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STUDY ON THE ANTIBACTERIAL ACTIVITY OF *BERGENIA PURPURASCENS* EXTRACT

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Abstract

Background: *Bergenia purpurascens* has tonic, haemostatic and anti-tussive actions. Anti-inflammatory and anti-bacterial activities of *Bergenia purpurascens* have not been reported so far. The objective of this paper is to provide experimental basis for the clinical application of *Bergenia purpurascens* through the pharmacodynamic study on its anti-inflammatory and anti-bacterial effects.

Methods: Experimental models of xylene-induced ear edema in mice, cotton pellet granuloma in rats, and acetic acid-induced peritoneal capillary permeability in mice were used to investigate the anti-inflammatory effect of *Bergenia purpurascens*; bacteriostatic and bactericidal effects of *Bergenia purpurascens* extract on *Staphylococcus aureus* (SA), methicillin-resistant *Staphylococcus aureus* (MRSA), and β-lactamase positive *Staphylococcus aureus* (ESBLs-SA), were observed *in vitro*.

Results: The results show that *Bergenia purpurascens* extract could markedly inhibit xylene-induced mouse ear edema, cotton pellet granulation tissue hyperplasia, and increased capillary permeability. Bergenia *purpurascens* extract has an inhibitory effect on SA, MRSA and ESBLs-SA.

Conclusion: We conclude that *Bergenia purpurascens* extract has certain anti-inflammatory and anti-bacterial effects.

Keywords: *Bergenia purpurascens* extract; anti inflammation; SA; MRSA; ESBLs-SA; antibacterial

Introduction

Yan Bai Cai is the dried rhizome of *Bergenia purpurascens* (Hook. F. et Thoms.) Engl. is the genus Bergenia, of the family Saxifragaceae, which is mainly distributed throughout the northwest, central and northeast Yunnan. It is also grown in western Sichuan and southeastern Tibet. Yan Bai Cai was firstly recorded in the Qing Dynasty's "Classification of Properties of Herbal Medicine", which has tonic, haemostatic and anti-tussive actions (Yunnan, 2006; Lv et al., 2003). Literature search found that the anti-inflammatory and anti-bacterial activities of *Bergenia purpurascens* have not been reported so far.

In this paper, SA, MRSA and ESBLs-SA inhibitory activities as well as anti-inflammatory activity of *Bergenia purpurascens* extract were studied for the first time using K-B method and liquid culture method, providing a scientific basis for the research and development in the fields of food security and pharmaceutical production.

Materials and Methods

Instruments and reagents

Shimadzu UV-2450, UV-Vis spectrophotometer; Precisa XS-125A electronic analytical balance, Switzerland; Buchi R-200 rotary evaporator, Switzerland. Methanol, ethanol, ethyl ether, acetic acid (AR, Chengdu Weike Chemical Reagent Plant), dimethyl sulfoxide (Shanghai Jizhun Chemical Reagent Co., Ltd.), nutrient agar (Beijing Aoboxing Biotechnology Co., Ltd.), broth medium (Beijing Aoboxing Biotechnology Co., Ltd.), self-prepared deionized water, D-101 macroporous resin (Tianjin Meihong Co., Ltd.). JJ-CJ-2FD clean bench (Fushun Purification Equipment Factory), LDZX-305KB vertical pressure steam sterilizer (Guangzhou Shide Medical Equipment Factory), LRH-120 biochemical incubator (Shanghai Tongda Technology Co., Ltd.), ZWX-201B UV air sterilizer; electronic balance (Mettler-Toledo Instruments Co., Ltd., USA), CS-H1 mixer (Beijing Boliyang Technology Corporation); rotary evaporator (EYELA, Japan).

Drugs

Bergenia purpurascens sample was identified as the dried root of *Bergenia purpurascens* (Hook. F. et Thoms.) Engl. is the genus Bergenia, of family Saxifragaceae by associate Professor Pan Qingfu from our college, which was sifted through a 40 mesh sieve, and stored in a shady place for later use. Prednisolone acetate injection (specification: 125 mg/5 ml, Zhejiang Pharmaceutical Co., Ltd., batch number: 20120728);

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indomethacin enteric-coated tablets (25 mg/tablets, Guangdong Baiyunshan Pharmaceutical Group Co., Ltd., batch number: 20136589); benzathine benzylpenicillin injection (1.2 million u/injection, Northeast Pharmaceutical Co., Ltd.; batch number: S130120)

Experimental Strains

Staphylococcus aureus (SA), methicillin-resistant *Staphylococcus aureus* (MRSA), and β-lactamase positive *Staphylococcus aureus* (ESBLs-SA) (all the above strains were purchased from Beijing Tongheng Biological Information Co., Ltd., batch numbers were: SA-20133698, MRSA-20136589 and ESBLs-SA-2013212365, respectively), strains were all provided by the College of Pharmacy of Yunnan Medical University and identified routinely. All the bacteria used were kept at 37℃ for 18 h before the experiment. Before use, the bacteria solution was diluted to a final concentration of 10^5 CFU/ml, and set aside.

Animals

Kunming mice, weighing 20~22 g, animal inspection certificate No.: YNKM2013-0026; male SD rats, weighing 180~200 g, animal inspection certificate No.: YNKM2013-0125, were all provided by the Laboratory Animal Center of Kunming Medical University. All experimental procedures were approved by the Animal Research Ethics Committee.

Culture medium

① MH (Mneller-Hinon) broth: 1,000 ml of fresh beef extract solution, 1.5 g of soluble starch and 17.5 g of casein hydrolysate were autoclaved at 103.4 kPa for 17 min, then refrigerated at 4℃. ② MH agar plate medium: 2% agar was added with MH broth, autoclaved at 103.4 kPa for 15 min, and then poured into a sterile petri dish at about 5 mm thick, naturally cooled, and refrigerated for the cultivation of bacteria.

Preparation of *Bergenia purpurascens* extract

1 Kg of *Bergenia purpurascens* was taken, and extracted under reflux three times with 10-fold amount of 50% ethanol, each time lasted 0.5 h. The crude extract was adsorbed by D-101 macroporous resin, and eluted with water until the effluent was colorless, the water washed fraction was discarded, and the remaining was eluted with 6 column volumes of 20% ethanol. Eluent was removed, and the remaining was diluted to the required concentration with distilled water at the time of experiment.

Xylene-induced mouse ear edema test

50 mice were taken, and randomly divided into five groups of 10 each, namely *Bergenia purpurascens* extract high-, medium- and low-dose groups (67.6, 33.8 and 16.9 $g \cdot kg^{-1}$), positive control indomethacin group (0.65 $g \cdot kg^{-1}$) and blank control group (equal volume of saline). Mice in each group were intra-gastrically administered at the design dose (capacity of 0.2 ml/10 g) qd for 7 consecutive days, after the last administration, 0.05 ml of xylene was evenly applied on the right ear of each mouse, and left ear served as the control. 45 min later, left and right ears were cut off, round ear pieces in the corresponding parts were removed with a 4 mm radius punch, and weighed on an electronic balance, degree of edema was the weight of right ear piece subtracted by the weight of left ear piece, degree of edema among various groups was compared, and the edema inhibition rate was calculated: edema inhibition rate = (degree of edema of blank control group - degree of edema of treatment group) / degree of edema of blank control group × 100% (Xu et al., 2009).

Cotton pellet granuloma test

50 male rats weighing about 190 g were taken, anesthetized with ethyl ether, and abdominal incision was made under sterile conditions, cotton pellets were weighed, autoclaved, added with 10 $mg \cdot ml^{-1}$ benzathine penicillin per cotton pellet, dried at 50℃ in an oven, and then implanted subcutaneously in both auxiliary regions of rats. After operation, the rats were randomized into five groups of 10 each, namely the *Bergenia purpurascens* extract high-, medium- and low-dose groups (46.8, 23.4 and 11.7 $g \cdot kg^{-1}$), positive control indomethacin group (0.007

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g·kg^{-1}) and blank control group (equal volume of saline). From the next day, rats were intragastrically administered for 7 consecutive days, on the 8th day, the rats were sacrificed, and the cotton pellet granulation tissues were dissected and removed, dried at a 60~90℃ oven, and weighed, after subtracting the original weight of cotton pellets, net weight of granuloma was obtained, the weight of granuloma in each group was compared (Li et al., 1991).

Effect on acetic acid-induced mouse peritoneal capillary permeability

The mice were randomly divided into five groups of 10 each, namely the blank control group, *Bergenia purpurascens* extract low-, medium- and high-dose groups (16. 9, 33.8 and 67.6 g·kg^{-1}) and positive drug prednisolone group (8 mg·kg^{-1}), treatment groups were intragastrically administered (0.2 ml/10 g) qd for 7 consecutive days, blank control group was given (an equivalent volume of distilled water). After the last administration, the mice were given tail vein injection of 0.1 ml/10g 0.5% Evans Blue solution in saline, as well as intraperitoneal injection of 0.6% acetic acid at 0.2 ml per mouse, 30 min later, the animals were sacrificed, abdominal skin and muscle were removed, and abdominal cavity was washed with 6 ml of 0.9% NaCl solution, washing liquid was pipetted out, combined, then 0.9% NaCl solution was added to make the volume 10 ml, followed by centrifugation at 300 r·min^{-1}. The supernatant was taken and absorbance was measured at 590 nm, differences among groups were compared (Wang et al., 2000).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the drug against three bacterial strains were determined by test tube continuous dilution method. 2.0 ml of broth medium was taken, diluted to a certain concentration by addition of *Bergenia purpurascens* extract, liquid in the test tubes was diluted by double dilution method, 0.05 ml of bacterial solution was added to each test tube, and cultured at 37℃ for 24 h, a blank control was set up as well. The concentration of drug in the last clear test tube, which was the minimum inhibitory concentration, was observed (Zhang et al., 2009).

Determination of minimum bactericidal concentration (MBC)

After obtaining MIC values by test tube double dilution method, the liquid in the clear test tube was taken, inoculated into the plate medium, and cultured at 37℃ for 18 h, the concentration without bacterial growth was regarded as the minimum bactericidal concentration of the sample.

Statistical methods

Data were processed using SPSS 13.0 software, comparison of means among groups was performed by one-way ANOVA, and pair wise comparison among groups was performed by SNK test, $P<0.05$ was considered statistically significant.

Results

Inhibitory effect of *Bergenia purpurascens* extract on xylene-induced ear edema in mice

Bergenia purpurascens extract high- and medium-dose groups and indomethacin group could all antagonize xylene-induced mouse ear edema, compared with the blank control group, after treatment by *Bergenia purpurascens* extract high- and medium-doses and indomethacin, degree of ear edema markedly reduced, and the differences were statistically significant ($P<0.01$ or 0.05). Differences between *Bergenia purpurascens* extract low- and medium-dose groups and indomethacin group were statistically significant ($P<0.01$); differences between *Bergenia purpurascens* extract high-dose group and indomethacin group were not statistically significant ($P>0.05$). See Table 1.

Inhibitory effect of *Bergenia purpurascens* extract on cotton pellet granuloma in rats

The results are shown in Tab. 2, compared with the blank control group, granulomas in the *Bergenia purpurascens* extract medium- and low-dose groups were decreased, but the differences were not statistically significant ($P>0.05$); while the granuloma inhibitory effects of *Bergenia purpurascens* extract high-dose group and indomethacin group were significant, and the differences were statistically significant ($P<0.05$). Differences between the indomethacin group and the *Bergenia purpurascens* extract low-, medium- and high-dose groups were not statistically significant ($P>0.05$).

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Table 1: Effect of *Bergenia purpurascens* extract on xylene-induced mouse ear edema (x±s, n=10)

Group	Dose (g/Kg)	Degree of ear edema (mg)	Edema inhibition rate (%)
Blank control group	-	3.9±1.0	-
Indomethacin group	0.65	2.0±0.7**	45.9
Bergenia purpurascens Low-dose group	16.9	3.7±1.0	2.75
Medium-dose group	33.8	2.9±0.7*	23.12
High-dose group	67.6	1.8±0.1**	53.26

Note: Comparison with the blank control group, *P<0.05, **P<0.01.

Table 2: Effect of *Bergenia purpurascens* extract on rat cotton pellet granuloma (x±s, n=10)

Group	Dose (g/Kg)	Granuloma weight (mg)
Blank control group	-	98±40
Indomethacin group	0.007	61±11**
Bergenia purpurascens low-dose group	11.7	69±14
Medium-dose group	24.3	67±13
High-dose group	46.8	57±10**

Note: Comparison with the blank control group, *P<0.05, **P<0.01.

Inhibitory effect of *Bergenia purpurascens* extract on acetic acid-induced peritoneal capillary permeability in mice

Compared with the blank control group, *Bergenia purpurascens* extract high-, medium- and low-dose groups and the prednisolone group could all significantly inhibit 0.6% acetic acid-induced increased peritoneal capillary permeability in mice (P<0.05 or 0.01), the inhibitory effect was more significant (P<0.05) for prednisolone group. See Table 3.

Table 3: Effect of *Bergenia purpurascens* extract on 0.6% acetic acid-induced peritoneal capillary permeability in mice (x±s, n=10)

Group	Dose (g/Kg)	Absorbance (A)
Blank control group	-	0.615±0.082
Indomethacin group	0.008	0.353±0.052**
Bergenia purpurascens low-dose group	16.9	0.521±0.075*
Medium-dose group	33.8	0.458±0.036**
High-dose group	67.6	0.446±0.081**

Note: Comparison with the blank control group, *P<0.05, **P<0.01.

MIC determination results of *Bergenia purpurascens* extract

The results are shown in Table 4. *Bergenia purpurascens* extract had relatively good bacteriostatic and bactericidal effects on SA, MRSA, and ESBLs-SA.

Table 4: MIC of *Bergenia purpurascens* extract against three bacterial strains (mg/ml)

Drug	SA	MRSA	ESBLs-SA
Bergenia purpurascens extract	31.3	31.3	31.3

MBC determination results of *Bergenia purpurascens* extract#

Bergenia purpurascens extract has bactericidal effects on SA, MRSA, and ESBLs-SA, the distribution of MBC was 62.6, 93.9, 93.9

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mg/ml.

Discussion

SA, MRSA, and ESBLs-SA are common clinical bacteria, such bacteria are likely to cause infection, but long time use is prone to drug resistance. Modern pharmacological studies have shown that some Chinese medicines have a broad antibiotic spectrum (Lei, 2002; Editorial, 2001); the present study aims to search for drugs that can overcome bacterial resistance from traditional Chinese medicine.

In this experiment, *Bergenia purpurascens* extract was used to carry out in vitro antibacterial test, MIC was determined by dilution method, the study found that *Bergenia purpurascens* extract has certain bactericidal effect, the bacteriostatic and bactericidal effects may be by direct action of the drug on structure and metabolism of bacteria, the present study can provide the basis for clinical application. To mimic the acute and chronic inflammatory manifestations, the present study used foreign matters such as cotton pellets to implant into the animal skin, and to subsequently observe the effect of the drug on the weight of granuloma; ear edema test reflects whether the drug has an anti-alterative inflammation effect; while the capillary permeability test reflects whether the drug has an anti-exudative inflammation effect (Li et al., 2011). The pharmacodynamic experiments demonstrated that: *Bergenia purpurascens* extract has an apparent inhibitory effect on xylene-induced ear edema in mice; can inhibit agar granuloma in rats; and can markedly inhibit 0.6% acetic acid-induced increased peritoneal capillary permeability in mice. Its mechanisms may be associated with the inhibition of inflammatory cytokines such as histamine and serotonin.

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