

Determining Total Phenolic Content of *Paeonia sinjiangensis* K.Y. Pan and its Antimicrobial Activity Grown in Xinjiang, China

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Paeonia sinjiangensis K. Y. Pan is a perennial herb belonging to the family Ranunculaceae which is one of the most important crude drugs in traditional Chinese medicine, used as an anti-inflammatory, analgesic and sedative agent. This paper deals with the detailed total phenolic content of the crude drug *Paeonia sinjiangensis* K.Y. Pan, from Xinjiang, China. Meanwhile, it tested for antimicrobial activity in this study. The total phenolic content of *Paeonia sinjiangensis* K.Y. Pan was 9.58 ± 1.03 mg QE/g dry wt. The microscopic characteristics were investigated, which offer data to differentiate the drug from its other species. It showed strong inhibition against *Blastomyces albicans* and possessed considerable activity against *Staphylococcus aureus* and *Escherichia coli*.

Key words: Antimicrobial activity, *Paeonia sinjiangensis* K.Y. Pan, phenolics.

Paeonia sinjiangensis K.Y. Pan, belonging to Ranunculaceae family (Liu *et al.*, 2000), is a perennial herb. It is native of Xinjiang in China and naturalized in the Altai mountain area, especially mostly in the western Xinjiang region. In Traditional (Fulong *et al.*, 2001) inhibiting aggregation of platelet and stimulating hepatic cell regeneration (Yongyan, 2003; Meixue, 1998) removing thrombus, preventing coagulation (Hongmei *et al.*, 2000), avoiding hepatic atherosclerosis, protecting heart and liver and

antitumor (Jianhua *et al.*, 1998), etc. It is also frequently used as a remedy for diseases of women (Zhenyu, 1990).

Through the large numbers of consult literature found that few reports have been published regarding the¹ polysaccharides of this species and less has been published regarding its acidic polysaccharide properties (Masashi *et al.*, 1994). In that study, the authors demonstrated that an acidic polysaccharide, called peonan PA, was isolated from the root of *Paeonia lactiflora*.

It was homogeneous on eletrophoresis and gel chromatography, the polysaccharide exhibited remarkable reticuloendothelial system-potentiating activity in a carbon clearance test and considerable anti-complementary activity. Recently, a paper (Li X. *et al.*, 2007) was published which was concerning the chemical composition of *Paeonia anomala* sub sp. *veitchii* (Paeoniaceae). In recent years, large numbers of

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papers have been published about the study of content of *paeoniflorin* in extraction technology of *radix paeoniae rubra*. (Yu *et al.*, 2008) In recent years, our research group has studied the contents of *paeoniflorin* by rapid resolution liquid chromatography and polysaccharide with orthogonal test design from *P. sinjiangensis* K.Y. Pan (Zhou. *et al.*, 2011; Tian. *et al.*, 2011). Meanwhile, we have studied the pharmacognostical evaluation of the crude drug *P. sinjiangensis* K. Y. Pan. (Gong. *et al.*, 2012). The other studies concern on the contents of *paeoniflorin* from *Radix paeoniae rubra* (Yuanyuan *et al.*, 2008; Quan *et al.*, 2007; Zhenhua *et al.*, 2008; Xu *et al.*, 2008).

In spite of the numerous medicinal uses attribute to this plant, the total phenolic content information and antimicrobial activity about *P. sinjiangensis* K.Y. Pan in Xinjiang of China has not been published.

Hence, the present investigation is an attempt in this direction, determination of total phenolic content by the modified Folin-Ciocalteu method and its antimicrobial activity.

MATERIALS AND METHODS

Plant materials

The study was conducted with plants were collected in October 2010, locally from the Altai mountain area of Xinjiang, China. The voucher specimen was authenticated as *P. sinjiangensis* K.Y. Pan by Yonghe Li, a chief apothecary of the Traditional Chinese Medicine Hospital of Xinjiang and accessioned into the herbarium of Traditional Chinese Medicine Ethnical Herbs Specimen Museum of Xinjiang Medical University for future reference (the voucher specimen number: 2010-356.)

Reagents

Solvents

Folin-Ciocalteu phenol reagent, petroleum ether, chloroform, ethanol (95%), methanol; Reagents: ammonia, iodine, ferric chloride, acetic, nitric, sulfuric, silicowolframic, and hydrochloric acid, bromocresol green, β -naphthol, ninhydrin, gelatin, and so on, were purchased from Tianjin Fu-Yu Meticulous Chemical Reagent Company, China.

Test organisms

Organisms such as *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) were used for study. The organisms were maintained by serial sub-culturing every month on nutrient agar slants and incubating at 37° for 18-24 hours. The cultures were stored under refrigerated condition. The antifungal activity was tested against *Blastomyces albicans* (ATCC 10231). Penicillin (Zhongnuo Pharmaceutical Institute Company, H13021634), Gentamycin Sulfate Injection (Zhenzhou Linrui Pharmaceutical Co. Ltd, H41020318), Fluconazole (Tianjin Pharmaceutical Group Xinzheng Co. Ltd, 100108) were served as positive control to determine the sensitivity of *Staphylococcus aureus*, *Escherichia coli*, *Blastomyces albicans* tested, respectively.

Determination of total phenols

For the total phenols analyses, harvested plant samples were collected in room temperature. Total phenols content in the ethanol extract was determined by the modified Folin-Ciocalteu method (Wolfe *et al.*, 2003). An aliquot of extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate (20 %). The tubes were vortexed for 20 sec and allowed to stand for 10 min at 75° for color development. Absorbance was then measured at 760 nm using UV-VIS spectrophotometer. The amount of total polyphenols in the extract was calculated from the calibration curve in terms of gallic acid equivalents ($y=0.09221+137.25x$, $R=0.999$).

Test for antibacterial activity

Antibacterial activity of total phenols from *P. sinjiangensis* K.Y. Pan were studied against two bacterial strains viz. *Staphylococcus aureus*, *Escherichia coli*. A macrodilution broth susceptibility assay was used, as recommended by NCCLS (NCCLS, 1999) and described in Experiment technique of medical microbiology (Guan *et al.*, 2006). The samples were added aseptically to sterile melted Mueller Hinton Broth medium and determined MIC and MBC (Minimum Inhibitory Concentration and maximum bactericidal concentration), standard reference antibiotics (penicillin, gentamycin) were used as positive control.

All tests were performed in Mueller Hinton Broth and performed in triplicate.

Test for antifungal activity

The antifungal activity of total phenols from *P. sinjiangensis* K.Y. Pan against fungal isolates (*Blastomyces albicans*) was evaluated using the broth dilution method. The total phenols were added aseptically to sterile melted Sabouraud's Borth medium and Fluconazole was used as a reference antifungal drug. MIC value was determined as the lowest concentration of total phenols was absence of growth was recorded. Each test in this study was repeated triplicate and performed in Sabouraud's Borth.

Microscopic studies

Microscopic studies were done by transferring the plants to powder (# 60). Observe powder features of hand sample slides (State pharmacopeia committee of china, 2010).

Data were analysed using SAS software and procedure (Cary, 2005).

RESULTS AND DISCUSSIONS

Total phenolic content was 9.58 ± 1.03 mg QE/g dry wt. It is well-known that phenolic

compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and also in providing health beneficial effects.

As can be seen in Table 1-2, the total phenolic of *P. sinjiangensis* K.Y. Pan were found to have moderate antimicrobial activity. The results of MIC and MBC values indicated that it has strong inhibition against *Blastomyces albicans* and considerable activity against *Staphylococcus aureus* and *Escherichia coli*, compared with corresponding positive control.

In conclusion, the present study on pharmacognostical characters, total polyphenol content and antimicrobial activity of *P. sinjiangensis* K.Y. Pan may be useful to supplement information in regard to its identification.

The powder microscopy of the plant revealed the presence of fiber, non- glandular hairs, pollen grain, catheter, stomata, glandular scales and hairs, palisade cells (Fig. 1)

Table 1. Antimicrobial activity of standard antibiotics

Organisms	Penicillin		Gentamycin		Fluconazole	
	MIC ^a / MBC ^a		MIC ^a / MBC ^a		MIC ^a / MBC ^a	
<i>Staphylococcus aureus</i>	0.03	0.06				
<i>Escherichia coli</i>			0.031	0.063		
<i>Blastomyces albicans</i>					25	50

^a Values given as mg•ml⁻¹

Table 2. Antimicrobial activity of total phenolic from *P. sinjiangensis* K.Y. Pan

Organisms	Total phenolic from <i>P. sinjiangensis</i> K.Y. Pan	
	MIC ^a	MBC ^a
<i>Staphylococcus aureus</i>	16.254	32.508
<i>Escherichia coli</i>	16.736	32.647
<i>Blastomyces albicans</i>	4.064	8.127

^a Values given as mg•ml⁻¹

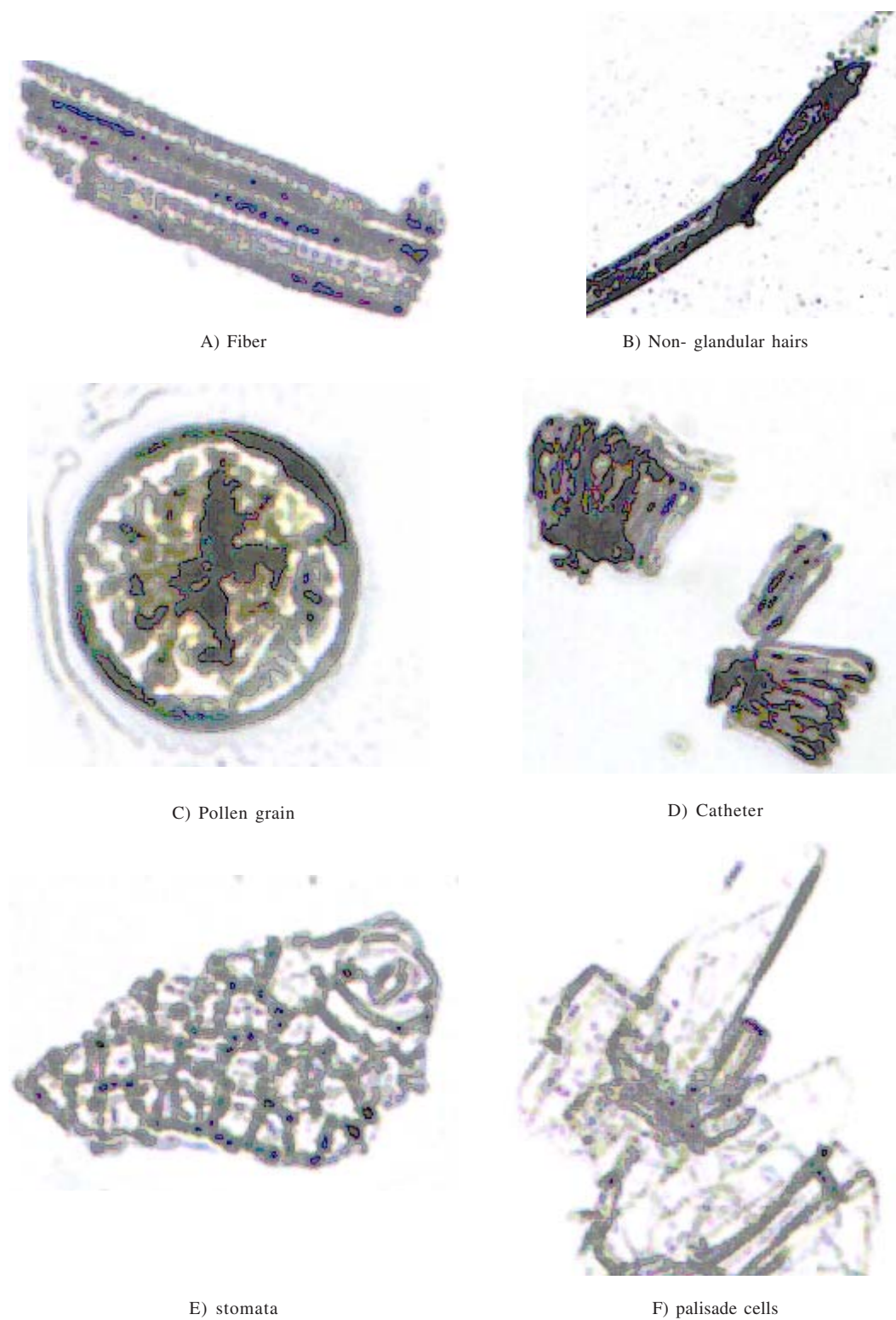


Fig. 1. Powder microscopy

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