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Quantitative estimation of tannins by HPLC

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ABSTRACT

A High Performance Liquid Chromatography method has been developed and quantitative estimation of tannins in Pueraria tuberosa (Fabaceae) using aqueous extract, very simple method has been performed. Nucleosil® C18 column was used with methanol (HPLC Grade) and water as mobile phase with ratio (50:50) with pH 4.5, at flow rate of 1 mL/min, UV detection performed at 270 nm. Total run time was 12 min and tannic acid was eluted at retention time of 3.1 minute. Calibration plots of tannic acid standard were linear over concentration range 0-60 µg/mL with respect to the mean area.

Keywords: *Pueraria tuberose*, tannins, HPLC

INTRODUCTION

Pueraria tuberosa (Roxb. ex Willd. DC, family: Fabaceae) is a perennial climber found throughout the Indian subcontinent in wet and damp areas. The tuberous roots of this plant are used in Indian traditional medicine (*Ayurveda*) in general debility, nervous breakdown, spermatorrhoea, burning sensation, heart diseases, intrinsic hemorrhage, tuberculosis etc. The chemical constituents have been identified as puerarin, diadzein, daidzin, â-sitosterol and sigmasterols. Puerarin (isoflavones) has been reported to possess anti-fertility, anti-hypertensive, anti-hyperglycemic, nootropic, and neuroprotective effects [1]. Considering the varied important activities reported in traditional system of medicine with this plant, it was planned to study the effects of tubers extracts of *P. tuberosa DC* for its antidiabetic activity [2]. Tannins (commonly referred to as tannic acid) play an important role and have wide applications. Tannins are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decrease in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their anti-oxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The anti-microbial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins. It has also been reported that tannic acid and propyl gallate, but not gallic acid, were inhibitory to food-borne bacteria, aquatic bacteria and off-flavor-producing microorganisms. Their anti-microbial properties

seemed to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. Tannins have also been reported to exert other physiological effects, such as accelerating blood clotting, reducing blood pressure, decreasing the serum lipid level, producing liver necrosis, and modulating immunoresponses [3].

MATERIALS AND METHODS

Tannic Acid (analytical grade), methanol and water (HPLC grade) were purchased from S D fine chemicals, Mumbai and tuber of *Pueraria tuberosa* was provided as a gift sample by Mr. Vivek Gourbroom.

Preparation of extract:

Aqueous Extract

About 100 g of tuber powder was taken in a round bottom flask (2000 mL capacity) and macerated with 500 mL distilled water and 10 mL of chloroform as preservative for 24 hr with occasional shaking every hour; then the marc was removed by filtering the extract, and was concentrated on water bath at 50 °C. It was then kept in refrigerator below 4 °C till experimental study.

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and water in ratio of 50:50 and filtered through 0.2-µm filter, using vacuum pump and sonicated for 30 min [4, 5, 6].

Preparation of calibration curve of tannic acid

Tannic acid (10 mg) was dissolved in 10 mL of mobile phase to prepare stock solution with concentration of 1000 µg/mL. A series of dilutions with concentration of 20, 30, 40, and 50 µg/mL were prepared by taking aliquots of 0.2, 0.3, 0.4, and 0.5 mL of stock solution (1000 µg/mL) and diluted up to 10 mL with mobile phase. Each dilution (20µL) was injected with the help of a syringe in triplicate and the area under curve at 270 nm was recorded. Calibration curve of mean area against concentration was plotted (*Figure 1*).

Preparation of sample

10 mg sample was dissolved in 10 mL of mobile phase and allowed to stand for 8 hr with occasional stirring and filtered through 0.2-µm filter and sonicated for 30 min. Quantification was carried out using an absolute calibration curve method with standard solutions of Tannic acid. The chromatographic conditions for analysis were as mentioned in *Table 1*.

***Table 1*: Chromatographic conditions for quantitative estimation of tannins**

Parameters	Chromatographic conditions
HPLC System	Agilent
Pump	LC-P-100
Detector	LC-UV-100 UV/VIS Detector
Column	Nucleosil® C18 5 µm particle size, L × I.D. = 25 cm × 3.2 mm
Column temperature	Ambient
Mobile phase	Methanol: Water (50:50)
Wavelength of detection	270
Flow rate	1mL/min
Sample volume	20 µL
Run time	12 min
Retention time	3.1 min

RESULTS AND DISCUSSION

Quantitative estimation of tannins by HPLC:

1. Calibration curve of Tannic acid

In the present study, a method was developed by using HPLC for quantitative estimation of tannins in aqueous extracts of *Pueraria tuberosa* using tanninc acid as standard and methanol water (50:50) with pH 4.5 as mobile phase. Calibration curve of mean area against concentration of varying concentrations of tannic acid standard was plotted (*Figure 1*) as per calculations stated in *Table 2*.

***Table 2*: Calculations for plotting the tannic acid standard curve**

Conc. (µg/mL)	Injection 1 Area	Injection 2 Area	Injection 3 Area	Mean Area	Std. dev.	% RSD	Mean % RSD
10	12995770	12773973	12975660	12915134	122662	0.94	1.168
20	21846410	19402462	20836590	20695154	1228098	0.54	
30	31302615	32310422	31302616	31638551	581857	1.83	
40	40421089	39923299	38922590	39755659	763186	1.91	
50	49633853	50979076	50289170	50306465	776695	1.53	
60	58533712	55507297	57547198	57196069	1543459	0.26	
Sample 50 µg/ml	13937665	14130444	14120272	14062794	108484	0.8	

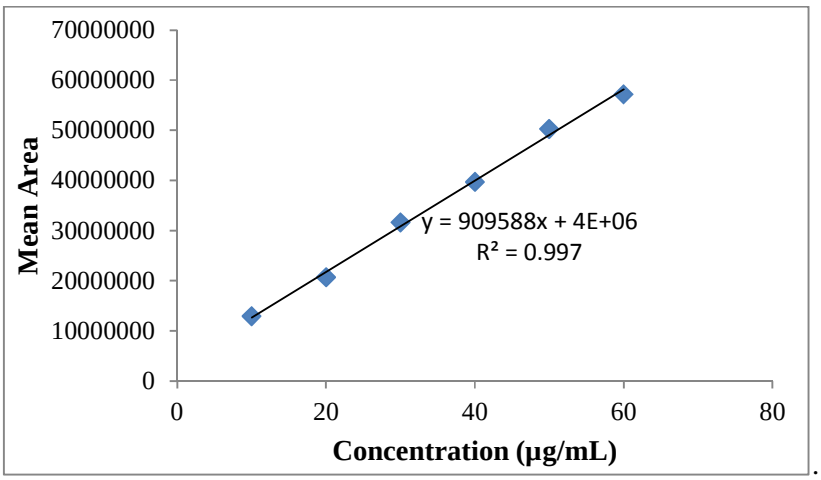

***Figure 1*: Calibration curve of mean area against concentration of tannic acid standards**

A representative chromatogram of tannic acid standard 50 µg/mL (injection # 3) has been given in *Figure 2*. The retention time for tannic acid standard was about 3.19 minutes with USP plate count of 562 and asymmetry factor of 1.644.

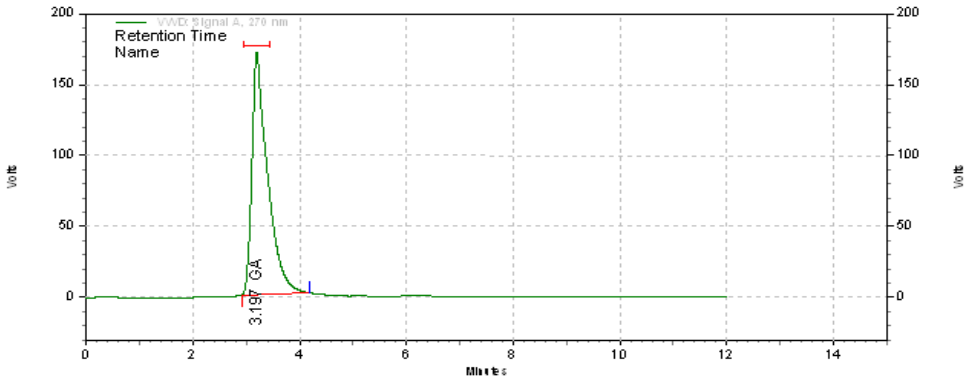

***Figure 2*: Chromatogram of tannic acid standard 50 µg/mL (injection # 3)**

AQUEOUS EXTRACT:

50 µg/mL of sample solution was prepared and 20 µL was injected in the same chromatographic conditions as the tannic acid standards. The chromatogram of injection # 1 has been represented in *Figure 3* below. The retention time of tannic acid peak was 3.047 and asymmetry factor was 1.067.

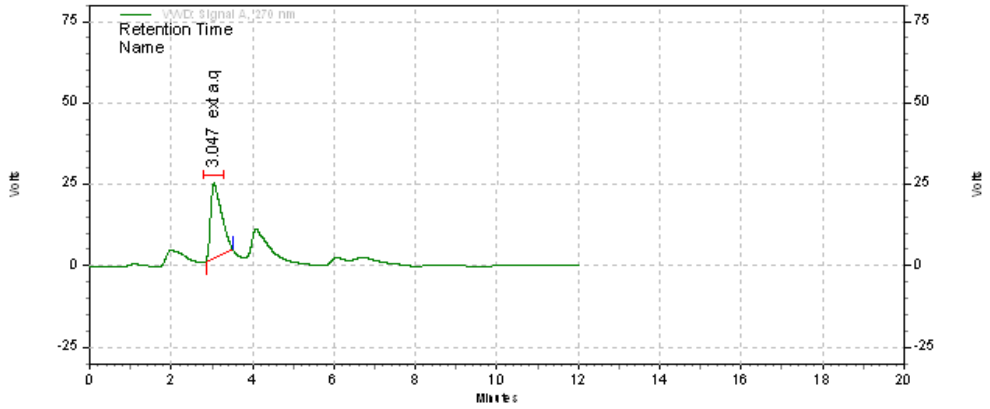

***Figure 3*: Chromatogram of aqueous extract injection # 1 (50 µg/mL)**

Calculation:

By using following formula the quantitative estimation of tannins by HPLC is calculated. All the values considered are mean values of triplicate injections of standard (50 µg/mL) and sample (50 µg/mL) mentioned in *Table 2*.

$$\frac{\text{Conc. of standard}}{\text{Conc. of sample}} = \frac{\text{AUC of standard}}{\text{AUC of sample}}$$

$$\therefore \text{Conc. of sample} = \text{Conc. of standard}/\text{AUC of standard} \times \text{AUC of sample}$$

$$\therefore \quad \text{Conc. of sample} = \frac{0.05\ \text{mg/mL}}{50306465} \times 14062794$$

$$\text{Conc. of sample} = 0.01398\ \text{mg/mL of tannic acid}$$
$$= 13.98\ \mu\text{g/mL of tannic acid}$$

According to the mentioned data, 50 µg/mL of aqueous extract of *Pueraria tuberosa* contained 13.98 µg/mL of tannic acid. To further investigate the HPLC-based method, it can be validated preferably using ICH guidelines and mass spectrometric confirmation can be done.

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