Pharmacognostical Evaluation and Determination of Total Phenols of *Paeonia sinjiangensis* K. Y. Pan

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Abstract: *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 pharmacognostical evaluation of the crude drug *P. sinjiangensis* K. Y. Pan. The microscopic, physico-chemical, preliminary physicochemical parameters, total phenols contents presented in this paper may be proposed as parameters to establish the authenticity of *P. sinjiangensis* K. Y. Pan and can possibly help to differentiate the drug from its other species.

Keywords: *Paeonia sinjiangensis* K. Y. Pan, Pharmacognosy, Phytoconstituents, Total phenols.

Introduction

Paeonia sinjiangensis K. Y. Pan is belong to family Ranunculaceae which is a perennial herb. The plant is native of Xinjiang in China and naturalized in the Altai mountain area, mostly in the western Xinjiang region. As one of the most important crude drugs in traditional Chinese medicine, it has functions of inhibiting aggregation of platelet¹ and stimulating hepatic cell regeneration^{2,3}, stabilizing erythrocyte membrane structure⁴, removing thrombus, preventing coagulation⁵, avoiding hepatic fibrosis⁶, stopping atherosclerosis, protecting heart and liver and antitumor⁷, etc. It is also frequently used as a remedy for diseases of women⁸.

In recent years, a large number of articles have been published on the study of the contents of paeoniflorin from *Radix paeoniae rubra*⁹⁻¹². 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^{13,14}. However, from the large amount of concerned literature, it was found that few reports have been published regarding the pharmacognostical evaluation and total phenols content of *P. sinjiangensis* K. Y. Pan from

Xinjiang of China. So the aim of this study was to carry out a more exhaustive analysis of *P. sinjiangensis* K. Y. Pan. Thus, pharmacognostical evaluation and determination of total phenols of *P. sinjiangensis* K. Y. Pan as the research object. In the present study, we initiated this study to phytochemical characterize and total phenols content of *P. sinjiangensis* K. Y. Pan from Xinjiang and reported the results in this paper.

Experimental

The plants were collected in October 2010, locally from the Altai mountain area of Xinjiang Province, 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.)

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

Microscopic studies

Microscopic studies were done by transferring the plants to powder (# 60). Observe powder features of hand sample slides¹⁵.

Physicochemical analysis

Ash values

Physicochemical Analysis was performed using standard procedures which are helpful in determining the quality and purity of crude drugs, especially in powder form¹⁶.

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 weight¹⁶.

Acid insoluble ash

The ash obtained as described above was boiled with 10 ml of 2N HCl for ten minutes. 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 weight¹⁶.

Water soluble ash

The ash obtained as described in the determination of total ash was boiled for 5 minutes 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 minutes 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 ash¹⁶. Fluorescence analysis was carried out according to the method of¹⁶ and

Chase and Pratt¹⁷.

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, there values indicate the nature of the constituents present in a crude drug¹⁸. After concentration and drying of each extract in vacuum desiccator, identification of phytoconstituents was carried out using chemical test.

Determination of polyphenols

Total phenols content in the ethanol extract was determined by the modified Folin-Ciocalteu method¹⁹. 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°C 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 ($y=0.09221+137.25x$, $R=0.999$).

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 (Figure 1). The proximate analysis result shown that the total ash value, acid insoluble ash value, water soluble ash value were $9.54\pm0.03\%$, $0.83\pm0.14\%$ and $5.59\pm0.03\%$, respectively (Table 1).

Figure 1a

Figure 1b

Figure 1c

Figure 1d

Figure 1e

Figure 1f

Figure 1. Powder microscopy A: Fiber, B: Non- glandular hairs, C: Pollen grain, D: Catheter E: stomata, F: palisade cells.

Table 1. Physicochemical 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 extract were than subjected to study chemical nature of the drug (Table 3). Preliminary physicochemical studies revealed that petroleum ether fraction contain flavonoids, triterpenoids, fixed oils and fats. Ethanol fraction contain tannins, carbohydrates, glycosides or polysaccharide, phenols, alkaloids, organic acids. Methanol fraction contain tannins, glycosides or polysaccharide, organic acids, alkaloids, phenols, flavonoids, fixed oils and fats. Benzene fraction contain organic acids and carbohydrates. Chloroform fraction contain carbohydrates, glycosides or polysaccharide and organic acids.

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	+	—	+	+	—	—
Carbohydrates	+	—	+	—	+	+
Glycosides or polysaccharide	+	—	+	+		+
Saponins	—	—	—	—	—	—
Flavonoids	—	+	—	+	—	—
Alkaloids	+	—	+	+	—	—
Phenols	+	—	+	+	—	—
Triterpenoids	—	+	—	—	—	—
Proteins and Amino acids	—	—	—	—	—	—
Fixed oils and fats	—	+	—	+	—	—
Organic acids	+	—	+	+	+	+

+ = Present, — = Absent.

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

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+1N H ₂ SO ₄	Light brown	Cinnamon	maroon
Power+1N Dil. HCl	Dark brown	Brown	cream soda
Power+Conc.HCl	Dark brown	Brown	Brown
Power+Conc.H ₂ SO ₄	Dark purple	Light purple brown	Dark purple
Power+1N 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

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

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