In vitro antimicrobial activity screening of ethanol extracts from 19 common Chinese medicinal herbs: a postprint

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Abstract

To investigate the in vitro antimicrobial activity of ethanol extracts from 19 traditional Chinese medicinal herbs against clinically common pathogenic bacteria. The coarse powder of traditional Chinese medicinal materials was extracted by maceration with 80% ethanol, the extract was vacuum-concentrated to prepare soft extracts, the inhibition zones of the extracts were determined by agar well diffusion method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC/MFC) were determined by microbroth dilution method. The results demonstrated that the screened ethanol extracts from 19 traditional Chinese medicinal herbs exhibited varying degrees of inhibitory effects against different strains. The inhibition zones of ethanol extracts from 14 traditional Chinese medicinal herbs against SA, EC, PA, and CA ranged from 8–27 mm, among which the inhibition zones of Dijincao, Sikuaiwa, Sankezhen, Maweihuanglian, and Tudahuang against SA and EC ranged from 10.3–26.6 mm. The ethanol extracts of Maweihuanglian, Ziran, Dijincao, Guangxi Ezhu, Chuanxinlian, Yimucao, Wuzhuyu, Tudahuang, Yeshanghua, Tulianqiao, Fengweicao, and Sankezhen showed significant antimicrobial activity against both MRSA and drug-resistant Pseudomonas aeruginosa, with MIC/MBC values ranging from 391–6,250 $g \bullet mL^{-1}$. Dijincao and Sankezhen exhibited the lowest MIC values against MRSA at 391 and 781 $g \bullet mL^{-1}$, respectively, and the lowest MIC values against drug-resistant PA were both 1,562.5 $g \bullet mL^{-1}$. The ethanol extracts of Maweihuanglian, Ziran, and Sankezhen had moderate inhibitory effects on drug-resistant Candida albicans, with no significant fungicidal effect. These findings provide a reference for subsequent research on these plants as potential sources of antimicrobial compounds and adjuvants for antimicrobial drugs.

Full Text

Screening of Antimicrobial Activity of Ethanol Extracts from 19 Chinese Herbal Medicines In Vitro

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Abstract: This study investigated the antimicrobial activities of ethanol extracts from 19 Chinese herbal medicines against common clinical pathogens. The crude powders were extracted with 80% ethanol at room temperature, and the extracts were concentrated under reduced pressure to prepare extracts. Antimicrobial activity was screened using the agar diffusion method, and minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined by serial microdilution. The results demonstrated that the ethanol extracts exhibited varying degrees of inhibition against different strains. Fourteen extracts showed inhibition zones of 8–27 mm against *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), and *Candida albicans* (CA). Notably, *Euphorbia humifusa*, *Chloranthus japonicus*, *Berberis sargentiana*, *Thalictrum petaloideum*, and *Rumex madaio* exhibited inhibition zones of 10.3–26.6 mm against SA and EC. Ethanol extracts from *Thalictrum petaloideum*, *Cuminum cyminum*, *Euphorbia humifusa*, *Curcuma kwangsiensis*, *Andrographis paniculata*, *Leonurus artemisia*, *Evodia rutaecarpa*, *Rumex madaio*, *Helwingia japonica*, *Hymenodictyon flaccidum*, *Pteris multifida*, and *Berberis sargentiana* showed significant antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and drug-resistant *P. aeruginosa*, with MIC/MBC values ranging from 391 to 6,250 $g \cdot mL^{-1}$. *Euphorbia humifusa* and *Berberis sargentiana* demonstrated the lowest MIC values against MRSA (391 and 781 $g \cdot mL^{-1}$, respectively) and against drug-resistant PA (both 1,562.5 $g \cdot mL^{-1}$). Ethanol extracts from *Thalictrum petaloideum*, *Cuminum cyminum*, and *Berberis sargentiana* showed moderate inhibitory effects against drug-resistant *C. albicans*, though fungicidal activity was not evident. These findings provide a reference for further investigation of these plants as potential sources of antibacterial compounds and antimicrobial adjuvants.

Keywords: Chinese herbal medicines, pathogens, antibacterial activity, minimum inhibitory concentration, minimum bactericidal/fungicidal concentration

Introduction

The discovery of penicillin by Alexander Fleming in 1929 ushered in the golden age of antibiotics, yet this glory lasted merely four decades. The primary driver

of resurgent infectious diseases is the dramatic increase in microbial multidrug resistance due to the irrational use of antimicrobial agents. Pathogen resistance to antibiotics can lead to clinical treatment failure and increased mortality. Identifying novel antimicrobial agents and resistance-modifying agents that can block or reverse microbial resistance has emerged as a major research focus worldwide, offering a promising solution to the resistance crisis (Pages et al., 2011; Reens 2018; Mouwakeh et al., 2019). Traditional Chinese medicine has long been used to treat infectious diseases (Mohanta et al., 2012; Li and Zhu, 2013; Mohanta et al., 2014; Zhang et al., 2017; Jiang et al., 2019). Compared with synthetic antibiotics, bioactive compounds derived from plants offer tremendous therapeutic potential with fewer side effects, making the development of effective and safe natural products to control multidrug-resistant (MDR) pathogens an urgent priority.

Numerous studies have reported the antimicrobial screening of Chinese herbal medicines. Panda et al. (2016) evaluated the antimicrobial activity of extracts from different parts of 222 plant species, demonstrating inhibitory effects against both Gram-positive and Gram-negative bacteria. Pauw and Eloff (2014) randomly screened hundreds of South African tree species for antimicrobial activity to identify potent inhibitors. Li (2017) investigated the antifungal activity of different parts of *Euphorbia humifusa* against *Trichophyton rubrum*, finding that the ethanol extract showed superior efficacy compared to fractionated components. Zhang et al. (2008) examined the inhibitory effects of *Curcuma kwangsiensis* oil against six phytopathogenic fungi, revealing strong antifungal activity. However, most plant-based antimicrobial studies remain preliminary, and reports on activity against resistant strains are scarce. Building on our group' s previous work and leveraging China' s biodiversity, combined with phytochemical taxonomic considerations, this study evaluated the in vitro inhibitory effects of 19 Chinese herbal medicines against four standard strains (*S. aureus*, *E. coli*, *C. albicans*, *P. aeruginosa*) and their drug-resistant counterparts (MRSA, drug-resistant *P. aeruginosa*, and drug-resistant *C. albicans*). The objective was to identify promising herbal candidates for further chemical investigation, active component tracking, isolation, and structural identification of lead compounds, thereby providing scientific evidence to help alleviate bacterial resistance.

1.1 Materials and Reagents

1.1.1 Herbal Materials: The following 19 medicinal herbs were purchased from the Luosifen Traditional Chinese Medicine Market in Kunming, Yunnan, and authenticated by the Phytochemistry Research Center of the Pharmacy Department at the 920th Hospital of PLA Joint Logistic Support Force: *Radix Stemona*, *Asparagus officinalis*, *Thalictrum petaloideum*, *Cuminum cyminum*, *Euphorbia humifusa*, *Andrographis paniculata*, *Curcuma kwangsiensis*, *Leonurus artemisia*, *Chloranthus japonicus*, *Evodia rutaecarpa*, *Taraxacum mongolicum*,

Rumex madaio, *Aster tataricus*, *Euphorbia pekinensis*, *Atractylodes macrocephala*, *Helwingia japonica*, *Hymenodictyon flaccidum*, *Pteris multifida*, and *Berberis sargentiana*. Voucher specimens were retained.

1.1.2 Culture Media and Reagents: Nutrient agar (Beijing Sanyao Technology Development Co., batch No. 180503), Sabouraud agar (Qingdao Hi-Tech Industrial Park Haibo Biotechnology Co., batch No. 20180515), liquid Sabouraud medium (Qingdao Hi-Tech Industrial Park Haibo Biotechnology Co., batch No. 20160822), and nutrient broth (Beijing Sanyao Technology Development Co., batch No. 171110) were used. Reagents included NaCl (Sichuan Xilong Chemical Co., batch No. 20151219), dimethyl sulfoxide (DMSO, Rianlon Bohua (Tianjin) Pharmaceutical Chemical Co., batch No. 20151009), and industrial-grade ethanol (redistilled before use), all purchased from Kunming Fuhaida Chemical Glass Instrument Co.

1.1.3 Test Strains: Standard strains of *Staphylococcus aureus* (ATCC 29213, CMCC(B) 26003), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (CMCC(B) 44102), and *Candida albicans* (ATCC Y0109, ATCC SC5314) were obtained from the National Institutes for Food and Drug Control and Guangdong Huankai Microbial Technology Co. Drug-resistant strains, including *P. aeruginosa* resistant strains (PA 135, PA 204, PA 216, PA 238, PA 244, PA 276, PA 283, PA 294, PA 314, PA 319), methicillin-resistant *S. aureus* (MRSA 8, MRSA 23, MRSA 40, MRSA 82, MRSA 98, MRSA 115, MRSA 128, MRSA 166, MRSA 187, MRSA 331), and *C. albicans* resistant strains (CA 100, CA 152, CA 632, CA 649, CA 819, CA 953, CA 956), were isolated from clinical specimens of severely infected patients at the 920th Hospital and identified by morphological and biochemical methods.

2.1 Preparation of Herbal Extracts

The 19 herbal medicines were ground into coarse powder and extracted with 80% ethanol at room temperature for six cycles: the first extraction lasted 7 days, the second and third lasted 5 days each, the fourth and fifth lasted 3 days each, and the sixth lasted 1 day. Each extraction was filtered through eight layers of gauze, and the filtrates were combined and concentrated under reduced pressure (temperature maintained below 40°C) to obtain extracts. The concentrated extracts were transferred to sterile glass bottles with a spatula and stored at 4°C until use.

2.2 Preparation of Test Solutions and Inocula

Drug Solutions: Fifty milligrams of each extract were weighed into a 2 mL EP tube, and 10% DMSO was added as a solubilizer. The mixture was sonicated to dissolve the drug, then sterile physiological saline was added in a laminar flow hood to prepare a 50 $mg \cdot mL^{1}$ stock solution.

Bacterial and Fungal Inocula: Strains were inoculated onto appropriate agar media (M-H agar for bacteria, Sabouraud agar for fungi) and incubated at 35°C for 20 h. Bacterial suspensions were adjusted to 1.5 × 10 CFU · mL ^{1} using a 0.5 McFarland standard, while fungal suspensions were adjusted to 1.0 × 10 CFU · mL ^{1} using a hemocytometer for drug susceptibility testing and agar diffusion assays. For MIC and MBC/MFC determinations, bacterial suspensions were diluted 300-fold to 5 × 10 CFU · mL ^{1}, and fungal suspensions were diluted 100-fold to 1.0 × 10 CFU · mL ^{1}.

2.3.1 Agar Diffusion Assay for Inhibition Zones

Agar plates were punched with 6 mm wells (five wells per plate). Bacterial suspensions (1.5 × 10 CFU · mL ^{1}) were evenly spread onto M-H agar plates using sterile swabs, while fungal suspensions (1.0 × 10 CFU · mL ^{1}) were spread onto Sabouraud agar plates. Fifty microliters of the 50 mg · mL ^{1} drug solution were added to each well without overflow. Plates were incubated at 35°C for 20 h, and inhibition zone diameters were measured with calipers. Experiments were performed in triplicate, and mean values were calculated. According to pharmacological testing guidelines: <10 mm indicates resistance or no effect; 10 mm indicates mild sensitivity; 11–15 mm indicates moderate sensitivity; 16 mm indicates high sensitivity. Antibiotic inhibition zones were evaluated according to Clinical and Laboratory Standards Institute (CLSI) criteria.

2.3.2 Determination of MIC and MBC/MFC

MIC and MBC/MFC were determined by serial microdilution in liquid medium (Hu et al., 2018), following previously described procedures.

3.1 Inhibition Zone Results for Herbal Ethanol Extracts

Inhibition zones were measured by agar diffusion (Table 1). Against standard *S. aureus*, *Thalictrum petaloideum*, *Euphorbia humifusa*, and *Berberis sargentiana* showed high sensitivity with zones >16 mm, indicating strong inhibition. *Andrographis paniculata*, *Leonurus artemisia*, *Rumex madaio*, *Helwingia japonica*, *Hymenodictyon flaccidum*, and *Pteris multifida* exhibited mild to moderate sensitivity with zones of 10–16 mm. Against standard *E. coli*, *Euphorbia humifusa*, *Chloranthus japonicus*, *Berberis sargentiana*, and *Rumex madaio* showed mild to moderate sensitivity (10–16 mm). Against standard *P. aeruginosa*, *Chloranthus japonicus*, *Aster tataricus*, *Cuminum cyminum*, *Leonurus artemisia*, *Pteris multifida*, and *Thalictrum petaloideum* showed mild sensitivity (10 mm). Against standard *C. albicans*, *Berberis sargentiana* demonstrated high sensitivity (16 mm), while *Chloranthus japonicus* and *Thalictrum petaloideum* showed mild to moderate sensitivity (10–16 mm).

Note: "–" indicates no inhibition zone.

3.2 MIC and MBC/MFC Determination Results

Based on inhibition zone screening, selected herbs were tested against standard and resistant strains by microdilution. Negative controls showed robust bacterial growth, excluding interference from 10% DMSO, while blank controls remained sterile, confirming proper aseptic technique.

Standard Strains: Ethanol extracts from 17 herbs exhibited varying inhibitory effects against four standard bacterial strains, with MIC/MBC values primarily ranging from 3,125 to 12,500 g · mL ^{1} (Table 2). *Cuminum cyminum* showed inhibitory activity against two standard *C. albicans* strains with MIC/MFC values of 3,125–12,500 g · mL ^{1}.

Resistant Strains: Extracts from *Thalictrum petaloideum*, *Cuminum cyminum*, *Euphorbia humifusa*, *Curcuma kwangsiensis*, *Andrographis paniculata*, *Leonurus artemisia*, *Evodia rutaecarpa*, *Rumex madaio*, *Helwingia japonica*, *Hymenodictyon flaccidum*, *Pteris multifida*, and *Berberis sargentiana* demonstrated significant antimicrobial activity against MRSA and drug-resistant *P. aeruginosa*, with MIC/MBC values of 391–6,250 g · mL ^{1} (Tables 3 and 4). *Euphorbia humifusa* and *Berberis sargentiana* showed the lowest MIC values against MRSA (391 and 781 g · mL ^{1}, respectively) and against drug-resistant *P. aeruginosa* (both 1,562.5 g · mL ^{1}). Against drug-resistant *C. albicans*, extracts from *Thalictrum petaloideum*, *Cuminum cyminum*, and *Berberis sargentiana* exhibited moderate inhibition without clear fungicidal effects (Table 5).

Note: "–" indicates no activity. The same below.

Discussion

Our results demonstrate that extracts from *Thalictrum petaloideum*, *Euphorbia humifusa*, *Andrographis paniculata*, *Leonurus artemisia*, *Rumex madaio*, *Hymenodictyon flaccidum*, and *Pteris multifida* produced inhibition zones at concentrations 50 mg · mL ^{1} against standard strains, with MIC values ranging from 391 to 6,250 g · mL ^{1}. Notably, half of the tested plant extracts showed antimicrobial activity at MIC 6,250 g · mL ^{1}. *Euphorbia humifusa* exhibited the lowest MIC against MRSA (391 g · mL ^{1}) and drug-resistant *P. aeruginosa* (1,562.5 g · mL ^{1}). *Berberis sargentiana* showed strong activity against MRSA and drug-resistant *P. aeruginosa* (MIC 781–3,125 g · mL ^{1}) and moderate inhibition of drug-resistant *C. albicans* (MIC 3,125 g · mL ^{1}).

Literature reports indicate that *Thalictrum petaloideum*, *Cuminum cyminum*, *Euphorbia humifusa*, *Curcuma kwangsiensis*, *Andrographis paniculata*, *Leonurus artemisia*, *Evodia rutaecarpa*, *Rumex madaio*, *Helwingia japonica*, *Hymenodictyon flaccidum*, *Pteris multifida*, and *Berberis sargentiana* contain primarily terpenoids, phenolics, and alkaloids. The promising antimicrobial activity observed in our study aligns with Zacchino et al. (2017), who reported that natural

low-molecular-weight compounds enhancing antimicrobial activity are concentrated in phenolic and terpenoid classes. These findings provide a valuable reference for selecting plant species as potential antibacterial compounds and adjuvants.

Interestingly, extracts from *Aster tataricus*, *Atractylodes macrocephala*, and *Taraxacum mongolicum* showed no inhibition zones in agar diffusion but exhibited sensitivity at MIC 3,125 $g \cdot mL^{1}$ in broth dilution, suggesting that inhibition zone size does not always correlate with MIC values. This discrepancy may arise from the complex composition of herbal extracts, differential solubility of components, and distinct mechanisms of action—for instance, phenolics strongly bind macromolecules, while terpenoids, being lipophilic, efficiently penetrate cell walls. Variations in sensitivity between strains of the same species likely reflect differences in plant composition and strain-specific resistance mechanisms. Unlike single-target synthetic drugs, Chinese herbal medicines contain compound groups with interconnected components that induce complex biochemical changes in cells, exhibiting holistic dose-effect relationships rather than simple single-target actions (Han et al., 2016). Additionally, test strains possess varying intrinsic tolerance levels to antimicrobial agents.

Combination therapy involving natural products and conventional antimicrobials offers highly promising prospects for novel drug regimens in combating bacterial and fungal resistance (Zacchino et al., 2017). As "green antibiotics" from traditional medicine enter a new era, fundamental challenges remain: resistance mechanisms are unclear, material basis studies are insufficient, molecular-level research is limited, and targets are poorly defined. Before further development and commercialization, safety evaluation of plant extracts through animal and human studies to determine efficacy in whole organisms is essential. Research on antimicrobial resistance in Chinese herbal medicine remains in its infancy, with many questions awaiting investigation.

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Note: Figure translations are in progress. See original paper for figures.