In Vitro Antibacterial Activity Screening of 23 Chinese Herbal Medicines: Postprint

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Abstract

This study investigated the in vitro antimicrobial activity of 80% ethanol extracts from 23 traditional Chinese medicinal herbs against four clinically common pathogenic microorganisms. The agar diffusion method was used to determine the diameter of inhibition zones, while the micro-broth dilution method was employed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). The results showed that 16 extracts, including Gentiana rigescens, Hypericum patulum, and Isodon lophanthoides, exhibited strong antibacterial activity against Staphylococcus aureus with MIC/MBC values ranging from 0.19 to 3.12 mg · ml ^{1}. Fourteen extracts, including Polygonum capitatum, Lophatherum gracile, and Scutellaria barbata, demonstrated relatively strong antibacterial activity against Pseudomonas aeruginosa with MIC/MBC values between 1.56 and 6.25 mg · ml ^{1}. Except for Sophora japonica fruit, all other extracts showed relatively strong antibacterial activity against Escherichia coli with MIC/MBC values ranging from 3.12 to 12.5 mg · ml ^{1}. Against Candida albicans: Fibraurea recisa and Agastache rugosa extracts exhibited relatively strong antifungal activity with MIC/MFC values between 0.78 and 6.25 mg · ml ^{1}; extracts of Gentiana rigescens, Hypericum patulum, Adina rubella, Sophora flavescens, Piper nigrum, Penthorum chinense, Piper longum, and Lophatherum gracile had MIC/MFC values ranging from 6.25 to 12.5 mg · ml ^{1}, also demonstrating certain antifungal activity. Therefore, all selected traditional Chinese medicinal herbs exhibited good antimicrobial effects, with most possessing broad-spectrum antimicrobial activity. Among them, Agastache rugosa and Fibraurea recisa with strong antifungal activity against Candida albicans, and Hypericum patulum, Adina rubella, Agrimonia pilosa, Sophora flavescens, Penthorum chinense, and Isodon lophanthoides with strong antibacterial activity against Staphylococcus aureus, may provide valuable references for further investigation of their active monomeric compounds and mechanisms of action.

Full Text

Screening of Antibacterial Activity of 23 Chinese Herbal Medicines In Vitro

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Abstract

This study investigated the in vitro antibacterial activity of 80% ethanol extracts from 23 Chinese herbal medicines against four common clinical pathogens. The agar diffusion method was used to measure inhibition zone diameters, while micro-broth dilution was employed to determine minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). The results demonstrated that 16 extracts, including *Gentiana rigescens*, *Hypericum patulum*, and *Rabdosia serra*, exhibited strong antibacterial activity against *Staphylococcus aureus* with MIC/MBC values ranging from 0.19 to 3.12 mg · ml ^{1}. Fourteen extracts, including *Polygonum capitatum*, *Lophatherum gracile*, and *Scutellaria barbata*, showed strong activity against *Pseudomonas aeruginosa* with MIC/MBC values between 1.56 and 6.25 mg · ml ^{1}. Except for *Sophora japonica*, all extracts displayed moderate to strong activity against *Escherichia coli* with MIC/MBC values of 3.12–12.5 mg · ml ^{1}. Against *Candida albicans*, *Agastache rugosa* and *Daemonorops margaritae* extracts showed strong antifungal activity (MIC/MFC: 0.78–6.25 mg · ml ^{1}), while *Gentiana rigescens*, *Hypericum patulum*, *Geum japonicum*, *Sophora flavescens*, *Piper nigrum*, *Penthorum chinense*, *Piper longum*, and *Lophatherum gracile* exhibited moderate activity (MIC/MFC: 6.25–12.5 mg · ml ^{1}). These findings indicate that the selected herbal medicines possess good broad-spectrum antimicrobial effects. Notably, *A. rugosa* and *D. margaritae* with potent anti-*Candida* activity, and *H. patulum*, *G. japonicum*, *Agrimonia pilosa*, *S. flavescens*, *P. chinense*, and *R. serra* with strong anti-*Staphylococcus* activity warrant further investigation to isolate active monomeric compounds and elucidate their mechanisms of action.

Keywords: Chinese herbal medicine extracts; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Escherichia coli*; *Candida albicans*; in vitro antimicrobial activity

Introduction

Since the discovery of penicillin, nearly ten thousand antibiotics have been identified. However, the irrational use of antibiotics in anti-infective therapy has continuously intensified microbial resistance. Resistant bacteria were first identified in 1945, followed by methicillin-resistant *Staphylococcus aureus* (MRSA) in 1961, vancomycin-resistant *S. aureus* in 2002, and pan-drug resistant bacteria between 1994 and 2016. These developments underscore the escalating crisis of antimicrobial resistance, multidrug resistance (MDR), and pan-drug resistance, which have created therapeutic challenges and, in some cases, left no effective treatment options. Furthermore, resistant strains can emerge concurrently with the introduction of new antibiotics.

Chinese herbal medicine has a long history in treating infections in China, offering advantages such as diverse and complex chemical constituents, low toxicity, multiple targets, and reduced potential for resistance development. Numerous bioactive components with antimicrobial properties have been identified, including flavonoids, alkaloids, organic acids, volatile oils, polysaccharides, saponins, anthraquinones, coumarins, and tannins. Screening Chinese herbs containing these components for antimicrobial activity and tracing their active constituents is therefore crucial for delaying or reversing microbial resistance and developing effective, low-toxicity antimicrobial agents or adjuvants. This study evaluated the in vitro antimicrobial activity of 23 Chinese herbal medicines against standard and resistant strains of *S. aureus*, *P. aeruginosa*, *C. albicans*, and *E. coli* to provide a foundation for subsequent isolation of active compounds and mechanistic studies.

Materials and Methods

1.1 Strains and Materials

1.1.1 Microbial Strains Standard strains including *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC Y0109, ATCC SC5314) were purchased from the National Institutes for Food and Drug Control. *S. aureus* (CMCC(B) 26003) and *E. coli* (CMCC(B) 44102) were obtained from Guangdong Huankai Microbial Technology Co., Ltd. Resistant strains comprising ten MRSA isolates (MRSA8, MRSA15, MRSA23, MRSA40, MRSA42, MRSA82, MRSA166, MRSA187, MRSA202, MRSA440), ten resistant *P. aeruginosa* isolates (PA87, PA120, PA129, PA216, PA244, PA250, PA281, PA307, PA314, PA319), and six resistant *C. albicans* isolates (CA100, CA152, CA649, CA819, CA953, CA956) were clinically isolated and identified by the microbiology laboratory of the 920th Hospital of PLA Joint Logistic Support Force.

1.1.2 Materials and Reagents Culture media including nutrient agar, nutrient broth, Sabouraud dextrose agar, and liquid Sabouraud medium

were purchased from Beijing Solarbio Science & Technology Co., Ltd. and Qingdao Hope Bio-Technology Co., Ltd. Chemical reagents such as sodium chloride and dimethyl sulfoxide (DMSO) were obtained from Kunming Fuhaida Chemical Glass Instrument Co., Ltd. Antibiotic susceptibility discs for bacteria included fluoroquinolones (levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin), -lactams (penicillin, oxacillin, ampicillin, aztreonam), cephalosporins (cefazolin, cefuroxime, ceftazidime, cefoperazone, cefepime), carbapenems (imipenem), cephamycins (cefoxitin), glycopeptides (vancomycin, teicoplanin), fosfomycin, aminoglycosides (tobramycin, amikacin, netilmicin), polymyxin B, macrolides (azithromycin), tetracyclines (minocycline), and combination agents (cefoperazone/sulbactam, piperacillin/tazobactam, clindamycin, rifampicin). Antifungal agents included polyenes (amphotericin B, nystatin), allylamines (terbinafine), azoles (econazole, ketoconazole, clotrimazole, fluconazole, itraconazole, voriconazole), 5-fluorocytosine, and miconazole. All antibiotic discs were provided by the National Institutes for Food and Drug Control.

Herbal materials including *Gentiana rigescens*, *Polygonum capitatum*, *Fallopia multiflora*, *Agrimonia pilosa*, *Hypericum patulum*, and *Verbena officinalis* were collected from Fucun Town, Fuyuan County, Yunnan Province (105°0’ 20” E, 25.5°–35°N, altitude 1,980 m) between August 1–8, 2018. The remaining herbs were purchased from the Luosifen Chinese Herbal Medicine Market in Kunming, Yunnan. All specimens were authenticated by the Natural Medicine Research Center, Department of Pharmacy, 920th Hospital of PLA Joint Logistic Support Force.

1.2 Experimental Methods

1.2.1 Preparation of Herbal Extracts Twenty-three herbs were pulverized into coarse powder. Each sample (80 g) was soaked in 80% ethanol at room temperature for 7, 5, 5, 4, and 3 days sequentially. Each extraction was filtered through eight layers of gauze, and the combined filtrates were concentrated under reduced pressure at 40°C to obtain crude extracts, which were sealed and stored at room temperature.

1.2.2 Determination of Resistance Spectra Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method, with results interpreted according to CLSI guidelines (CLSI:M02-A12, 2017).

1.2.3 Preparation of Drug and Microbial Solutions Drug solutions were prepared by dissolving 50 mg of each herbal extract in 10% DMSO and sterile saline to a final concentration of 50 mg · ml ^{1}. For bacterial and fungal inocula, strains were cultured on appropriate agar plates at 35°C for 24 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. These were used for susceptibility testing and agar diffusion assays. For MIC/MBC determination, 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}.

1.2.4 Agar Punch Method for Inhibition Zones Nutrient agar plates were prepared with five 6-mm diameter wells per plate. Standard bacterial suspensions (1.5×10 CFU·ml ^{1}) or fungal suspensions (1.0×10 CFU·ml ^{1}) were evenly spread onto the agar surface. Each well received 100 µl of herbal extract solution (50 mg·ml ^{1}) without overflow. Plates were incubated at 35°C for 24 h, and inhibition zone diameters were measured with calipers. Experiments were performed in triplicate and results averaged. Interpretation criteria: diameter <10 mm indicated resistance or no activity; 10 mm indicated low sensitivity; 11–15 mm indicated moderate sensitivity; 16 mm indicated high sensitivity.

1.2.5 Determination of MIC and MBC/MFC MIC values were determined by micro-broth dilution according to CLSI guidelines (CLSI:M07-A10, 2017). For MBC/MFC determination, cultures from the three to five wells preceding the MIC value were subcultured onto agar plates and incubated at 35°C for 24 h. The lowest drug concentration yielding <5 colonies on agar was defined as the MBC/MFC. All experiments were performed in triplicate.

Results

2.1 Resistance Spectra of Resistant Strains

The antimicrobial susceptibility patterns of resistant strains are summarized in Tables 1–3 . MRSA isolates showed resistance to multiple antibiotic classes including -lactams, macrolides, and fluoroquinolones, while remaining susceptible to glycopeptides (vancomycin, teicoplanin) and some aminoglycosides. Resistant *P. aeruginosa* isolates exhibited extensive resistance to cephalosporins, carbapenems, and fluoroquinolones, with variable susceptibility to polymyxin B and aminoglycosides. Resistant *C. albicans* strains demonstrated resistance to azoles and 5-fluorocytosine, but remained susceptible to amphotericin B and nystatin.

2.2 Inhibition Zone Determination

Inhibition zone results are presented in Table 4 . Against standard *S. aureus* strains, extracts of *Agrimonia pilosa*, *Polygonum capitatum*, *Rabdosia serra*, *Agastache rugosa*, and *Penthorum chinense* produced zones >16 mm, indicating high sensitivity. *Rauvolfia verticillata*, *Sophora japonica*, *Gentiana rigescens*, *Hypericum patulum*, *Verbena officinalis*, *Fallopia multiflora*, *Geum japonicum*, *Sophora flavescens*, and *Uncaria rhynchophylla* showed moderate activity (11–15 mm). Against standard *E. coli* and *P. aeruginosa*, extracts of *Gentiana rigescens*, *Hypericum patulum*, *Fallopia multiflora*, *Geum japonicum*, *Agrimonia pilosa*, *Sophora flavescens*, *Penthorum chinense*, *Lophatherum gracile*, *Agas-*

tache rugosa, and *Scutellaria barbata* exhibited moderate sensitivity (11–15 mm). For standard *C. albicans*, *Agastache rugosa*, *Hypericum patulum*, *Lophatherum gracile*, and *Daemonorops margaritae* showed moderate activity (11–15 mm). Most other extracts displayed low sensitivity or no activity.

2.3 MIC and MBC/MFC Determination

Sterile blank controls confirmed absence of contamination, and negative controls (10% DMSO) showed no inhibitory effect, validating the experimental reliability. Against standard and MRSA *S. aureus* strains (Table 5 and Table 6), 16 extracts including *Gentiana rigescens*, *Hypericum patulum*, and *Rabdosia serra* demonstrated strong antibacterial activity with MIC/MBC values of 0.19–3.12 mg · ml ^{1}. Seven extracts (*Rauvolfia verticillata*, *Sophora japonica*, *Daemonorops margaritae*, *Trollius chinensis*, *Piper nigrum*, *Uncaria rhynchophylla*, *Piper longum*) showed moderate activity (MIC/MBC: 6.25–12.5 mg · ml ^{1}).

Against standard and resistant *P. aeruginosa* (Table 5 and Table 7), 14 extracts including *Rauvolfia verticillata*, *Gentiana rigescens*, *Daemonorops margaritae*, *Hypericum patulum*, *Verbena officinalis*, *Fallopia multiflora*, *Geum japonicum*, *Crotalaria ferruginea*, *Agrimonia pilosa*, *Sophora flavescens*, *Agastache rugosa*, *Polygonum capitatum*, *Lophatherum gracile*, and *Scutellaria barbata* exhibited strong activity (MIC/MBC: 1.56–6.25 mg · ml ^{1}). Most remaining extracts showed moderate activity (6.25–12.5 mg · ml ^{1}), except *Piper longum* which was inactive at 12.5 mg · ml ^{1}.

For standard *E. coli* (Table 5), all extracts except *Sophora japonica* showed moderate activity with MIC/MBC values of 3.12–12.5 mg · ml ^{1}. Against standard and resistant *C. albicans* (Table 5 and Table 8), *Daemonorops margaritae* and *Agastache rugosa* displayed strong antifungal activity (MIC/MFC: 0.78–6.25 mg · ml ^{1}), while *Gentiana rigescens*, *Hypericum patulum*, *Geum japonicum*, *Sophora flavescens*, *Piper nigrum*, *Penthorum chinense*, *Piper longum*, and *Lophatherum gracile* showed moderate activity (MIC/MFC: 6.25–12.5 mg · ml ^{1}).

Discussion

The results demonstrate that most herbal extracts, particularly *Agrimonia pilosa* and *Hypericum patulum*, exhibit inhibitory activity against both standard and resistant strains. Notably, *Gentiana rigescens*, *Daemonorops margaritae*, *Hypericum patulum*, *Geum japonicum*, *Sophora flavescens*, *Agastache rugosa*, *Piper nigrum*, *Penthorum chinense*, *Piper longum*, and *Lophatherum gracile* show inhibitory effects against all four tested pathogens, indicating broad-spectrum antimicrobial activity.

Interestingly, some extracts such as *Sophora japonica* and *Trollius chinensis* showed no obvious inhibition zones but demonstrated measurable MIC/MBC

values (primarily 6.25–12.5 mg · ml [1]). This discrepancy may arise from the diffusion characteristics of the agar punch method, where drug components diffuse radially from the well but only contact the basal layer of the bacterial lawn, potentially limiting interaction area. In contrast, the broth dilution method allows complete mixing of drug and microorganism, enabling full expression of antimicrobial effects. Additionally, some extracts (*Humulus lupulus*, *Buddleja officinalis*, *Rabdosia serra*) showed better activity against certain MRSA isolates (e.g., MRSA8, 23, 166) than against standard strains, possibly reflecting differences in resistance mechanisms among strains and the multifaceted mechanisms of herbal components, which may include inhibition of protein and nucleic acid synthesis, disruption of cell membranes/walls, and enzyme inhibition.

In an era where resistance development outpaces antibiotic discovery, researchers have identified novel anti-resistance strategies including bacteriophages, antimicrobial peptides, probiotics, and herbal monomers. Combination therapy represents a particularly effective approach. For instance, sophoraflavanone G synergizes with ampicillin against MRSA, gallic acid enhances -lactam activity by binding peptidoglycan, piperine shows synergy with gentamicin, and psychorubrin demonstrates additive effects with chloramphenicol. While most combination studies are limited to in vitro testing, challenges remain in replicating the dual effects on host and pathogen observed in vivo. Furthermore, highly active compounds may fail to achieve therapeutic plasma concentrations or may undergo significant first-pass metabolism, potentially limiting in vivo efficacy. Therefore, enhanced research on pharmacokinetics and mechanisms of antibiotic-herb combinations is essential to develop reliable therapeutic regimens.

The herbal extracts evaluated in this study demonstrate significant antimicrobial activity, particularly *Agastache rugosa* and *Daemonorops margaritae* against *C. albicans*, and *Hypericum patulum*, *Geum japonicum*, *Agrimonia pilosa*, *Sophora flavescens*, *Penthorum chinense*, and *Rabdosia serra* against MRSA. These promising extracts warrant further investigation to isolate novel antimicrobial monomers and evaluate their efficacy and mechanisms, both alone and in combination with conventional antibiotics, with the ultimate goal of developing effective therapeutic strategies to shorten treatment duration for resistant infections.

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