Differences in Antibacterial Activity of Eight Medicinal and Edible Traditional Chinese Medicines Produced in Zhaoqing

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Abstract [**Objectives**] To study the differences in antibacterial activity of eight medicinal and edible traditional Chinese medicines Produced in Zhaoqing. [**Methods**] This study selected eight common medicinal and edible traditional Chinese herbs from Zhaoqing region (*Centipeda minima*, *Lonicera japonica*, *Vitex negundo*, *Plantago asiatica*, *Houttuynia cordata*, *Hedyotis diffusa*, *Hylocereus undatus*, and *Bombax ceiba*) to compare their antibacterial activity differences through *in vitro* antibacterial experiments, and explored the effects of extraction methods and different solvents. For *H. undatus* and *B. ceiba*, the antibacterial effects of decoction and ultrasound-assisted extraction on *Escherichia coli* and *Staphylococcus aureus* were compared. For *H. diffusa* and *H. cordata*, three different solvents (n-butanol, ethyl acetate, and dichloromethane) were used for extraction to analyze the influence of polarity on antibacterial activity (inhibition zone). The remaining four herbs were directly compared for the inhibitory differences of their crude extracts against Gram-positive bacteria. [**Results**] (i) The ultrasound-assisted extracts of *H. undatus* and *B. ceiba* exhibited significantly superior antibacterial effects compared to traditional decoction. (ii) The n-butanol extract of *H. diffusa* showed 7.5% and 4.5% higher inhibition rate against *S. aureus* than the ethyl acetate and dichloromethane extracts, respectively. The ethyl acetate extract exhibited weak inhibitory effects on *E. coli*, while extracts from other solvents showed no inhibition. The ethyl acetate extract of *H. cordata* demonstrated good inhibitory effects against both bacterial strains and outperformed the extracts of *H. diffusa*. (iii) The crude extracts of *C. minima*, *L. japonica*, *V. negundo*, and *P. asiatica* all exhibited good inhibitory effects against *S. aureus*, with *C. minima* showing the strongest antibacterial activity. Correlation analysis revealed a positive relationship between antibacterial effects and extract concentration. [**Conclusions**] This study provides optimization strategies for the differential extraction and antimicrobial applications of medicinal and edible herbs.

Key words Medicinal and edible herbs, Traditional Chinese medicine, Antibacterial, Differential analysis

1 Introduction

With the increasing severity of antibiotic misuse, the global demand for novel antimicrobial agents continues to rise[1]. Chinese herbal medicine has garnered growing attention and recognition for its unique pharmacological mechanisms and lower resistance potential[2]. The development of plant-derived preservatives holds particular significance for China. Compared with developed nations, China's research in this field remains insufficient, with limited products and patents available. However, the country possesses abundant medicinal plant resources with antibacterial activity, providing a solid foundation for screening plant-based preservatives from traditional Chinese medicines. The primary active antimicrobial components in Chinese herbs include polysaccharides, flavonoids, alkaloids, *etc.* In-depth studies on these compounds have elucidated their pharmacological mechanisms and antimicrobial spectra[3-5]. He Fuyin *et al.*[6] demonstrated that Chinese herbal combinations exhibit significant antibacterial effects. Li Ping *et al.*[7] investigated the bacteriostatic and bactericidal effects of *Lonicera japonica* aqueous extracts and alcohol extracts at varying concentrations against *Staphylococcus aureus*, *Escherichia coli*, and other strains *in vitro*. Li Jihua[8] revealed through antimicrobial tests that *Centipeda minima* extracts showed no activity against *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae*, but exhibited inhibitory effects against other tested strains, with particularly remarkable activity against *S. aureus*.

Research on the antibacterial properties of traditional Chinese medicine (TCM) has opened new avenues for addressing antibiotic resistance[9]. China's abundant medicinal plant resources and centuries of clinical experience provide a solid foundation for developing highly effective, low-resistance plant-derived preservatives[10]. Future efforts should focus on strengthening fundamental research and technological translation to expand the application prospects of TCM-based antimicrobial solutions[11].

2 Materials and methods

2.1 Materials and reagents The following medicinal materials were purchased from commercial sources: *L. japonica*, *P. asiatica*, *C. minima*, and *V. negundo* were obtained from Zhaoqing Dashenlin Pharmacy Co., Ltd. (Duanzhou Helenburg Branch). *O. diffusa* and *H. cordata* were purchased from Zhaoqing University Hengda Pharmacy. *S. undatus* and *B. ceiba* were sourced from Zhaoqing Dongjiang Market. All eight herbal materials were authenticated by professor Teng Xifeng from Guangdong Pharmaceutical University, and voucher specimens were deposited in the storage room of the Pharmaceutical Engineering De-

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partment, Zhaoqing University. 95% ethanol, n-butanol, ethyl acetate, and dichloromethane (all of analytical grade). Shuanghuanglian oral solution was used as the positive control, and DMSO served as the negative control.

2.2 Instruments The following instruments and equipment were used in this study: ultrasonic cleaner (Shanghai Boxun), Rotary evaporator (Tokyo Rikakikai, N-1300V-W), autoclave sterilizer (BXY-30R, Shanghai Boxun Medical & Biological Instruments Co., Ltd.), laminar flow cabinet (Suzhou Antai Air Technology Co., Ltd.), biochemical incubator (Shanghai Pudong Rongfeng Scientific Instruments Co., Ltd.), pipettes, test tubes, conical flasks, beakers, and other glassware were provided by the Pharmaceutical Engineering Laboratory, Zhaoqing University.

2.3 Strains and culture media Microbial strains: *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were provided by the Food Microbiology Laboratory, Zhaoqing University. Preparation of nutrient agar medium: 18 g of beef extract peptone culture medium (containing beef extract, peptone, and sodium chloride), 15 g of agar dissolved in 500 mL of distilled water, sterilized in a high-pressure steam sterilization pot at 121 ℃ for 30 min to obtain a solid culture medium. Transferred to the ultra clean workbench, turned on the purple light lamp and fan, ignited the alcohol lamp for sterilization for 15 min, transferred the solid culture medium to the ultra clean workbench, inverted the solid culture medium in the culture dish, turned on the purple light lamp for sterilization for 15 min, observed whether there are any foreign bacteria growing in the culture dish, took the non foreign bacteria culture dish and prepared it for use.

2.4 Sample preparation using different extraction methods for two medicinal materials

2.4.1 Decoction method. 50 g of fresh sword flower was cut into small pieces. 40 g of the processed sample was accurately weighed and placed in a 500 mL round-bottom flask. Water (320 mL, 1∶8 solid-to-liquid ratio) was added for decoction. The mixture was heated and refluxed for 30 min, with continuous replenishment of evaporated water to maintain volume. After extraction, the solution was filtered, and the filtrate was collected. The residue was re-extracted twice under the same conditions. All filtrates were combined and concentrated to a thick paste under reduced pressure. The concentrated extract was freeze-dried (lyophilized) to obtain the final dried aqueous extract. The extract was stored in a sealed container at −20 ℃ for further use.

2.4.2 Ultrasonic-assisted extraction method. 50 g of fresh sword flower was cut into small pieces. 40 g of processed sample was accurately weighed and placed in a 500 mL round-bottom flask. Added 320 mL distilled water (1∶8 solid-to-liquid ratio). The mixture was subjected to ultrasonic extraction at 60 ℃ for 30 min (40 kHz, 250 W). Evaporated water was replenished during the process to maintain a constant volume. After extraction, the solution was filtered, and the filtrate was collected. The residue was re-extracted twice under identical conditions. All filtrates were combined and concentrated under reduced pressure to obtain a thick paste. The concentrate was freeze-dried (lyophilized) to yield the final dried aqueous extract. The extract was stored in airtight containers at −20 ℃ for subsequent experiments. The extraction method for the water extract sample of cotton flowers is the same as above.

2.5 Sample preparation using different extraction solvents for two medicinal materials Took 50 g of *H. diffusa* sample, cut it into pieces, weighed 40 g into a 500 mL beaker, and added 320 mL of extraction solvent (solid-to-liquid ratio of 1∶8). To prevent solvent evaporation, sealed the mouth of the beaker with a sealing film. After soaking for 2 h, extracted with ultrasonic waves at 40 ℃ for 30 min, filtered and collected the filtrate, operated the same method twice on the filter residue, combine the filtrate, concentrate to liquid extract, freeze dry, and obtain the extract sample of *H. diffusa*. Prepared the extract samples of *H. diffusa* using n-butanol, ethyl acetate, and dichloromethane according to this method. The method for extracting samples of *H. cordata* is the same as above.

2.6 Sample preparation of crude extracts of four medicinal herbs Took 50 g of *L. japonica* sample, cut it into pieces, weighed 40 g into a 500 mL beaker, added 320 mL of ethanol (solid-to-liquid ratio of 1∶8), soaked for 2 h, and extracted with ultrasound at 40 ℃ for 30 min. Filtered and collected the filtrate, operated the filtered residue twice using the same method, combined the filtrate, concentrated to a liquid extract, frozen dry, and obtained the extract sample of *H. diffusa*. The preparation method of ethanol extraction samples for *C. minima*, and *P. asiatica* is the same as above.

2.7 Experimental bacterial culture According to the *S. aureus* culture guidelines (ATCC 25923), *S. aureus* was inoculated into 10 mL liquid medium at a concentration of 10^{-1} CFU/mL. Then, 1 mL of the bacterial suspension was taken and placed in 9 mL liquid medium at a concentration of 10^{-2} CFU/mL. The suspension was then placed in a constant temperature incubator and incubated at 37 ℃ for 24 h. The bacterial suspension was taken and set aside for later use.

E. coli (ATCC 25922) culture protocol. Aseptically inoculated *E. coli* into 10 mL sterile nutrient broth to achieve an initial concentration of 10^{-1} CFU/mL. Transferred 1 mL of the primary suspension into 9 mL fresh sterile broth (1∶10 dilution) to obtain 10^{-2} CFU/mL working suspension. Cultured in a thermostatic shaker incubator at: temperature (37 ± 1) ℃, agitation 150 rpm (if applicable), duration (24 ± 2) h. Visually inspected for turbidity ($OD_{600} \approx 0.6-0.8$), immediately used for subsequent experiments or stored at 4 ℃ ≤24 h if needed.

2.8 Preparation of test and reference solutions

2.8.1 Preparation of test samples for sword flower and *B. ceiba*. Took 4 g of the ultrasonic extract and decoction extract of *H. undatus* in Section **2.4.1**, and placed them in two test tubes. Added 10 mL of dimethyl sulfoxide (DMSO) to each tube, vortexed for 5 min and sonicated (40 kHz, 300 W) for 10 min to ensure complete dissolution. Final concentration: 400 mg/mL (*W/V*, calcu-

lated as crude extract). The preparation of the test solution for *B. ceiba is the same as above*.

2.8.2 Preparation of test samples for *H. diffusa* and *H. cordata*. Accurately weighed 200 mg each of: n-Butanol extract, ethyl acetate extract, Dichloromethane extract (all from Section **2.4.2**), transferred separately into 3 labeled 5 mL centrifuge tubes. Added 2 mL DMSO to each tube to achieve a final concentration of 100 mg/mL. Vortexed for 3 min, followed by ultrasonic bath treatment (40 ℃, 40 kHz, 10 min) for complete dissolution. The preparation of test solutions for different solvents of *H. cordata* is the same as above.

2.8.3 Preparation of four types of medicinal materials for testing high concentration solution (400 mg/mL): accurately weighed 0.8 g of *L. japonica* extract. Dissolved in 2 mL pure DMSO (vortexed for 2 min, sonicated at 40 ℃ for 5 min); medium concentration solution (200 mg/mL): pipetted 1 mL of 400 mg/mL solution. Mixed with 1 mL pure DMSO (vortexed 1 min); Low concentration solution (100 mg/mL): Pipetted 1 mL of 200 mg/mL solution. Mixed with 1 mL pure DMSO (vortexed 1 min). Concentrated Shuanghuanglian into a paste, took 0.8 g of Shuanghuanglian oral liquid extract and dissolved it in 2 mL of pure DMSO to prepare a positive control solution. Took 2 mL of pure DMSO as the negative control solution. The preparation of the other three medicinal materials for testing is the same as above.

2.9 Antibacterial activity experiment of traditional Chinese medicine extract This experiment employed the filter paper disc diffusion method[13] to evaluate the antibacterial activity of traditional Chinese medicine extracts, using *L. japonica* as an example. The following procedures were conducted in a biosafety cabinet: Turned on the UV lamp and blower of the biosafety cabinet, and ignited an alcohol burner. Placed the spreading rod, pipetted, pipette tips, and sterilized filter paper discs into the cabinet for sterilization (15 min). Took the prepared solid culture medium and evenly spread 1 mL of *S. aureus* suspension (at a specified concentration) using the spreading rod. Divided the solid medium into five sections: high concentration test group (400 mg/mL *L. japonica* extract), medium concentration test group (200 mg/mL *L. japonica* extract), low concentration test group (100 mg/mL *L. japonica* extract), positive control group, negative control group. Soaked the sterilized filter paper discs in the respective solutions, then transferred them onto the bacterial-coated medium. Incubated the plates at 37 ℃ in a constant-temperature incubator for 24 h. Measured the diameter of the inhibition zones in each Petri dish. Each antibacterial test for the herbal extract was repeated three times, and the average diameter of the inhibition zones was calculated.

3 Results and analysis

3.1 Antibacterial differences of different extraction methods for two types of floral medicinal materials As shown in Table 1, both herbal extracts exhibited stronger antibacterial effects against *E. coli* when extracted via ultrasonic-assisted extraction compared to water decoction. The maximum inhibition zone diameter for *H. undatus* was 8.17 mm, while that for *B. ceiba* was 7.19 mm, indicating that *H. undatus* had a stronger inhibitory effect on *E. coli* than *B. ceiba*. For *S. aureus*, *B. ceiba* showed a weak inhibitory effect with an inhibition zone of 6.26 mm, whereas *H. undatus* exhibited almost no inhibition. The results suggest that: different extraction methods lead to variations in the antibacterial efficacy of the two herbal extracts. Both flower-derived extracts demonstrated inhibitory effects against Gram-positive bacteria (*S. aureus*), while showing little to no inhibition against Gram-negative bacteria (*E. coli*). Ultrasonic extraction consistently outperformed decoction, likely because heat-sensitive bioactive compounds in flower-based herbs may degrade under high-temperature conditions. This may explain why many floral herbs in traditional medicine are added later in the decoction process to preserve their active components.

Table 1 Differences in antibacterial effects (average inhibition zone diameter) of different extraction methods mm

Bacterial strain	Decoction method		Ultrasonic method		Positive control	Negative control
	Hylocereus undatus	*Bombax ceiba*	*Hylocereus undatus*	*Bombax ceiba*		
Escherichia coli	6.15 ± 0.13	6.95 ± 0.17	8.17 ± 0.21	7.19 ± 0.18	6.50 ± 0.11	–
Staphylococcus aureus	–	6.26 ± 0.11	6.10 ± 0.04	6.23 ± 0.07	6.45 ± 0.13	–

NOTE The numbers in the table represent the mean ± standard deviation of three replicates, and " – " indicates no antibacterial activity. The same below.

3.2 Antibacterial differences of different extraction solvents for two types of herbal medicines As shown in Table 2, the antibacterial effects of both medicinal materials against *S. aureus* followed the trend of n-butanol extract > ethyl acetate extract > dichloromethane extract. The maximum inhibition zone of the n-butanol extract of *H. diffusa* was 7.49 mm, while that of *H. cordata* was 8.98 mm, indicating that *H. cordata* exhibited superior inhibitory effects against *S. aureus* compared to *H. diffusa*. As an extraction solvent, n-butanol demonstrated the best antibacterial performance in both medicinal materials, which may be attributed to its ability to more effectively enrich antibacterial active components such as flavonoids, anthraquinones, and flavonoid glycosides.

Regarding the antibacterial effects against *E. coli*, *H. diffusa* showed only weak inhibition, whereas the ethyl acetate extract of *H. cordata* produced the largest inhibition zone (7.12 mm), indicating its strong inhibitory effect against *E. coli*. The experimental results suggest that both *H. diffusa* and *H. cordata* exhibit optimal inhibitory effects against Gram-positive bacteria. However, for Gram-negative bacteria, *H. diffusa* showed almost no inhibitory effect, while *H. cordata* demonstrated some inhibition, though significantly weaker than its effect on Gram-positive bacteria.

The differences in the antibacterial effects of these two medicinal materials against the two bacterial strains indicate variations in their antimicrobial spectra, highlighting the selective inhibitory effects of different medicinal materials on different bacterial species.

Table 2 Differences in antibacterial effects of different solvent extracts

Sample		Inhibition zone diameter (average) // mm			
		Staphylococcus aureus	*Escherichia coli*	Positive control	Negative control
White *Hedyotis diffusa*	n-butanol	7.49 ± 0.17	6.17 ± 0.08	6.50 ± 0.06	–
	Ethyl acetate	6.96 ± 0.13	6.11 ± 0.06	6.50 ± 0.08	–
	Dichloromethane	7.37 ± 0.16	6.08 ± 0.08	6.50 ± 0.06	–
Houttuynia cordata	n-butanol	8.98 ± 0.26	6.69 ± 0.11	6.49 ± 0.11	–
	Ethyl acetate	7.84 ± 0.21	7.12 ± 0.13	6.52 ± 0.08	–
	Dichloromethane	7.35 ± 0.15	6.69 ± 0.12	6.50 ± 0.06	–

3.3 Differences in antibacterial activity of crude extracts from four medicinal materials As shown in Table 3, all four medicinal materials exhibited strong inhibitory effects against *S. aureus*, demonstrating a concentration-dependent trend, higher concentrations correlated with enhanced antibacterial activity. Among them, the three herbaceous medicinal materials (*C. minima*, *V. negundo*, and *P. asiatica*) showed superior antibacterial activity compared to *L. japonica* (a floral medicinal material). This suggests that herbaceous materials may possess particularly effective inhibitory properties against Gram-positive bacteria, potentially due to their lipophilic components (such as alkaloids and flavonoid glycosides) more readily penetrating bacterial cell walls.

The observed variations in antibacterial efficacy among the different medicinal materials may be attributed to differences in their chemical compositions. The positive correlation between inhibitory effects and extract concentration indicates that optimizing extraction processes to increase the content of active constituents could further enhance antibacterial performance.

Table 3 Differences in the antibacterial activity of alcohol extracts from four medicinal materials against *Staphylococcus aureus*

Sample	Inhibition zone diameter (average) // mm				
	0.4 mL	0.2 mL	0.1 mL	Positive control	Negative control
Centipeda minima (L.) A. Braunet Aschers.	9.56 ± 0.26	8.24 ± 0.16	7.85 ± 0.17	7.80 ± 0.17	–
Lonicera japonica Thunb.	8.01 ± 0.18	7.63 ± 0.13	7.30 ± 0.11	7.57 ± 0.12	–
Vitex negundo var. *cannabifolia* (Sieb. et Zucc.) Hand.-Mazz.	8.50 ± 0.21	7.85 ± 0.20	7.83 ± 0.15	7.65 ± 0.14	–
Plantago asiatica L.; *Plantago depressa* Willd	8.25 ± 0.18	7.35 ± 0.08	7.13 ± 0.08	7.35 ± 0.11	–

4 Conclusions and prospects

4.1 Significant effect of extraction methods on antibacterial efficacy The ultrasound-assisted extracts of *H. undatus* and Bombax ceiba (*B. ceiba*) demonstrated significantly superior antibacterial effects compared to traditional water decoction, indicating that ultrasonic extraction can more efficiently release active components. This method proves particularly suitable for enriching antibacterial compounds from such medicinal materials.

4.2 Significant effect of solvent polarity on antibacterial activity of medicinal herbs The n-butanol extract of *H. diffusa* exhibited optimal inhibitory effects against *S. aureus*, demonstrating 7.5% and 4.5% higher efficacy compared to its ethyl acetate and dichloromethane extracts, respectively. This suggests that its primary antibacterial components are likely medium-polarity compounds.

In contrast, the ethyl acetate extract of *H. cordata* showed strong antibacterial activity against both *S. aureus* and *E. coli*, outperforming *H. diffusa*. These results highlight that: species-specific solvent affinity: Different medicinal herbs require tailored extraction solvents for optimal antibacterial component recovery; broad-spectrum potential: *H. cordata*'s ethyl acetate extract may contain multi-target bioactive substances effective against both Gram-positive and Gram-negative bacteria.

The findings underscore the importance of polarity-guided solvent selection in maximizing the antibacterial potential of medicinal plant extracts.

4.3 Antibacterial activity variation among different categories of crude extracts The crude extracts of *C. minima*, *L. japonica*, *V. negundo*, and *P. asiatica* all exhibited significant inhibitory effects against *S. aureus* (a Gram-positive bacterium). Among them, *C. minima* demonstrated the strongest antibacterial activity, and the three herb-based medicinal materials outperformed the flower-based *L. japonica*. This suggests that differences in chemical composition among different categories of medicinal materials may be the key factor contributing to variations in their antibacterial efficacy.

Facing the serious challenge of antibiotic resistance, there is an urgent societal demand for novel antimicrobial agents, which has led to increasing attention on the antibacterial properties of Chinese herbal medicines[14]. As a vital component of traditional Chinese medicine (TCM), herbal medicines boast a long history and extensive applications. Research on their antibacterial effects holds profound significance for exploring natural antimicrobial resources[15]. This study investigated the differential antibacterial activities of eight medicinal and edible herbs from Zhaoqing, aiming to: explore potential applications of these herbs in antimicrobial products; provide scientific foundations for developing natural preservatives in the food industry; support diversified utilization of

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cells are evaluated by cell culture and other methods to reduce the potential harm of drugs to human body.

5 Conclusions

As an important component of modern drug research and development, small molecule inhibitors play a key role in treating many diseases. With the in-depth study of these inhibitors, although they face numerous technical challenges during process development, such as difficulties in synthesis efficiency, solubility, bioavailability, manufacturing scalability, and side effect control, advances in technology continue to drive continuous improvements in their solutions. The efficiency and quality of small molecule inhibitor development have been significantly enhanced by optimizing synthesis processes, improving solubility and stability, refining manufacturing processes and quality control, and predicting side effects more accurately through computer-aided design. In the future, with the ongoing advancement of new technologies, the R & D of small molecule inhibitors are expected to become more efficient and precise, further ensuring their therapeutic efficacy and safety profiles. Overcoming these technical hurdles not only provides a theoretical foundation for drug R&D but also has far-reaching implications for the practical application of drugs and therapeutic outcomes.

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medicinal herbs; offer references for optimizing the development and use of Zhaoqing's herbal resources.

Through this study, there are following key implications: addressing antibiotic resistance through natural alternatives; bridging traditional knowledge with modern scientific validation; promoting local herb resources for sustainable antimicrobial solutions. The findings will contribute to both pharmaceutical development and food safety innovation while enhancing the value of regional medicinal plants.

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