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Research Article

The Antibacterial Activity and Antibacterial Mechanism of *Bergenia scopulosa* T.P. Wang Extract

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Abstract: The extract from *Bergenia scopulosa* T.P. Wang (pan long qi) was obtained with the help of methanol and tested for antibacterial activity against three bacteria through methods of inhibition zone, MIC and MBC. Then, the antibacterial mechanism of the extract was studied based on the growth curve and cell membrane permeability. The results showed that the extract of *Bergenia scopulosa* T.P. Wang could antagonize *S. aureus*, *P. aeruginosa* and *E. coli* respectively, but the inhibitory impact on *S. aureus* was stronger than *P. aeruginosa* and *E. coli*. The antibacterial mechanism of the extract against three pathogen is as follow: drug cause a decrease in the number of cell divisions in logarithmic phases and induce the voids of cell walls to become, thus the materials in the cell to leak out.

Keywords: Antibacterial activity, antibacterial mechanism, *Bergenia scopulosa* T.P. Wang (pan long qi) extract, pathogen

INTRODUCTION

Since the penicillin was found, the antibiotics had been the first line of treatment against many bacteria infections and prokaryotic system since the early twentieth century. Applying antibiotic excessively will not heal faster or better results and caused inhibition of probiotics, imbalance of intestinal flora, or toxic reaction (Newman and Cragg, 2007). So it is very important to find the new drug.

Chinese Herbal Medicine (CHM) is an important part of chinese medicine and has been used widely in China for more than thousands of years. The CHM used in this study which was called *Bergenia scopulosa* T.P. Wang (pan long qi) was cllected from Taibai Mountain, the main peak of Qinling Mountains, which harbours more than 600 kinds of Chinese herbal medicine. The *Bergenia scopulosa* T.P. Wang (pan long qi) is a tradition herbal medicine in folk. It could treat many illnesses, such as bone-setters of the injury, dysentery and common cold (Zhao, 1998). The methanol extract from *Bergenia scopulosa* T.P. Wang (pan long qi) was evaluated for its antimicrobial activity and mechanism against three kinds bacteria *S. aureus*, *P. aeruginosa* and *E. coli*, respectively.

MATERIALS AND METHODS

Microorganisms and media: The indicator strains (one Gram-positive bacterium (G+), two Gram-negative bacteria (G-)) tested for antimicrobial activity and mechanism were Staphylococcus aureus (ATCC 25923; G+; pathogen), Pseudomonas aeruginosa (ATCC 27853; G-; pathogen) and Escherichia coli (ATCC 25922; G-; pathogen), respectively. The Nutrient Agar (NA) medium consisted of peptone 10.0 g, agar 15.0 g, beef extract 3.0 g and NaCl 5.0 g/L of distilled water was used for strain storage and The nutrient agar (LB) medium consisted of peptone 10.0 g, beef extract 3.0 g and NaCl 5.0 g/L of distilled water was used for strain storage, both of them were all autodaved at 121°C for 20 min and were used for the antibacterial assay (Yuan, 2010).

Fig. 1: (A) The plant of *Bergenia scopulosa* T.P. Wang (pan long qi), (B) the dried *Bergenia scopulosa* T.P. Wang (pan long qi), (C) the extract of *Bergenia scopulosa* T.P. Wang (pan long qi)

Valuable components extraction: The *Bergenia scopulosa* T.P. Wang (pan long qi) was collected from Taibai Mountain, China in September, 2012 (Fig. 1). It was healthy rhizomes and stored after being dried in the shade. After crushed the herb, 500 mL methanol and 100 g powdery of the herb was put into the 1000 mL round-bottomed flask and reflux condensation was achieved through spherical condensers. The complete setup was heated with thermostat water bath cauldron in order to reach constant temperature 55°C lasted for 2 h. Then the vacuum extraction filtering method was used

for herb solution to get the filtrate. The filter cake and 500 mL methanol were put into the 1000 mL round-bottomed flask for reflux condensation for 2 h. This process was repeated three times (Gao, 2012). Then merge the obtained filtrate which was under rotary evaporation in vacuum under the condition of 0.04 Mpa and 55°C. After the MeOH was evaporated, added distilled water and waved under super sonic to get the 100 mg/mL turbid liquid to proceed for the tests.

The methods of experiment:

Determination of bacterium numbers: Aseptically diluted bacterium liquid of the original sample achieved 1.5×10^{-8} spores/mL when the bacteria solution was regulated to the exact level with the Mesh 0.5 tube (Harley, 2011). After the determination of bacteria numbers, the experiment about antimicrobial activity and mechanism would begin.

Antimicrobial activity: Antimicrobial activity of the *Bergenia scopulosa* T.P. Wang extract was studied through the microbial technology of measuring inhibition zone (Harley, 2011) of herbal solution. The inhibition zone diameter formed by the extract at concentration of 100 mg/mL against the tested microbial strains was used to determine their antimicrobial activities. The 1.0 mL of pathogens was transferred to Nutritional agar by pipette gun aseptically. The swab was then taken and streaked on the surface of the Nutritional agar plate three times, rotating the plate after each streaking. Dispensed the extract onto the plates which diameter were 6 mm made by punch and the herb was 10 μL every disk with pipette gun. Make sure that contact was made between the extract disk and the culture by gently pressing the disk with aseptically tweezer. The plates were incubated for 18 to 20 h at 35°C. The inhibition zone diameter was the average of three measured replicates.

After the experiments of inhibition zone for each microbial strain, determined the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) by double broth dilution method with microdilution checkerboard techniques (Katsura *et al*., 2001). Steps as follows, inoculated 100 μL the extract liquid and 100 μL LB broth into the first orifice of A row in orifice plate and also got fully blowing-suction by pipette gun more than three times in order to let them uniform mixture. After that, inoculated 100 μL the liquid from first orifice into the second orifice of A row and mixed 100 μL LB broth together, fully blowing-suction again. Repeated this operation till the last orifice of A row, added 100 μL bacterium fluid to each orifice of A row. The microorganism was incubated in an inverted position for 20 h at 35°C to observe whether it turned red after an increase of 5% MTT. The concentration of the last no-reddened well plate was MIC. After MIC experiment, 200 μL taken from each well with the concentration being no more than MIC bacterial suspension was inoculated in blood agar plates for 24 h for observation. The lowest drug concentration that yielded no growth was documented as MBC. All kinds of bacteria were conducted three times.

Antimicrobial mechanism: After the determination of bacteria numbers and antimicrobial activity test, the antibacterial mechanism experiments would begin. The bacterial growth curve was drawn by classical method (Liu *et al*., 2012). First, the ultraviolet spectrophotometer would be adopted to measure the absorbance whose wavelength is 610 nm at each time-point and then a curve of absorbance would be drawn accordingly. Studied on cell membrane permeability of extract was mainly done through the conductivity (Zhang *et al*., 2008) and reducing sugar content (Zhang *et al*., 2011). In the experiment of conductivity, each kind of bacteria was divided into two groups: a control group (without drug, CK group) and a dosing group. In each group, there were seven test tubes, which were marked 0, 5, 15, 30, 60, 90 and 180 min, respectively. Each tube contained 18 mL sterile water and 2ml bacteria. In the dosing group 1ml liquid at a concentration of 100 mg/mL was added in. Both of the tubes were then cultivated at a constant temperature of 35°C. Later, corresponding samples were picked out to measure the conductivity at each time point. When measuring the reducing sugar content, every kind of bacteria was divided into two groups: a control group (without drug, CK group) and a dosing group. In each group, there were seven test tubes, which were marked 0, 1, 2, 3, 4, 5 and 6 h, respectively. Each tube contained 18 mL sterile water and 2 mL bacteria. One mL liquid at a concentration of 100 mg/mL was added into the dosing group, the groups cultivated at a constant temperature of 35°C. Later, corresponding samples were picked out to measure the reducing sugar content at each time point through Fehling's solution (He and Li, 2013).

RESULTS AND DISCUSSION

Antimicrobial activity: The experimental results of the inhibition zone in the antimicrobial activity test are shown in Fig. 2.

The figure shown that the extract of *Bergenia scopulosa* T.P. Wang (pan long qi) can antagonize the both three kinds of bacteria *S. aureus*, *P. aeruginosa*

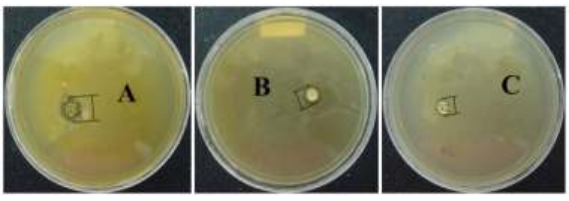

Fig. 2: (A) The inhibition zone of *S. aureus*, (B) the inhibition zone of *P. aeruginosa*, (C) the inhibition zone of *E. coli*

and *E. coli.* Moreover, the *Bergenia scopulosa* T.P. Wang extract (pan long qi) not only show strong antagonistic action to pathogenic bacteria *S. aureus* (Gram-positive bacteria (G-)), but also have inhibitory impact on *P. aeruginosa* and *E. coli* (two Gram-negative bacteria (G+)). The inhibitory impact on *S. aureus* was stronger than *P. aeruginosa* and *E. coli*. After the inhibition zone test, the MIC and MBC of the *Bergenia scopulosa* T.P. Wang extract (pan long qi) extract was measured through double broth dilution method. Results are shown in the Table 1. And then compared the antibacterial effect between antibiotics and the herb extract, the result showed that the extract had the better inhibitory effect on *S. aureus* than Penicillin, the inhibition zones was 12 mm, MIC was 0.15 mg/mL and MBC was 0.3 mg/mL, respectively.

Antimicrobial mechanism: After the antimicrobial activity test, the antimicrobial mechanism of the *Bergenia scopulosa* T.P. Wang (pan long qi) was studied against *S. aureus*, *P. aeruginosa* and *E. coli*, respectively.

Normal *S. aureus*, *P. aeruginosa* and *E. coli* were used as the control group (CK group). And a comparison experiment was made with the herb extract added. The growth curve in Fig. 3 shows that the *Bergenia scopulosa* T.P. Wang extract inhibited the growth of bacteria, whose logarithmic growth stage did not reach the number in normal conditions. So the *Bergenia scopulosa* T.P. Wang extracts could inhibit division of cells in logarithmic growth phase. The permeability changes of *S. aureus*, *P. aeruginosa* and *E. coli* outer membrane were studied with the cell membrane permeability test. It could be found that under the influence of the extract, the conductivity of *S. aureus*, *P. aeruginosa* and *E. coli* liquid reached the maximum within an hour and the reducing sugar content could significantly increase after 4 h. Thus, it

Table 1: The inhibition zones width, MIC and MBC of *Bergenia scopulosa* T.P. Wang (pan long qi) and *Penicillin*

Microorganisms	Drugs	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)
S. aureus	*Bergenia scopulosa* T.P. Wang	12.00	0.15	0.29
	Penicillin	8.50	0.29	0.88
P. aeruginosa	*Bergenia scopulosa* T.P. Wang	7.70	9.38	18.80
	Gentamicin	9.70	0.59	0.59
E. coli	*Bergenia scopulosa* T.P. Wang	7.66	1.17	1.17
	Gentamicin	9.40	0.29	0.59

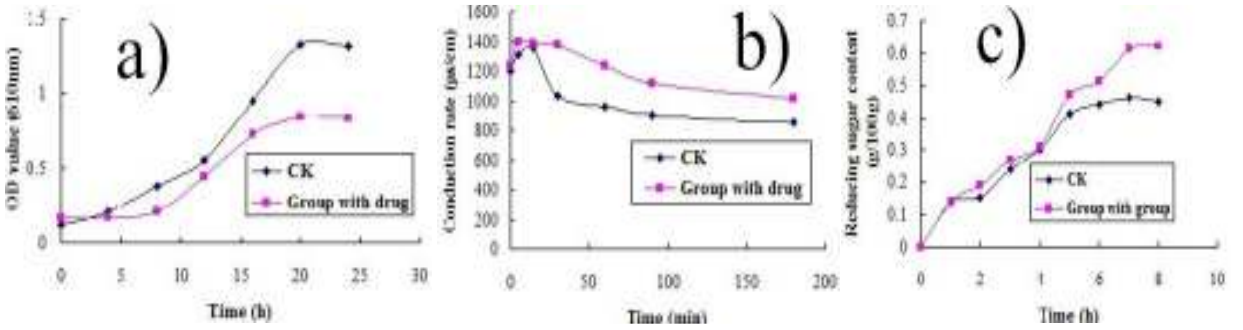

Fig. 3: The figure of antimicrobial mechanism about *S. aureus*, (a) the growth curve of microorganisms, (b) the conduction rate of microorganisms, (c) reducing sugar content of microorganisms

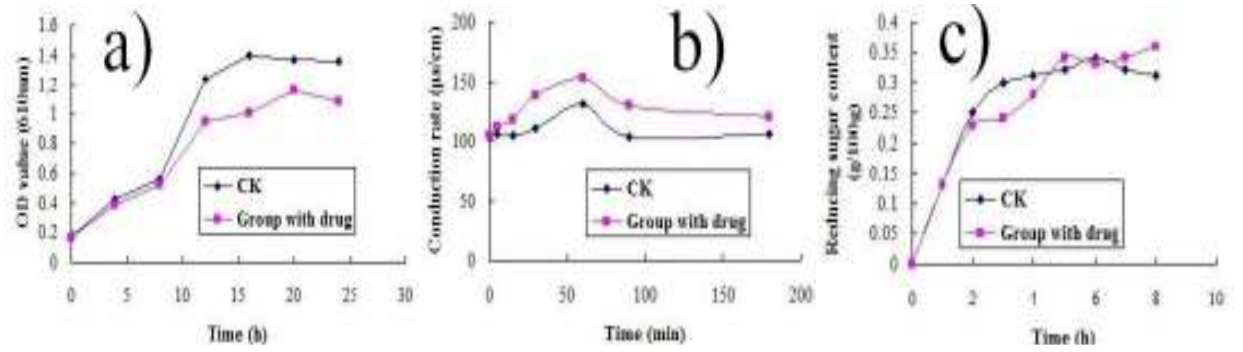

Fig. 4: The figure of antimicrobial mechanism about *P. aeruginosa*, (a) the growth curve of microorganisms, (b) the conduction rate of microorganisms, (c) reducing sugar content of microorganisms

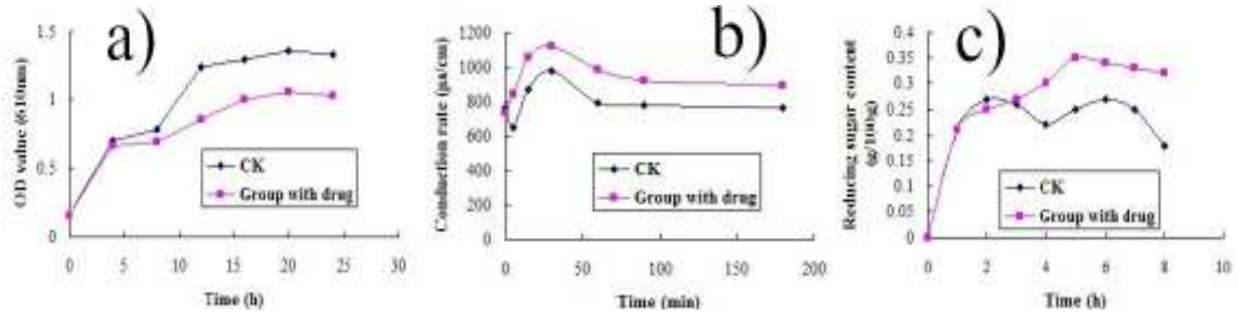

Fig. 5: The figure of antimicrobial mechanism about *E. coli*, (a) the growth curve of microorganisms, (b) the conduction rate of microorganisms, (c) reducing sugar content of microorganisms

can be concluded that after drug was added, the micro-molecules seeped quickly from cell of pathogens and leaded the conduction rate to increase, then as time passed by, reducing sugar content of macromolecules gradually seeped as well. The results were shown on Fig. 3 to 5.

In summary, the inhibitory mechanism of *Bergenia scopulosa* T.P. Wang extract can be deduced as follows: the drug destroy the cell membrane structure of the three pathogens (*S. aureus*, *P. aeruginosa* and *E. coli*), which can increase the permeability of the cell membrane or the formation of membrane pores, leading to intracellular material leakage, the inhibition of cell growth and finally the death of cells.

Discussion: In this stage, we found that the *Bergenia scopulosa* T.P. Wang (pan long qi) could antagonize the both three kinds of bacteria *S. aureus*, *P. aeruginosa* and *E. coli* and the inhibitory impact on *S. aureus* was stronger than *P. aeruginosa* and *E. coli*. The antibacterial effect was compared between antibiotics and the extract of herb, the result showed that the extract had the better inhibitory effect on *S. aureus* than *Penicillin*.

From the results of antimicrobial mechanism, they were shown that the extract of herb had more powerful inhibition of the *S. aureus* growth relative to the *P. aeruginosa* and *E. coli*. When the extract of *Bergenia scopulosa* T.P. Wang was used for *S. aureus*, the failure time was shorter and the damaged condition was more destructiveness in the cell membrane permeability test. Maybe it was why the inhibitory impact on *S. aureus* was stronger than others bacteria and why in some herb books, *Bergenia scopulosa* T.P. Wang (pan long qi) can treat lots illness (Shaanxi Public Health Bureau, 1971).

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