterial effect of most plants is difficult to be presented [12-13].

Table 2: The Extraction rate of methanol and ethanol extract

The name of the plant	Weight of methanol extract (g)	Yield of methanol concentrate /%	Yield of ethanol concentrate /%	Weight of methanol extract (g)
C. rotundus	5.8	11.6	5.0	10.0
B. papyrifera (L.)	5.6	11.2	6.9	12.8
C.camphora	8.2	16.4	12.6	25.2
P. nigra (Lodd.)	5.8	11.6	7.1	14.2

C.acuminate	5.1	10.2	--	--
P.fortuneana	12.7	25.4	--	--
G. biLoba	6.4	12.8	10.3	20.6
E. japonica (Thumb)	8.1	16.2	14.6	29.2
R. communis L	3.7	7.4	9.7	19.4
C. grandis (Linn.)	3.6	7.2	5.0	10.0
Eucalyptus	5.2	10.4	10.4	20.8
M.azedarch	5.6	11.2	5.5	11.0
N.indicum	8.4	16.8	15.5	31.0
L. japonica	3.9	7.8	6.9	13.8
P. massoniana	7.7	15.4	10.0	20.0
H.Paederiae	7.0	14.0	8.9	17.8
S. japonica cv	6.2	12.4	8.9	17.8
E.. purpurea	2.9	5.8	4.1	8.2

Note:"--"means do not test

3.2 Antibacterial activity of methanol extract

The results showed that *Pyracantha fortuneana*, *Eucalyptus spp* and *Lonicera japonica* Thunb showed good antibacterial activity against E. coli; *Camptotheca acuminate* Decne and *Eucalyptus spp* showed good antibacterial activity against Bacillus subtilis; *Cyperus rotundus* Linn, *Camptotheca acuminate* Decne and *Eucalyptus spp* showed good antibacterial activity against Staphylococcus aureus. The remaining plants, which were not listed, did not show antimicrobial activity against any of the three bacteria.

Table 2: Antibacterial activity of methanol extract

The name of the plant	Bacillus subtilis/ mm	Escherichia coli/ mm	Staphylococcus aureus/ mm
P.fortuneana	12.8	7.3	15
Eucalyptus	17.8	14.2	15.2
L.japonica Thunb	--	--	8.8
C.rotundus	--	--	18

Note:"--"means no inhibition zone was observed.

3.3 The minimum inhibitory concentration of methanol extract

The results showed that, *EucaLyptus spp* and *Camptotheca acuminate* Decne had antibacterial effect on the three kinds of bacteria, among which,

The MIC of *Eucalyptus spp* was very small and had good antibacterial activity. Meantime, *Cyperus rotundus* Linn only had antibacterial effect on Staphylococcus aureus, while *Lonicera japonica* Thunb only had antibacterial effect on Escherichia coli.

Table 3: The minimum inhibitory concentration of methanol extract

Plants/Bacteria	Bacillus subtilis (mg/ml)	Escherichia coli (mg/ml)	Staphylococcus aureus(mg/ml)
EucaLyptus	1.25	2.50	1.25
C. acuminate	5.00	5.00	5.00
C. rotundus	--	--	2.50
L. japonica Thunb	--	5.00	--

Note:"--"means greater than the maximum test concentration of 10.0 g/ml.

3.4 Antibacterial activity of ethanol extract

Through experimental observation, the extracts of *Eucalyptus* spp, *Surattensis japonica* cv and *Ginkgo biloba* Linn showed strong bacteriostatic effect on E. coli, and the bacteriostatic circle was obvious. The extract of *Eucalyptus* spp had the strongest inhibitory effect on Staphylococcus aureus, and the inhibitory circle diameter reachied 21.7mm. Next, the extract of *Lonicera japonica Thunb* had good bacteriostatic effect on S. aureus. However, we found that the extracts of *Phyllostachys nigra (Lodd*.).

Munro, *Ginkgo biloba* Linn, *Eucalyptus* spp, *and Pinus massoniana* Lamb had a relatively strong inhibitory effect on Bacillus subtilis.

Table 4: Antibacterial activity of ethanol extract

The name of the plant	Escherichia coli	Bacillus subtilis	Staphylococcus aureus
C. rotundus	--	8.4	8.9
B.papyrifera(L.)	7.8	12.6	--
C. camphora	8.3	11.1	--
G.biloba	12.3	12.4	10.5
E.japonica(Thumb)	8.3	8.8	9.8
R. communis	--	10.2	8.6
C. grandis (Linn.)	--	8.8	--
Eucalyptus	14.1	15.5	21.7
M. azedarch	--	10.0	9.8
T. peruviana (Pers.)	--	12.9	--
L. japonica Thunb	9.1	9.1	9.1

P.massoniana Lamb	12.1	11.0	7.8
S. japonica cv.	--	17.5	8.9
E. purpurea	--	10.9	9.3
P. nigra (Lodd.)	11.7	--	--
P. scandens(Lour.)	--	--	9.3

Note:"--"means no inhibition zone was observed.

3.5 The minimum inhibitory concentration of ethanol extract

From the results, we concluded that the ethanol extract of *Eucalyptus* spp had the strongest antibacterial activity, and it also had the best antibacterial effect on all three kinds of bacteria.

The antibacterial effects of *Ginkgo biloba* Linn, *Melia azedarch* L, *Thevetia peruviana (Pers.)* Schum and *Surattensis. japonica* cv also had a good effect on the three bacteria. In general, presenting a great effect, the minimum antibacterial concentration of ethanol extract was lower than others.

Table 6: Minimum inhibitory concentration of ethanol crude extract (MIC)

The name of the plant	Bacillus subtilis mg/ml	Escherichia coli mg/ml	staphylococcus aureus mg/ml
B. papyrifera(L.)	--	1.25	--
C.camphora	--	1.25	--
G. biloba	1.25	0.625	0.313
*E. japonica(*Thumb*)*	1.25	1.25	--
Eucalyptus	0.0156	0.0156	0.156
M.azedarchL	0.625	0.625	--
T.peruviana (Pers.)	0.625	0.625	--
L. japonica Thunb	--	0.25	--
P.massoniana	--	--	1.25
S. japonica cv	2.50	0.625	--

Note:"--" means that greater than the maximum test concentration of 10.0 mg/ml

4. Discussion

From the above process, we saw that ethanol was a great solvent to extract antibacterial substances, and the antibacterial ability of ethanol extract was generally stronger than methanol. There were three reasons to explain the phenomenon. First, we considerd that probably because ethanol had a smaller polarity than methanol, and antibacterial substances were more likely to dissolve in ethanol. Second, according to the study of Li [15], we speculated the results might because we diluted the methanol extract with sterile water, while diluted the ethanol extract with dimethyl sulfoxide solvent. However, dimethyl sulfoxide is a very polar organic solvent, and many organic substances have a high solubility in dimethyl sulfoxide. Third, according to Zhao's paper [15], we found that the water extract of Honeysuckle had an antibacterial effect on the three kinds of bacteria. Nevertheless, the boiling point of methanol is lower than water. So, we speculated that the temperature also affects the dissolution of the antibacterial substance. The extraction temperature adopted in this experiment was 55 degrees, while the temperature adopted by Zhao's experiment was 100 degrees.

5. Conclusion

Through the experiment, we concluded that methanol and ethanol extracts of *Eucalyptus* spp had the best inhibitory effect on the three bacteria. Meanwhile, methanol extract of *Camptotheca acuminate* Decne had an inhibitory effect on the three bacteria, while ethanol extract did not. Besides, the methanol extracts of *Lonicera japonica Thunb* and *Cyperus rotundus* Linn showed inhibition against Escherichia coli, and Staphylococcus aureus respectively. The ethanol extracts of *Broussonetia papyrifera (L.)* Vent, *Ginkgo biloba* Linn, *Eriobotrya japonica* (Thumb) Lind and other plants showed inhibitory activity to Escherichia coli, while the ethanol extracts of *Ginkgo biloba* Linn, *Eucalyptus* spp and *Pinus massoniana* Lamb showed the inhibitory effect to Bacillus subtilis. In general, as far as the solvents were concerned, ethanol worked well in extracting the antibacterial substances from those plants tested in this experiment, and ethanol extract had a better antibacterial effect on the bacteria. As for materials, *Eucalyptus* spp had shown its strong antibacterial ability and broad-spectrum antibacterial property, which made a natural medicinal plant with development value.

6. Ackownledgement

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