

Antimicrobial Activity of Fourteen Chinese Herbal Extracts



Huan Chen¹, Lan Lin¹, Renlin Zheng¹, Liangchun Li¹, Wenjiao Zhao², Tianzhi Dai² and Dequn Sun^{1*}

¹School of Life Science and Engineering, Southwest University of Science and Technology, China

²Marine College, Shandong University at Weihai, China

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*Corresponding author: Dequn Sun, School of Life Science and Engineering, Southwest University of science and technology, Mianyang, 621010, PR China

Abstract

Drug resistance has drawn great attention from all over the world since it was put forward in 1900s. Infections caused by drug resistance have cost billions of dollars in clinic. Chinese herbs have been used as medicine in China for thousands of years with few adverse effects and drug resistance, it is very likely to find new drug candidates and medicine from Chinese herbs. In our work, fourteen commonly used Chinese herbal extracts were tested for their antibacterial activity against six typical bacteria. Most of them showed good inhibition to test bacteria. Especially, extracts 6, 14 exhibited very strong inhibition (MIC = 50, 10 µg/mL respectively) against *S. aureus* and *S. epidermidis*, which deserves further investigation in order to find potential activity compounds from the extracts.

Keywords: Antimicrobial activity; Chinese herbs; Extract; *Gentiana scabra*; *Sophorae Flavescentis*; *Artemisia annua*; *Polygonum bistorta* L; *Agrimonia Pilosa*; *Chrysanthemum indicum*; *Hottuynia cordata* Thunb; *Gardeniae jasminoides*; *Viola philippica* Cav; *Gardeniae jasminoides* Ellis; *Astragalus membranaceus* Bunge

Introduction

Nowadays, using antibiotics is the main method to protect people from infection of bacteria [1]. But the antimicrobial agents were limited, and resistance caused by antibiotics abuse has become a public health problem. The abuse of antibiotics which not only used in human life, but also widely used in aquaculture and livestock breeding has induced the production of super bacteria, meanwhile it has caused the water body of pollution, which is further harmful to human health [2]. And China is one of the countries with the most serious problem of antibiotic abuse, therefore, it is always meaningful to find new effective and low toxic antimicrobial candidates. Traditional Chinese Medicine (TCM) is a valuable resource in China, which has a long history in the treatment of various infectious diseases. And it also plays a fundamental role in traditional medicine in Korea, Japan, India, Egypt and other countries [3]. With the development of economy, Chinese herbal medicine has also gone abroad, which is favored by countries because of its low adverse reactions and non-pollution. After Tu You you won the Nobel Prize for discovering artemisinin which has a good anti-malaria effect, traditional Chinese herb has received more attention in the scientific community. For example, Ma, Feng et al researched the activity of *H. pylori* using 50 kinds of Chinese herbal medicine [4]. Su Pai-Wei et al. [5] studied the antibacterial activity and mechanism of *Polygonum cuspidatum* extracts direct

ing at resistant pathogenic bacteria. In fact, many Chinese herbs have significant and broad-spectrum antibacterial activity. Some plants are also proved to be capable of improving health and be a gentle therapy of infection caused by virus without any significant side effects [6].

Here are some of the advantages of Chinese herbs

- a. Traditional Chinese medicine possess good therapeutic effect for human according to the symptoms of the disease, especially for chronic disease or enhancing the body's resistance to diseases. And complementary and alternative medicine use was more and more prevalent.
- b. Herbal medicines could be used for complementary therapies, which aims to treat the entire person, because of its high value of studies [7].
- c. The herbal extracts used as medicines have many potent or synergistic ingredients with diverse chemical structures. When they are used to treat disease, less resistance may be found than synthesized drugs such as antibiotics [8].
- d. During the history of drug discovery, novel drug candidates were often exploited from herbal medicines [9]. In antibacterial area, the vast majority of the new chemical entities are natural products or derived molecules.



Figure 1: Pictures of Herbs from which the Extracts were Obtained.

1. *Magnolia officinalis* Rehd. et Wils;
2. *Radix Sophorae Flavescentis*; 3.
3. *Gentiana scabra* Bunge;
4. *Artemisia annua* Linn;
5. *Polygonum bistorta* L.;
6. *Ilex chinensis* Sims;
7. *Prunella vulgaris*;
8. *Agrimonia pilosa* Ledeb;
9. *Chrysanthemum indicum*;
10. *Artemisia capillaris* Thunb;
11. *Epimedium brevicornum* Maxim;
12. *Houttuynia cordata* Thunb;
13. *Viola philippica* Cav.;
14. *Gardeniae jasminoides* Ellis;
15. *Astragalus membranaceus* Bunge.

The long history of Chinese herbal medicine demonstrates the potential of plants as important sources of lead compounds. In order to have a further explore of Chinese medicinal herbs for the treatment of bacterial infections, it is highly necessary to sep-

arate the active components and find new lead compounds, or develop new Chinese medicine preparation, which could be used as anti-bacteria reagent. In this work, the antibacterial activity of 14 Chinese medicinal herbal extracts on six typical bacteria were

measured. Extract of *Magnolia officinalis* Rehd. et Wils (Figure 1) is widely used clinically with many effects such as calming the central nervous and working as clinical antimicrobial agents for bacterial dysentery or inflammation with high safety, low side effects and easy to be excreted from the body [10]. Kyu reported the significant inhibitory activities of Magnolol and Honokiol against several human pathogenic fungi [11]. It could be used as lead compounds for the development of novel antifungal agents. Magnolol and Honokiol also have a marked antimicrobial effect against *Micrococcus luteus* and *Bacillus subtilis* [12]. We chose Magnolol (98%) as the positive control in our initial test. In order to look for agents with good antimicrobial activity, 14 commonly used Chinese medicinal herbs with great distribution in Sichuan Province were chosen for their antimicrobial activity evaluation. As a part of a research program directed at the isolation of active agents from plants grown in Sichuan Province, this paper describes the preliminary investigations on the antimicrobial activity of the extracts.

Material and Methods

Extracts preparation

All herbs: (Figure 1) and magnolol (98%) were gained from Sichuan Sanxingdui Pharmaceuticals Co., Ltd.

Preparation of extract from *Radix Sophorae Flavescentis*: Raw roots of *Radix Sophorae Flavescentis* (5.0 g) were wetted with 10.0 mL of 2.0% ammonium hydroxide for 4 h. Then the wet roots were crushed by a high-speed disintegrator. The crushed *Radix Sophorae Flavescentis* was extracted three times with 10.0 mL of the mixture of chloroform and methanol (5:5, v/v) successively. The combined extracts were evaporated to dryness under reduced pressure.

Preparation of extract from *Gentiana scabra* Bunge: Pulverized herbal *Gentiana scabra* Bunge was extracted with the appropriate amount of 50% aqueous ethanol (being capable of immersing the solid sample) by stirring at room temperature for 30 min, then centrifugated at a speed of 4000 rpm for 10 min. Extraction was repeated three times. The extracts were combined and filtered through a 0.45 mm cellulose acetate membrane filter. The final combined extracts were evaporated to dryness under reduced pressure.

Preparation of extract from *Artemisia annua* Linn: The air-dried powdered herbs of *Artemisia annua* Linn (5 kg) were extracted three times with methanol under refluxing. The resultant extract was combined and concentrated under reduced pressure to afford the residue.

Preparation of extract from *Polygonum bistorta* L.: The plants were finely powdered and 100 g of powder was extracted with 70% aqueous ethanol (500 mL) using a Soxhlet extraction apparatus. The extracts were filtered hot using a Whatman No. 1 filter paper and then concentrated under vacuum (40-60 °C) and finally freeze-dried to yield extract.

Preparation of extract from *Ilex chinensis* Sims: The fresh leaves of *Ilex chinensis* Sims (2 kg) were extracted three times with MeOH (50 L) at room temperature for a week, and the solvent was removed under reduced pressure.

Preparation of extract from *Prunella vulgaris*: The dry inflorescence (250 g) was washed with water and then soaked in distilled water (2.5 L) in two 5.0 L flasks and boiled under refluxing for 2 h. The mixture was cooled to ambient temperature, left to be separated, and the aqueous extract was filtered through a Whatman No. 1 filter paper to give 1.6 L of clear solution. Freeze drying of 200 ml of this extract gave a fibrous dark brown residue (2.20 g) accounting for 7.1% of the total dry weight of the herb.

Preparation of extract from *Agrimonia pilosa* Ledeb: The dried leaves of *Agrimonia pilosa* Ledeb (10 kg) were cut into small pieces and extracted three times with 80% MeOH at room temperature for 7 days. The MeOH extract were filtered and concentrated under reduced pressure.

Preparation of extract from *Chrysanthemum indicum*: Dried flowers of *Chrysanthemum indicum* (5.8 kg) were finely cut and extracted with methanol under refluxing. Evaporation of the solvent under reduced pressure gave the MeOH extract (1650 g, 28.4%).

Preparation of extract from *Artemisia capillaris* Thunb: The flowers of *Artemisia capillaris* Thunb were collected and crushed with a grinder, 0.1993 g was extracted in 30 ml methanol for 30 min, and the extract evaporated was dried using nitrogen gas.

Preparation of extract from *Epimedium brevicornum Maxim*: The dried powder of *Epimedium brevicornum Maxim* (30 g) was refluxed three times for 1.5 h with 75% ethanol. The extracted solutions were combined, ethanol was removed under reduced pressure to give extract.

Preparation of extract from *Hottuyia cordata* Thunb: *Hottuyia cordata* Thunb 50% ethanol extracts (yield: 6.73% of dry wt.) were obtained by 48 h maceration at room temperature. The ethanol extract was filtered through a 0.45µm filter, lyophilized and kept at 4°C.

Preparation of extract from *Violaphilippica* Cav: Air-dried and powdered plant material of *Violaphilippica* Cav. (874 g) was extracted with 1:1 CH₂Cl₂/MeOH at room-temperature and filtered through a cotton wool plug, CH₂Cl₂ and MeOH were removed under reduced pressure.

Preparation of extract from *Gardenia jasminoides* Ellis: Stir-baked *Gardenia jasminoides* Ellis (1 kg) was boiled in water three times, and the resulting decoctions pooled. This solution was then clarified by centrifugation and filtration, lyophilized and kept at 4°C.

Preparation of extract from *Astragalus membranaceus* bunge: The fresh plant material (300 g) was extracted twice with water (2 L) for 2.5 h at 100°C. The combined extracts were con-

centrated to 250 mL using a rotary evaporator at 65°C under vacuum. The proteins in the extract were removed by Savage reagent. After removal of the Savage reagent, 100 mL of anhydrate ethanol was added before the mixture was maintained overnight at 4°C to precipitate polysaccharides. The crude polysaccharides (25 g) was obtained by centrifugation at a speed of 3860 rpm for 15 min. The herbal extracts included Radix *Sophorae Flavescentis* Extract (2, 98% matrine), [13] *Gentiana scabra* Bunge Extract (3, main ingredients were terpenoids, containing 2.5% gentiopicroside), [14] *Artemisia annua* Linn Extract (4, 99% Artemisinin), [15] *Polygonum bistorta* L. Extract (5, 99% Ginsenoside), [16] *Ilex chinensis* Sims Extract (6, contains the volatile oil, the flavanone, the original catecha phenol and so on), [17] *Prunella vulgaris* Extract (7, 90% prunellin), [18] *Agrimonia pilosa* Ledeb Extract (8, 90% Agrimophol), [19] *Chrysanthemum indicum* Extract (9, contains Buddleog Lucside, Essential oil, Flavone and so on), [20] *Artemisia capillaris* Thunb Extract (10, Capillarisin ,Chlorogenic acid and so on), [21] *Epimedium brevicornum* Maxim Extract (11, 50% Icarin), [22] *Houttuynia cordata* Thunb Extract (12, contains Decanoy acetaldehyde, lauric aldehyde, a-pinene, linlool and so on), [23] *Violaphilippica* Cav. Extract (13, mainly contains organic acid, flavonoids and their glycosides, phenols, sugars, amino acids, peptides and proteins, saponins, phyosterols, tannins and ten other

active ingredients), [23] *Gardeniae jasminoides* Ellis Extract (14, mainly contains gardenoside, crocetin and crocin), [24] Astragali Poly saccharoses (15, 80%). [25] The tested clinical microbes included four representative gram-positive bacteria (*S. aureus*, *S. epidermidis*, *S. lutea* and *Bacillus*), two representative gram-negative bacteria (*E. coli* and *Pseudomonas*)

Bioactivity Assay

All of the extracts were dissolved in dimethylsulfoxide (DMSO), H₂O milliQ and the mixture of DMSO/H₂O milliQ (1:1, v: v) respectively for test. The antimicrobial activity was evaluated in Laboratory for Medicinal Chemistry of Katholieke Universiteit Leuven using a growth-inhibition plate assay. Bacterial cell cultures were grown overnight in Luria Bertani (LB) broth at 37°C. The next morning, 5µL of these cultures were used to inoculate in 5 mL of LB medium. Bacterial cultures were grown at 37°C until OD600 = 0.1 (5×10⁷ cells/mL) and diluted 1/10 (OD600 = 0.01, 5×10⁶ cells/mL). 10 µL of the diluted bacterial cultures was spotted on agar plates containing tested extracts. Controls containing solvent were also given. After overnight incubation of the plates at 37°C, the growth of the bacterial were observed. The MIC value was defined as the lowest concentration of each extract that showed no detectable bacterial growth.

Results

Table 1: The Preliminary Tests of Antibacterial Activity.

		Gram-Positive Bacteria				Gram-Negative Bacteria	
	Extracts	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. lutea</i>	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>E. coli</i>
1	Ia	++	++	++	++	+	+
	IIb	++	++	++	++	++	+
	IIIc	++	++	++	++	++	++
2	Ia	++	++	+	-	-	++
	IIb	++	++	-	-	-	+
	IIIc	++	++	-	-	++	++
3	Ia	-	++	-	-	-	-
	IIb	-	-	-	-	-	-
	IIIc	++	+	++	++	-	+
4	Ia	-	++	-	-	-	+
	IIb	+	++	-	-	++	++
	IIIc	++	++	+	+	-	++
5	Ia	++	++	++	++	-	-
	IIb	++	++	++	++	++	++
	IIIc	++	++	++	++	++	++
6	Ia	++	++	++	++	++	++
	IIb	++	++	++	++	++	++
	IIIc	++	++	++	++	++	++
7	Ia	-	+	-	-	-	++
	IIb	-	++	-	-	-	-
	IIIc	+	+	+	-	++	++

8	Ia	++	++	-	++	++	-
	IIb	++	++	++	++	+	+
	IIIc	++	++	++	++	+	++
9	Ia	-	+	-	-	-	-
	IIb	-	-	-	-	-	-
	IIIc	-	-	++	+	+	++
10	Ia	++	++	++	++	++	++
	IIb	-	++	-	-	++	-
	IIIc	++	++	++	++	++	++
11	Ia	-	-	-	-	-	+
	IIb	-	-	-	-	-	-
	IIIc	-	-	-	-	-	-
12	Ia	++	++	+	++	+	-
	IIb	++	++	++	++	+	++
	IIIc	++	++	+	++	+	+
13	Ia	+	+	++	++	-	-
	IIb	+	+	++	++	-	-
	IIIc	++	+	+	++	++	+
14	Ia	++	++	++	++	++	+
	IIb	++	++	++	++	++	++
	IIIc	++	++	++	++	++	++
15	Ia	-	++	++	-	++	++
	IIb	-	++	+	-	-	+
	IIIc	-	-	++	+	++	++

- a. The extracts were dissolved in H₂O milliQ;
 b. The extracts were dissolved in the mixture of DMSO/H₂O milliQ (1:1, v:v);
 c. The extracts were dissolved in dimethylsulfoxide (DMSO);
 -: no inhibition; +: intermediate inhibition; ++: total inhibition.

The results of the preliminary tests were listed in Table 1. The results exhibited the certain inhibition of all the extracts to tested bacteria's except 9 and 11, which displayed very weak inhibition to the tested bacteria's. Six extracts (2,3,4,7,15) demonstrated low to intermediate inhibition. Seven extracts (5, 6, 8, 10, 12, 13, 14) displayed moderate to strong inhibition to all tested bacteria's. Remarkably, extracts 6 and 14 exhibited total inhibition to most of the tested bacteria both dissolved in water and DMSO. For most

of the tested extracts (3, 4, 5, 7, 8, 9, 12, 13), better inhibition rates were obtained when the extracts were dissolved in DMSO than in H₂O milliQ. Since two herbal extracts (6 and 14) inhibit the growth of the tested bacteria's well. The MIC of these two extracts were tested. The results were showed in Table 2, indicating that 6 and 14 had better activity to gram-positive bacteria (especially *S. aureus* and *S. epidermidis*) than to gram-negative bacteria (*Pseudomonas*, *E. coli*).

Table 2: The MIC (µg/mL) of the Extracts Against Six Bacterias.

Extract	Gram-Positive Bacteria				Gram-Negative Bacteria		
		<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. lutea</i>	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>E. coli</i>
1	Ia	>100	>100	100	100	100	>100
	IIb	50			100		
	IIIc	>100			50		
6	Ia	50	50	>100	>100	>100	>100
	IIb	50	50				
	IIIc	50	50				

14	Ia	10	50	>100	>100	>100	>100
	IIb	10	10	50	50		
	IIIc	10	10	10	50		

The extracts were dissolved in H₂O milliQ;

The extracts were dissolved in the mixture of DMSO/H₂O milliQ (1:1, v:v);

The extracts were dissolved in Dimethyl sulfoxide (DMSO).

Discussion

Four extracts (3, 5, 12, 13) Table 1 displayed stronger inhibition to gram-positive bacteria than to gram-negative bacteria's, while for extract 15, having better inhibition rate for gram-negative bacteria, which means Astragalus membranous Bunge 15 might contains some ingredients specifically for gram-negative bacteria's. Eight extracts (3, 4, 5, 7, 8, 9, 12, 13,) had better inhibition rates when they were dissolved in DMSO than in H₂O milliQ, this might revealed their active ingredients have better solubility in DMSO than in water.

From the information's of MIC for 6 and 14 extracts, Extract 6 displayed strong inhibition (MIC = 50 µg/mL) to gram-positive bacteria (*S. aureus* and *S. epidermidis*) whatever it was dissolved in water or in DMSO, while it showed weak activity (MIC > 100 µg/mL) to gram-negative bacteria's (*Pseudomonas*, *E. coli*). This result might reveal that extract 6 contains some ingredients with good inhibition activity to gram-positive bacteria's. While magnolol had much less effect on *S. epidermidis* (MIC > 100 µg/mL), and it had a little better effect on *S. aureus* (MIC = 50 µg/mL) when it was dissolved in mixture of DMSO/H₂O. Extract 14 exhibited low inhibition (MIC >100 µg/mL) to the tested gram-negative bacteria (*Pseudomonas* and *E. coli*). when 14 was dissolved in DMSO or in the mixture of DMSO/H₂O, it had much excellent antimicrobial activity (MIC = 10 µg/mL) to gram-positive bacteria (*S. aureus* and *S. Epidermidis*) than magnolol (MIC ≥ 50 µg/mL) and 6 (MIC ≥ 10 µg/mL), but activity decreased sharply when it was dissolved in water, this could explains that active ingredients in 14 are more liposoluble.

Through the preliminary antibacterial experiment, we screened out the two Chinese herbal medicine extracts 6 and 14 with the best antibacterial activity. However, the Chinese herbal medicine extract generally contains a variety of effective components, whether one of them plays an antibacterial role or the joint synergistic effect of multi-components, it needs to be further studied. *Ilex chinensis* Sims Extract 6 contains the volatile oil, the flavanone, the original captcha phenol and so on. Specially the flavanone has efficacy of antioxidant [26] and anti-tumor activity [27]. The main components of *Fructus Gardenia* Extract 14 has gardenoside, crocetin and crocin. *Gardenia* has been used in many preparations of Chinese patent medicine with anti-inflammatory effect, [28] anti-metastatic and anti-angiogenic activities, [29] anti-diabetic [30] and so on. The action and mechanism of *gardenia* was preliminary reported [31]. Crocetin and crocin possess hypolipidemic effect [32] and a significant anti-tumor effect both in vitro and in vivo on pancreatic cancer [24]. But there are few studies on antibacterial activity. So, it is meaningful that we separate and

purify the active ingredients and study their activity respectively to discover the lead compounds. Screening of the compounds or their derivatives may be of great importance to develop novel drug for preventing infection of bacteria with low side-effect.

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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