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Phamacognostical Evaluation and Antimicrobial Activity of *Paeonia sinjiangensis* K.Y. Pan in Xinjiang, China

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This paper deals with the phamacognostical evaluation of the crude drug *Paeonia sinjiangensis* K.Y. Pan, which is a perennial herb belonging to the family Ranunculaceae and an important crude drug in Traditional Chinese Medicine. The microscopic, physico-chemical, preliminary physicochemical parameters were presented in this work may be to establish the authenticity of *Paeonia sinjiangensis* K.Y. Pan and can possibly help to differentiate the drug from its other species. Meanwhile, it was determined total phenols and tested for its antimicrobial activity in this study. The total phenols content of *Paeonia sinjiangensis* K.Y. Pan was 9.58 ± 1.03 mg QE/g dry wt. It showed strong inhibition against *Blastomyces albicans* and possessed considerable activity against *Staphylococcus aureus* and *Escherichia coli*.

Key Words: *Paeonia sinjiangensis* K.Y. Pan, Phamacognostical evaluation, Total phenols, Antimicrobial activity.

INTRODUCTION

Paeonia sinjiangensis K.Y. Pan, belonging to Ranunculaceae family[1], 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 Chinese medicine, as an important crude drug, it has functions of stabilizing erythrocyte membrane structure[2], inhibiting aggregation of platelet[1] and stimulating hepatic cell regeneration[3,4], removing thrombus, preventing coagulation[5], avoiding hepatic stopping atherosclerosis, protecting heart and liver and antitumor[6], *etc*. It is also frequently used as a remedy for diseases of women[7].

In recent years, our research group have studied the contents of paeoniflorin by rapid resolution liquid chromatography and polysaccharide with orthogonal test design from *P. sinjiangensis* K.Y. Pan[8]. The other studies concern on the contents of paeoniflorin from Radix paeoniae rubra[9,10].

In spite of the numerous medicinal uses attribute to this plant, the pharmacognosy information and antimicrobial activity about *P. sinjiangensis* K.Y. Pan in Xinjiang of China has not yet been published. Hence, the present investigation is an attempt to determine total phenols and its antimicrobial activity.

EXPERIMENTAL

The 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).

Folin-Ciocalteu phenol reagent, petroleum ether, chloroform, ethanol (95 %), methanol; reagents: ammonia, iodine, ferric chloride, acetic, nitric, sulphuric, silicowolframic and HCl, bromocresol green, α-naphthol, ninhydrin, gelatin, *etc*. were purchased from Tianjin Fu-Yu Meticulous Chemical Reagent Company, China.

Test organisms: Organisms such as *Staphylococcus aureus* (ATCC 25923) and *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 ºC for 18-24 h. 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.

Microscopic studies: Microscopic studies were done by transferring the plants to powder (# 60). Observe powder features of hand sample slides[11].

Ash values: Physico-chemical analysis was performed using standard procedures which are helpful in determining the quality and purity of crude drugs, especially in powder form[12].

Total ash: About 3 g powder was accurately weighed and taken in a crucible, which was previously ignited and weighed. The powder was spread as a fine, even layer on the bottom of the crucible. The crucible was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight[12].

Acid insoluble ash: The ash obtained as described above was boiled with 10 mL of 2N HCl for 10 min. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight[12].

Water soluble ash: The ash obtained as described in the determination of total ash was boiled for 5 min with 25 mL of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into crucible, ignited for 15 min and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water-soluble ash[12]. Fluorescence analysis was carried out according to the method of[12] and Chase and Pratt[13].

Preliminary physicochemical screening: The powder of dried plants was subjected to continuous soxhlet extraction with various organic solvent such as petroleum ether, benzene, chloroform, methanol and ethanol, respectively. Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug can not be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug[14].

After concentration and drying of each extract in vacuum desiccator, identification of phytoconstituents was carried out using chemical test.

Determination of poly phenols: Total phenols content in the ethanol extract was determined by the modified Folin-Ciocalteu method[15]. 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 s and allowed to stand for 10 min at 75 ºC for colour 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 (1999)[16] and described in Experiment technique of medical microbiology[17]. 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.

RESULTS AND DISCUSSION

The powder microscopy of the plant revealed the presence of fiber, non-glandular hairs, pollen grain, catheter, stomata, glandular scales and hairs, palisade cells. The proximate analysis result shown that the total ash value, acid insoluble ash value, water soluble ash value were 9.54 ± 0.03, 0.83 ± 0.14 and 5.59 ± 0.03 %, respectively (Table-1).

TABLE-1
PHYSICO-CHEMICAL ANALYSIS OF *P. sinjiangensis* K.Y. PAN

Ash values	Percentage* (%) w/w
Total ash value	9.54 ± 0.03
Acid insoluble ash	0.83 ± 0.14
Water soluble ash	5.59 ± 0.03

*Average of three determinations ± SEM.

Successive solvent extractions were shown in percentage of yield. The percentage for ethanol, methanol, petroleum ether, chloroform, benzene and aqueous were 34.35, 24.13, 2.59, 1.15, 2.03 and 36.36 %, respectively (Table-2).

TABLE-2
SUCCESSIVE SOLVENT EXTRACTIONS OF *P. sinjiangensis* K.Y. PAN

Extractive values	Percentage* (%) w/w
Ethanol solute extractive	34.35
Methanol solute extractive	24.13
Petroleum ether solute extractive	2.59
Chloroform solute extractive	1.15
Benzene solute extractive	2.03
Aqueous solute extractive	36.36

*Average of three determinations.

All extracts were than subjected to study chemical nature of the drug (Table-3). Preliminary physicochemical studies revealed that different fraction contains different components.

Fluorescence analysis of the plant powdered and extract were observed under UV (254 and 366 nm) and visible right. The results are shown in Table-4.

Total phenols 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 colour, taste, aroma and also in providing health beneficial effects.

Tables 5 and 6 showed that the total phenols 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.

TABLE-3
PHYTOCHEMICAL TESTS OF THE SUCCESSIVE EXTRACTS OF *P. sinjiangensis* K.Y. PAN

Chemical constituents	Aqueous extraction	Petroleum ether extraction	Ethanol extraction	Methanol extraction	Benzene extraction	Chloroform extraction
Tannins	Present	Absent	Present	Present	Absent	Absent
Carbohydrates	Present	Absent	Present	Absent	Present	Present
Glycosides or polysaccharide	Present	Absent	Present	Present	Absent	Present
Saponins	Absent	Absent	Absent	Absent	Absent	Absent
Flavonoids	Absent	Present	Absent	Present	Absent	Absent
Alkaloids	Present	Absent	Present	Present	Absent	Absent
Phenols	Present	Absent	Present	Present	Absent	Absent
Triterpenoids	Absent	Present	Absent	Absent	Absent	Absent
Proteins and amino acids	Absent	Absent	Absent	Absent	Absent	Absent
Fixed oils and fats	Absent	Present	Absent	Present	Absent	Absent
Organic acids	Present	Absent	Present	Present	Present	Present

TABLE-4
FLUORESCENCE ANALYSIS OF THE SUCCESSIVE EXTRACTS OF *P. sinjiangensis* K.Y. PAN

Drug & reagents	UV light		Visible light
	Short (254 nm)	Long (366 nm)	
Powder as such	Brown	Khaki	Brown
Power + Glacial acetic acid	Cream soda	Yellowish brown	Cinnamon
Power + 1 N H_2SO_4	Light brown	Cinnamon	Maroon
Power + 1 N Dil. HCl	Dark brown	Brown	Cream soda
Poer + conc. HCl	Dark brown	Brown	Brown
Power + Conc. H_2SO_4	Dark purple	Light purple brown	Dark purple
Power + 1 N NaOH	Cocoa	Khaki	Khaki
Power + Methanol	Yellowish-brown	Dark khaki	Khaki
Power + Diethyl ether	Khaki	Khaki	Khaki
Power + ethanol	Cocoa	Dark cocoa	Cocoa
Power + chloroform	Light cocoa	cocoa	Cream soda
Power + hexane	Khaki	Light khaki	Brunette
Power + ammonia	Dark cocoa	Brown	Light brown
Power + toluene	Cocoa	Light khaki	Cocoa
Power + benzene	Light brown	Dark khaki	Dark brown
Power + *n*-butanol	Dark brown	Light brown	Dark brown

TABLE-5
ANTIMICROBIAL ACTIVITY OF STANDARD ANTIBIOTICS

Organisms	Penicillin	Gentamycin	Fluconazole
	MIC[a]/MBC[a]	MIC[a]/MBC[a]	MIC[a]/MBC[a]
Staphylococcus aureus	0.03	0.06	
Escherichia coli	0.031	0.063	
Blastomyces albicans	25	50	

[a]Values given as mg mL^{-1}.

TABLE-6
ANTIMICROBIAL ACTIVITY OF TOTAL PHENOLS FROM *P. sinjiangensis* K.Y. PAN

Organisms	Total phenols from *P. sinjiangensis* K.Y. Pan	
	MIC*	MBC[a]
Staphylococcus aureus	16.254	32.508
Escherichia coli	16.736	32.647
Blastomyces albicans	4.064	8.127

[a]Values given as mg mL^{-1}.

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