

Bacteriostatic Effect of Self-designed Chinese Herbal Formula and Its Application in Preparing Traditional Chinese Medicine Acne-removing Soap

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Abstract [Objectives] This study was conducted to investigate the antibacterial effect of a self-designed acne-relieving Chinese herbal formula and prepare a kind of acne-removing soap according to the antibacterial effect. [Methods] Chinese herbs *Lonicera japonica*, *Taraxacum mongolicum*, *Scutellaria baicalensis*, *Angelica dahurica*, *Centella asiatica* and *Aloe vera*, were decocted with water, and the antibacterial effects on *Staphylococcus aureus*, *Staphylococcus albus*, *Candida albicans* and *Propionibacterium acnei* were tested by inhibition zone experiments. The soap base was prepared by saponification of coconut oil, palm oil, hazelnut oil and sodium hydroxide, and then the herbal liquid was added into the soap. [Results] The liquid had bacteriostatic effect on *S. aureus*, *S. albus* and *P. acnes*, but no inhibitory effect on *C. albicans*. The best bacteriostatic effect on *S. aureus* and *S. albus* was achieved at 330 mg/ml, and the best bacteriostatic effect on *P. acnes* was at 160 mg/ml. [Conclusions] The Chinese herbal formula has antibacterial effect. The handmade soap made from the Chinese herbal formula has the characteristics of smooth surface, moderate hardness, fine texture, rich foam and fresh smell.

Key words Chinese medicine formula; Acne; Antibacterial effect; *Staphylococcus aureus*; *Staphylococcus albus*; *Propionibacterium acnes*

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Acne is a kind of chronic inflammation, which often occurs in the sebaceous glands of facial hair follicles. Besides the face, serious acne can also occur on the chest and back, the extensor sides of both upper limbs, buttocks and even the waist. The greatest harm of acne to human skin lies in its post-inflammatory sequelae. Mild cases may resolve with only superficial scars, erythema, or hyperpigmentation, while severe manifestations can lead to various forms of permanent scars. Both atrophic (depressed) and hypertrophic scars can significantly influence aesthetics, profoundly impacting patients' physical and mental health. Acne is particularly challenging due to its chronic nature and high recurrence rate, necessitating prolonged, consistent pharmacological intervention. However, many patients discontinue medicinal treatment prematurely for various reasons, resulting in disease relapse and suboptimal therapeutic outcomes. In response to these clinical challenges, scholars have made significant advancements in developing daily skincare products for acne management. Notable innovations include Acne Removing Cleanser^[1], Acne-eliminating Cream^[2], Pingcuo Cream^[3], Pudan Xiaocuo Facial Mask^[4], and Acne Removing and Cleansing Gel^[5]. These products have demonstrated clinically-validated efficacy in acne treatment.

The *Yellow Emperor's Inner Canon · Shengqi Tongtian Lun* states: "If the human body is invaded by dampness during sweating, it will cause acne and miliaria," and "After sweating during activities, exposure to wind and cold can affect the normal functioning

of the body and comedo. Over time, acne can develop." It demonstrates TCM's understanding of acne pathogenesis as involving both external invasion by wind-cold-damp evil affecting the skin and internal factors including constitutional deficiency and spleen impairment from alcohol/overeating. Western medicine also attributes acne etiology to both internal and external factors. Internally, consumption of excessively spicy, oily or high-sugar foods stimulates sebaceous gland hypersecretion, leading to follicular occlusion by lipids and subsequent inflammatory reactions. Externally, bacterial infections play major roles, with occasional fungal involvement. The bacteria related to acne formation are *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*, and the related fungi are *Candida albicans* and *Malassezia*^[6]. Therefore, in view of the external causes of acne formation, we intended to use antibacterial Chinese herbal medicines for prevention and treatment. By consulting ancient books, we found that the herbs in Wuwei Xiaodu Drink in *The Golden Mirror of Medicine* have the effects of clearing away heat and toxic materials, dissipating and treating sores, and clearing away pathogenic factors, and the formula has a good effect in treating sores caused by the accumulation of heat toxin in the skin. We therefore modified the original formula by removing *Chrysanthemum indicum*, *Viola yedoensis*, and *Malva verticillata*, while retaining *Lonicera japonica* and *Taraxacum mongolicum* for their potent heat-clearing, detoxifying, and abscess-dispersing effects. The optimized formula incorporates *Scutellaria baicalensis* having the effects of expelling toxins and pus and healing sores and promoting muscle growth, *Angelica dahurica* with wind-dispelling, itch-relieving and abscess-expelling effects, and *Centella asiatica*^[7] and *Aloe vera*^[8] effective in tissue regeneration. The six-herb formula was experimentally

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validated for its combined antibacterial, wound-healing, tissue-regenerating and freckle-reducing effects. Upon confirming significant antimicrobial efficacy in laboratory studies, we developed a medicated antibacterial soap with convenient portability and simplified application through scientific integration of the herbal extract with nourishing restorative base oils via saponification.

Materials and Methods

Materials

Experimental materials and reagents Five Chinese herbal medicines, *S. baicalensis*, *A. dahurica*, *L. japonica*, *T. mongolicum*, and *C. asiatica*, were purchased from Dashenlin Pharmacy in Nanning, Guangxi. *A. vera* was collected in Nanning. Coconut oil, palm oil and hazelnut oil were obtained from Weiai Daily Chemicals. Tryptic soy broth (TSB medium), LB medium and analytical-grade sodium hydroxide were acquired from Guangdong Huankai Microbial Technology Co., Ltd.

Major instruments Electronic balance; electric thermostatic blast drying oven; electric thermostatic water bath; autoclave sterilizer; biosafety cabinet; electric thermostatic incubator.

Bacterial strains *S. aureus*, *Staphylococcus albus*, *C. albicans* and *P. acnes* were obtained from the Guangzhou Microbial Culture Collection Center.

Methods

Herbal decoction preparation The dried medicinal materials including *S. baicalensis*, *A. dahurica*, *L. japonica*, *C. asiatica* and *T. mongolicum* were precisely weighed, 50 g each, and soaked in 3 500 ml of purified water for 30 min. The mixture was boiled, and then added with 50 g of fresh *A. vera* and decocted over a small fire for 30 min. The primary decoction was collected through filtration. The residue was added with 1 000 ml of purified water, and the obtained mixture were boiled and decocted over a small fire, yielding the secondary decoction. The two decoctions were merged and concentrated to 150 ml to obtain a herbal liquid with a concentration of 330 mg/ml. The herbal liquid was mixed with TSB medium to prepare following five concentration gradients by a two-fold dilution method: 160, 80, 40, 20, and 10 mg/ml.

Preparation of bacterial suspensions First, 4.50 g of tryptic soy broth (TSB) medium was precisely weighed using an analytical balance and transferred into a 250 ml conical flask. A graduated cylinder was used to add 150 ml of purified water for medium dissolution. The flask was aseptically sealed with sealing film, kraft paper, and rubber bands, and then sterilized in an autoclave at 121 °C for 2 h. After the TSB medium was cooled, the conical flask was opened in a biosafety cabinet. An inoculation loop was employed to pick up a small amount of bacterial strain, and then inserted into the conical flask to mix the strain with the TSB medium. The conical flask was resealed with sealing film, kraft paper, and rubber bands. Next, the flask was placed on a shaker and incubated at 37 °C, 150 rpm for 8 h to obtain a bacterial suspension. After 8 h, a 1 000 µl pipette was employed to transfer 1 ml of the cultured bacterial suspension into a 50 ml centrifuge tube in

a biosafety cabinet. Finally, 19 ml of TSB medium was added to the centrifuge tube to dilute the bacterial suspension 20 times for later use.

Preparation of culture medium First, 3.00 g of LB medium and 2.55 g of agar powder were weighed and added into a 250 ml conical flask together. Next, 150 ml of purified water was measured using a graduated cylinder to dissolve the LB medium and agar powder. The flask was sealed with sealing film, kraft paper, and rubber bands, and then sterilized in an autoclave at 121 °C for 2 h. When the LB medium was cooled to 65 °C, the conical flask was opened in a biosafety cabinet, and the LB medium was evenly poured into petri dishes for plating. After the medium was cooled and solidified, the dishes were sealed and stored at -5 °C for later use.

Preparation of filter paper discs Three blank drug sensitivity filter paper discs (6 mm in diameter) were placed in empty Petri dishes. Inside a biosafety cabinet, 10 µl of herbal solution at a selected concentration was pipetted onto one side of each filter paper disc using a 10 µl micropipette. After air-drying, the discs were flipped with sterile forceps, and another 10 µl of herbal solution was applied to the opposite side of each disc. The Petri dishes were then transferred to an electric thermostatic drying oven to dry the filter paper discs for subsequent use.

Bacterial inoculation A sterile cotton swab was used to collect an appropriate amount of corresponding diluted bacterial suspension prepared in "Preparation of bacterial suspensions", which was then streaked onto the prepared agar plates prepared in "Preparation of culture medium". After the liquid on the plates were visibly absorbed, sterile forceps were employed to place three herbal liquid discs of the same concentration prepared in "Preparation of filter paper discs" evenly on the same inoculated medium. The inoculated plates were subsequently transferred to an electric thermostatic incubator and cultured at 37 °C for 24 h before observation.

Preparation of medicinal soap First, 1.87 g of sodium hydroxide was weighed and added into a 50 ml beaker. Next, 4.36 ml of purified water was measured with a graduated cylinder and added to the beaker, followed by stirring with a glass rod to dissolve the sodium hydroxide. Palm oil (1 ml), coconut oil (2 ml), and hazelnut oil (7 ml)^[9] were measured in a 1 : 2 : 7 ratio and transferred to another 50 ml beaker. The beaker containing the oils was placed in an electric thermostatic water bath and heated at 80 °C for 15 min. The sodium hydroxide solution was then added in small portions while continuously stirring the oil mixture with a glass rod until saponification was achieved. When the oil mixture turned milky white and transitioned from liquid to semi-solid state, 1 ml of medicinal liquid with the concentration of 330 mg/ml concentration was added. The electric thermostatic water bath was then turned off while keeping stirring to evenly distribute the medicinal liquid throughout the saponified mixture using residual heat. The homogeneous saponified product was poured into molds for solidification. After cooling and demolding, an herbal

antibacterial soap with a size of 331 cm was obtained. pH testing with pH paper showed that the soap had a pH of 13.0. After curing in a cool ventilated area for 7 d, the soap was measured to show a pH value of 7.0.

Results and Analysis

Measurement results of inhibition zone diameter

After 24 h of cultivation, the diameters of the inhibition zones were measured using a vernier caliper. The average value of the inhibition zone diameters from three herbal liquid filter paper discs was calculated, and the results are shown in Table 1.

Table 1 Inhibition zone diameters					mm
Concentration of herbal liquid//mg/ml	<i>S. aureus</i>	<i>S. albus</i>	<i>C. albicans</i>	<i>P. acnes</i>	
330	13.9	7.1	6.0	6.0	
160	13.6	6.1	6.0	6.0	
80	10.8	6.0	6.0	6.0	
40	6.0	6.0	6.0	6.0	
20	6.0	6.0	6.0	6.0	
10	6.0	6.0	6.0	6.0	

As shown in the table above, the herbal liquids at concentrations of 330, 160, and 80 mg/ml exhibited antibacterial effect against *S. aureus*, and the results are shown in Fig. 1, Fig. 2, and Fig. 3. The herbal liquids at concentrations of 330 and 160 mg/ml demonstrated antibacterial activity against *S. albus*, as shown in Fig. 4 and Fig. 5, and the inhibitory effect increased proportionally with the concentration of the herbal liquid increasing. No antibacterial effect was observed against *C. albicans* or *P. acnes*.

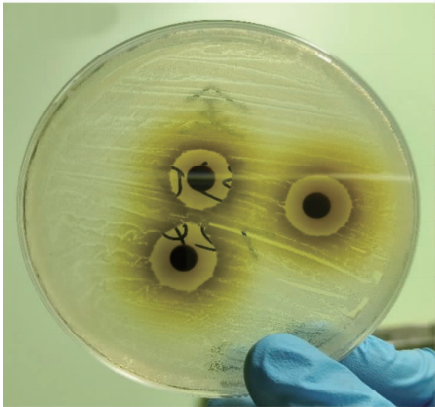


Fig. 1 Antibacterial effect of the 330 mg/ml herbal liquid on *S. aureus*

Measurement results of bacterial colony number

Since the herbal liquid showed no antibacterial effect against *C. albicans* and *P. acnes* in the inhibition zone experiment, the minimum bactericidal concentration (MBC) was determined for these two strains. A 100 μ l aliquot of the highest concentration herbal liquid (330 and 160 mg/ml) was mixed with 100 μ l of corresponding bacterial suspension at a 1 : 1 ratio, followed by streak cultivation on TSB medium plates. Meanwhile, a control group

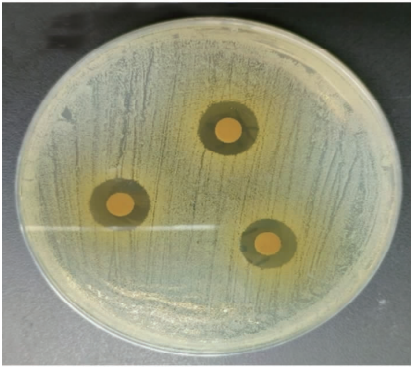


Fig. 2 Antibacterial effect of the 160 mg/ml herbal liquid on *S. aureus*

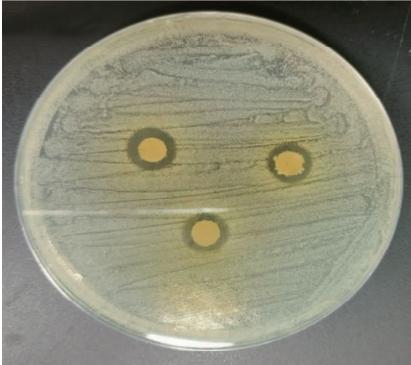


Fig. 3 Antibacterial effect of the 80 mg/ml herbal liquid on *S. aureus*

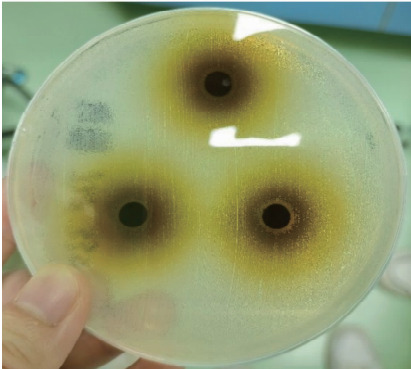


Fig. 4 Antibacterial effect of the 330 mg/ml herbal liquid on *S. albus*

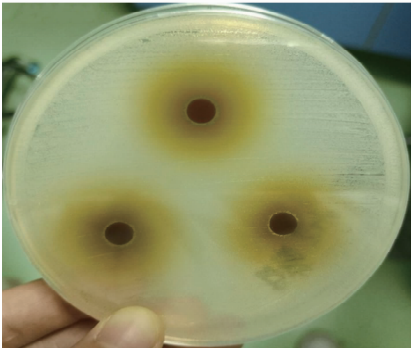


Fig. 5 Antibacterial effect of the 160 mg/ml herbal liquid on *S. albus*

was established by mixing liquid medium with corresponding bacterial suspension. The TSB medium was incubated in an electric thermostatic incubator at 37 °C for 24 h. After 24 h, bacterial growth was observed and colony number were recorded, and the results are shown in Table 2.

The results showed that the herbal liquids at concentrations of 330 and 160 mg/ml exhibited certain antibacterial effect against *P. acnes*, as shown in Fig. 6, Fig. 7 and Fig. 8. However, no inhibitory effect was observed against *C. albicans*.

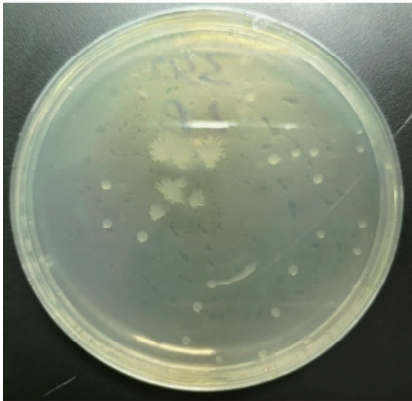


Fig. 6 Number of *P. acnes* colonies cultured with 330 mg/ml herbal liquid

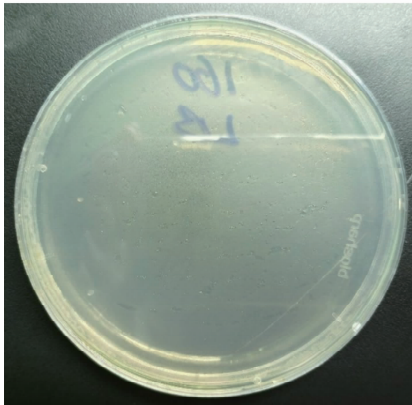


Fig. 7 Number of *P. acnes* colonies cultured with 160 mg/ml herbal liquid

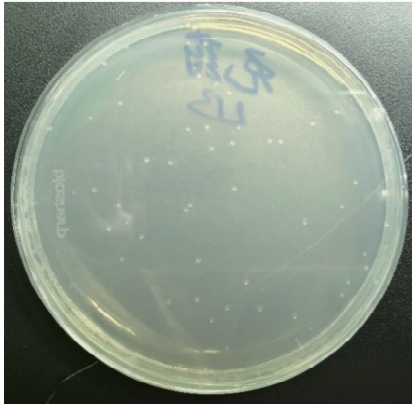


Fig. 8 Number of *P. acnes* colonies cultured with liquid medium

Table 2 Bacterial colony number

Concentration of herbal liquid	Number of <i>P. acnes</i> colonies	Number of <i>C. albicans</i> colonies
330 mg/ml	29	*
160 mg/ml	3	*
Liquid medium	53	*

* indicates that there are too many colonies to count.

Conclusions and Discussion

The experiment demonstrated that the herbal liquid exhibited a significant antibacterial effect against both *S. aureus* and *S. albus*, and the inhibitory effect was enhanced with the concentration increasing. For *P. acnes*, the liquid showed certain antibacterial activity, though the effect was not ideal, but no inhibitory effect was observed against *C. albicans*, which may be attributed to the anaerobic nature of *P. acnes* and the spore-reproduction mechanism of *C. albicans*. Therefore, the traditional Chinese medicine liquid has a certain therapeutic effect on suppurative acne mainly caused by *S. aureus* and *S. albus*.

Currently, with in-depth research on the components and efficacy of Chinese herbal medicine, an increasing number of consumers prefer medicinal products containing natural herbal ingredients. Chinese herbs contain multiple active compounds with complex pharmacological effects, making them applicable for treating various diseases and thus offering a broader therapeutic range. The experimental results demonstrated that the formulated herbal prescription exhibited significant efficacy against both *S. aureus* and *S. albus*, indicating promising potential for developing subsequent antimicrobial products. The herbal liquid has now been successfully prepared into an antibacterial herbal soap, featuring a smooth texture, ideal firmness, rich lather, and quietly-elegant herbal fragrance. The product will help to simplify the treatment process of patients and participate in the treatment as both therapeutic agent and adjunctive care product. Further development may reveal more unique medicinal values.

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