Antibacterial Activity of Gouteng Foot Bath Concentrated Liquid

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Abstract [**Objectives**] To explore the antibacterial activity of Gouteng Foot Bath Concentrated Liquid. [**Methods**] The inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Trichophyton rubrum* was determined using the mycelial growth rate method. The inhibitory effects of different concentrations of Gouteng Concentrated Liquid on these four microbial strains were analyzed. [**Results**] Gouteng Foot Bath Concentrated Liquid showed certain antibacterial activity against the above bacteria, and the antibacterial activity against *S. aureus*, *T. rubrum* and *C. albicans* was more significant. [**Conclusions**] This study provides experimental evidence supporting the application of Gouteng (Uncariae Ramulus Cum Uncis) in antimicrobial foot care products.

Key words Gouteng (Uncariae Ramulus Cum Uncis), *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, Antibacterial activity

0 Introduction

Gouteng (Uncariae Ramulus Cum Uncis) contains extremely extensive medicinal value and enormous potential, demonstrating quite considerable development prospects in both the pharmaceutical and healthcare fields. Over the past decade, China has achieved abundant results in research on the pharmacological effects and clinical applications of Gouteng. Numerous studies have not only deeply analyzed the mechanism of action by which Gouteng treats cardiovascular and cerebrovascular diseases, but have also successfully established the molecular regulatory network for its efficacy. These studies have further revealed the underlying principles of Gouteng's therapeutic effects, laying a solid foundation for its safe and rational clinical use. For instance, Gouteng is rich in various chemical components, mainly including alkaloids, triterpenoids, flavonoids, coumarins, and lignans; these components endow Gouteng with multiple pharmacological effects such as anti-inflammatory, antibacterial, antioxidant, sedative, and hypnotic activities[1]. Among the numerous components contained in Gouteng, rhynchophylline and isorhynchophylline are considered its most critical active ingredients, possessing exceptionally broad pharmacological activities. They exert therapeutic effects against common cardiovascular diseases such as primary hypertension, vasodilation, atherosclerosis, and arrhythmias by inhibiting overexcitation of sympathetic nerves and ganglia, promoting peripheral vasodilation, and suppressing intracellular Ca^{2+} release[2-5]. Gouteng also demonstrates significant efficacy in preventing and treating cerebral infarction, exhibiting anti-inflammatory and antithrombotic effects. Following cerebral infarction, increased IL-6 levels promote platelet aggregation, elevate blood viscosity, and accelerate thrombus formation[6]; however, these levels are effectively reduced and inflammatory responses are attenuated after treatment with Gouteng decoction[7]. Xiao Junfeng *et al.*[8] also confirmed that Gouteng can reduce IL-6 levels, improve blood coagulation in patients, and prevent disease progression. For instance, in patients taking Tianma Gouteng Decoction, indicators including high-cut viscosity (HCV), plasma viscosity (PV), and hematocrit values decreased compared to pre-treatment levels. This change reduced blood viscosity and increased arterial blood flow velocity, thereby effectively improving hemorheological parameters[9-10]. Whole blood viscosity (HCV), plasma viscosity (PV), and fibrinogen (FIB) are all key indicators reflecting hemorheological characteristics. Additionally, rhynchophylline can partially alleviate intestinal flora disorders and optimize the community composition and proportional structure of gut microbiota. After intervention with rhynchophylline, experimental results showed that the relative abundance of *Ruminococcus* significantly decreased in the high-dose rhynchophylline group; while in the low-dose group, the relative abundances of both *Oscillospira* and *Ruminococcus* were significantly reduced[11].

Current research on Gouteng mainly focuses on its antihypertensive, anti-inflammatory, and neuroprotective effects, with limited reports addressing its antibacterial properties. Thus, this study employs Gouteng Foot Bath Concentrated Liquid as the core component to validate its antibacterial activity through *in vitro* experiments, providing theoretical support for the modernization of traditional Chinese medicine in external use.

1 Materials and methods

1.1 Experimental materials The Foot Bath Concentrated Liquid was sourced from Guizhou Taihe Bencao Traditional Chinese Medicine Science & Technology Co., Ltd. The main ingredients include Uncariae Ramulus Cum Uncis, Clematidis Radix Et Rhizoma (Weilingxian), Zanthoxyli Pericarpium (Huajiao), Carthami Flos (Honghua), and Rosae Rugosae Flos (Meiguihua).

(i) Strains: *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Trichophyton rubrum*, all purchased from Shanghai Luwei Technology Co., Ltd.

(ii) Reagents: potato dextrose agar (PDA), Sabouraud dextrose liquid medium, Sabouraud dextrose agar medium, beef extract peptone medium dimethyl sulfoxide (DMSO), Tween-80, nutrient broth (NB), *etc.*

(iii) Equipment: microplate reader, pipette gun, punch,

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ultra-clean workbench, shaker, incubator, *etc.*

1.2 Determination of antibacterial activity

1.2.1 Antibacterial activity of Gouteng Foot Bath Concentrated Liquid against *T. rubrum*. With reference to findings of Chen Yuhuan *et al.*[12], *T. rubrum* was selected as the test strain to study the antibacterial activity of Gouteng Foot Bath Concentrated Liquid. Potato dextrose agar (PDA) solution was prepared, and *T. rubrum* was inoculated onto PDA medium for activation. The Gouteng Foot Bath Concentrated Liquid was dissolved in DMSO and diluted with 0.1% Tween-80 to concentration gradients of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 mg/mL. Equal amounts of DMSO and Tween-80 were used as blank controls. The mycelial growth rate method was employed. The medium was distributed into 50 mL conical flasks, sterilized at 121 ℃ for 20 min, and cooled to approximately 55 ℃ before being poured into petri dishes to solidify. Under sterile conditions, 100 μL of each prepared concentration was added to the sterilized PDA medium, with PDA medium containing DMSO serving as the blank control. Using a 5 mm punch, uniform fungal discs were taken from the edge of the activated test strain medium and transferred to the center of the medicated medium with an inoculating shovel. Each concentration was tested in triplicate and incubated at 28 ℃ in a constant temperature incubator for 5 d. The colony diameter of each medium was measured using the cross-cross method, and the inhibition rate was calculated. The calculation formula is as follows, using Formula (1) to calculate the inhibition rate.

1.2.2 Antibacterial activity of Gouteng Foot Bath Concentrated Liquid against *S. aureus* and *E. coli*. *S. aureus* and *E. coli* strains were activated using beef extract peptone medium. Individual colonies were then selected and cultured in nutrient broth (NB). When the optical density (OD) of the bacterial suspension reached between 0.6 and 0.7, NB was prepared and sterilized for later use. The Gouteng Foot Bath Concentrated Liquid was dissolved in DMSO and then diluted with NB to create concentration gradients of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 mg/mL. In a 96-well plate, test groups containing different concentrations of Gouteng Foot Bath Concentrated Liquid and a blank control group were set up, with six replicates for each group. The plate was incubated in a constant temperature shaker at 36 ℃ and 180 rpm for 24 h. The absorbance (OD_{600}) of each well was measured at 600 nm using a microplate reader. The inhibition rate was calculated using Formula (2).

1.2.3 Antibacterial activity of Gouteng Foot Bath Concentrated Liquid against *C. albican*. *C. albicans* strains were activated using SDA medium. Individual colonies were selected and cultured in SDB. When the OD of the fungal suspension reached between 0.6 and 0.7, SDB was sterilized and prepared for subsequent use. The Gouteng Foot Bath Concentrated Liquid was dissolved in DMSO and then diluted with SDB to create concentration gradients of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 mg/mL. In a 96-well plate, test groups containing different concentrations of Gouteng Foot Bath Concentrated Liquid and a blank control group were set up, with six replicates for each group. The plate was incubated in a constant temperature shaker at 30 ℃ and 180 rpm for 24 h. The absorbance (OD_{600}) of each well was measured at 600 nm using a microplate reader. The inhibition was calculated using Formula (2).

Net colony diameter = Measured colony diameter − Mycelial plug diameter

Inhibition rate (%) = (Control colony diameter − Treated colony diameter)/Control colony diameter × 100 (1)

Corrected *OD* = *OD* of inoculated medium − *OD* of sterile medium

Inhibition rate (%) = (Corrected *OD* of inoculated medium in blank control group − Corrected *OD* of inoculated medium)/Corrected *OD* of inoculated medium in blank control group × 100 (2)

1.3 Data analysis

The experimental data were expressed as mean ± standard deviation, and SPSS 26.0 was used for one-way analysis of variance (ANOVA), with $p < 0.05$ as significant difference.

2 Results and analysis

The results are listed in Table 1. It can be seen that Gouteng Foot Bath Concentrated Liquid had a certain degree of inhibitory activity on *S. aureus*, *E. coli*, *C. albicans* and *T. rubrum*, among which the inhibitory activity on *C. albicans*, *T. rubrum* and *S. aureus* was more significant. The highest bacteriostatic rates were 68.32%, 63.48% and 66.25%, respectively.

Table 1 Inhibitory activity of Gouteng Foot Bath Concentrated Liquid against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Trichophyton rubrum*

Concentration of Gouteng Foot Bath Concentrated Liquid//mg/mL	Inhibition rate//%			
	C. albicans	*E. coli*	*T. rubrum*	*S. aureus*
4	2.23 ± 3.72 e	5.27 ± 1.07 d	10.68 ± 7.33 e	12.55 ± 3.08 e
8	8.59 ± 2.58 de	6.02 ± 1.60 e	34.10 ± 5.78 c	22.65 ± 1.05 d
12	14.41 ± 1.96 d	18.31 ± 1.38 f	16.08 ± 8.28 de	36.77 ± 5.46 c
16	13.00 ± 7.79 d	32.62 ± 9.68 b	19.91 ± 13.31 d	53.27 ± 4.85 b
20	8.66 ± 1.82 de	44.13 ± 2.48 ab	11.99 ± 5.28 e	62.08 ± 2.12 ab
24	14.84 ± 2.87 d	46.69 ± 2.44 a	17.51 ± 3.26 de	61.51 ± 3.43 ab
28	40.40 ± 2.59 c	45.90 ± 7.88 a	39.74 ± 5.68 c	61.4 ± 4.99 ab
32	51.59 ± 1.85 b	48.02 ± 13.89 a	51.09 ± 4.23 b	60.94 ± 2.46 ab
36	57.01 ± 1.19 b	40.63 ± 6.85 ab	56.33 ± 2.73 ab	63.60 ± 2.88 a
40	55.13 ± 1.20 b	41.59 ± 8.32 ab	54.63 ± 2.37 b	66.25 ± 2.81 a
44	58.43 ± 1.65 b	38.19 ± 7.5 ab	57.43 ± 3.8 ab	61.64 ± 3.76 ab
48	68.32 ± 1.63 a	42.6 ± 4.47 ab	63.48 ± 5.46 ab	63.80 ± 2.14 a
CK	0.00 ± 0.00 e	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 f

NOTE Different letters in the same column indicate significant differences, $p < 0.05$.

3 Conclusions and discussion

The antibacterial assay results revealed that Gouteng Foot Bath Concentrated Liquid exhibited a relatively broad antimicrobial spectrum, demonstrating varying degrees of inhibitory effects against *S. aureus*, *E. coli*, *C. albicans*, and *T. rubrum*. These experimental findings provide critical data support and research direction for further investigation into the antibacterial activity of this concentrate, establishing a solid research foundation. As one of the most medicinally valuable species in China's ethnic medicine, Gouteng possesses exceptionally extensive pharmacological effects, garnering significant attention in both traditional medical applications and modern pharmacological research. For instance, Guo Xiaomin, Lee *et al.* discovered that compared to other types of tumor cells, the alkaloid rhyncholphylline exhibited more pronounced cytotoxic effects against human hepatocellular carcinoma HepG2 cells. Its mechanism of action lies in the precise modulation of multiple signaling cascade pathways within liver cancer cells, thereby effectively exerting dual anticancer and anti-metastatic effects. What is particularly noteworthy is that rhyncholphylline also reverses drug resistance in the hepatocellular carcinoma drug-resistant cell line HepG2/ADM, offering a novel potential strategy for liver cancer treatment[13-14]. Moreover, Zhang Xue *et al.* conducted research on the effects of isorhynchophylline on hippocampal neurocyte apoptosis and oxidative stress in depressed mice, along with its underlying mechanisms. The results demonstrated that isorhynchophylline alleviates depressive behaviors in mice by inhibiting neuronal apoptosis and oxidative stress, with its mechanism potentially associated with suppressing the activation of the JNK signaling pathway[15]. Wang Chen *et al.* investigated the effects of Gouteng-derived active compounds 297 and 315 on mice subjected to Chronic Unpredictable Mild Stress (CUMS). The study revealed that compound 297 significantly ameliorated anxiety and depression-like behaviors in CUMS mice while concurrently improving spatial learning and memory functions, suggesting its potential anti-stress effects[16]. Liu Songlin explored the antidepressant activity of total alkaloids from Gouteng and their possible mechanisms of action. The results demonstrated that the total alkaloids from Gouteng possess significant antidepressant effects, with their mechanism of action potentially associated with regulating hypothalamic-pituitary-adrenal (HPA) axis function, reducing serum levels of TNF-α and NO, and increasing the content of monoamine neurotransmitters in the brain[17]. This study revealed that Gouteng Foot Bath Concentrated Liquid exhibits significant antibacterial activity against common foot pathogens, such as *S. aureus*, indicating potential for development into an antimicrobial foot-soaking product. However, this research inevitably possesses certain limitations, and the current findings necessitate further in-depth verification to ensure scientific rigor and reliability.

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