Research Article

Quantitative Estimation of Tannins from *Pueraria tuberosa* by UV Spectrophotometry

Durgawale Trupti Pratik*[1], Durgawale Pratik Prakash[2], Khanwelkar Chitra Chandrashekhar[3]
[1]Ph.D. candidate, Department of Pharmacology, Krishna Institute of Medical Sciences Deemed University, Karad, Maharashtra, India.
[2]Research Officer, Molecular and Genetics Laboratory, Krishna Inst of Medical Sciences Deemed University, Karad, Maharashtra, India.
[3]Professor and Head of Dept, Dept of Pharmacology, Krishna Inst of Medical Sciences Deemed University, Karad, Maharashtra, India.
***Corresponding author's E-mail:** truptipdurgawale@gmail.com

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ABSTRACT

A simple and reproducible UV spectrophotometric method for the quantitative determination of tannins in *Pueraria tuberosa* root was developed and validated. Therefore, in the present study an attempt has been made to determine the tannin content in *Pueraria tuberosa* using Folin-Denis' method. A blue colored complex is formed by using tannic acid. Estimation was done on UV/Vis spectrophotometer.

Keywords: *Pueraria tuberosa*, tannic acid, UV spectrophotometer.

INTRODUCTION

Pueraria tuberosa, belonging to family Fabaceae, is a perennial climber commonly known as Vidarikand or Indian kudz.[1] This plant contains carbohydrates, steroids, tannins, triterpenes, phenolic compounds, flavonoids, proteins, amino acids, glycosides, etc.[2] The phytoconstituents like puerarin, daidzein, β-sitostreol, stigmasterol, puerarone and coumestan, isoflavone C-glycoside-4,6-diacetyl-puerarin, pterocarpintuberosin, puetuberosanol, and hydroxytuberosone etc. have been isolated and characterized from this species.[3] *Pueraria tuberosa*, popularly used in folklore medicine, was selected as it is reported for its nervetonic, galactogogue, anti-inflamation, brain tonic and as mind power syrup in Ayurvedic formulations. Earlier, the plant has been studied for its anti-staphylococcal, anti-tubercular, anti-fungal, anti-ischamic, anti-hepatotoxic, anti-implantation, anti-fertility, wound healing, estrogenic, contraceptive, hypoglycemic, and for adaptogenic activity.[4] Dry extract has been reported to contain flavonoids (5-10%), acids such as mallic acid (6%). *Pueraria tuberosa* is a very good adaptogenic.[5]

MATERIALS AND METHODS

U V Spectrophotometer (Shimadzu 1800) spectrophotometer with a pair of matched quartz cells was used to measure absorbance. Vidarikand root was a gift sample by Mr. Vivek Gourbroom. Folin-Denis' reagent (1N), Sodium carbonate (20%), tannic acid, polyvinylpolypyrrolidone (PVPP) and all other reagents used were of analytical grade and obtained from S.D Fine Chemicals, Mumbai.

Measurement of total tannins using Folin-Denis' method

Sample Preparation and Tannins Extraction

The sample was dried at 55 ±10 °C and ground to pass through a sieve of 1 mm diameter.[6,7] Tannins extraction was done using 400 mg ground sample in conical flask with 40 mL diethyl ether containing 1 per cent acetic acid (v/v) and mixed to remove the pigment material.

The supernatant was carefully discarded after 5 minute and 20 mL of 70 per cent aqueous acetone was added and the flask was sealed with cotton plug covered by aluminum foil and kept in electrical shaker for 2 hours for extraction. It was then filtered through Whatman filter paper No. 1 and sample was kept in refrigerator at 4 °C until analysis.

Preparation of standard curve

The standard curve for tannic acid solution was plotted by adding the reagents as mentioned in Table 1.

Immediately after the additions were completed, absorbance of the samples was measured at 715 nm and the tannic acid standard curve was plotted.

Validation of developed method

Linearity and range

The standard stock solution containing 100 µg/mL of tannic acid dilution was further diluted to get linearity concentration of 0.5-3.5 µg/mL for tannic acid. Each concentration was analyzed in triplicates. Calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The relation between drug and its absorbance is expressed by equation y = mx+b, where m=slope, and b= Y-axis intercept.

Limit of detection and limit of quantitation (LOD and LOQ)

LOD and LOQ of the drug were derived by calculating the signal-to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ) using the following equation designated by the International Conference on Harmonization (I.C.H.)

guidelines.[8,9] The residual standard deviation of regression line or standard deviation of Y intercept of regression lines was used to calculate LOD and LOQ.

$$LOD = 3.3 \times \frac{D}{S}$$

$$LOQ = 10 \times \frac{D}{S}$$

Where, D = Standard deviation of Y intercept of regression lines.

S = Slope of calibration curve.

Recovery studies

Recovery studies were carried out by standard addition method at three different levels.

A known amount of drug was added to pre-analyzed sample and percentage recoveries were calculated. The additions were done as mentioned in Table 2.

Precision

The intra-day precisions were determined by estimating the corresponding response three times on the same day for Tannic acid; whereas, the inter-day precision was determined by estimating the corresponding response on 3 different days over a period of 1 week.

The results were reported in terms of relative standard deviation (RSD).

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guideline. The method discussed in the present work provides convenient and accurate way for estimation of tannins.

Pueraria tuberosa extract obeys Beer-Lamberts' law in the concentration range of 2-18 µg/mL at the λ_{max} 725 nm. The correlation coefficient (R^2) was calculated, where the (R^2) value 0.998 for tannins indicated good linearity between the concentration and absorbance. The estimation of tannins in *Pueraria tuberosa* was carried out. The concentration of tannins present in raw material was found to be 1.27 ± 0.00 µg/mL in *Pueraria tuberosa*. In order to obtain precision and accuracy, the recovery study was performed at three levels by adding known amount of tannic acid with pre-analyzed sample of extract of *Pueraria tuberosa*.

The experiment was repeated three times at three levels and the result shows 98.04 ± 0.44, 98.14 ± 0.42, 98.56 ± 0.54% recovery of tannins at all three levels with a mean value of 98.24 ± 0.46 which prove reproducibility of the result. The percent relative standard deviation (%RSD) value was found to be inter-day precision 1.145 ± 0.0015, intra-day precision 0.111 ± 0.1353 for tannins, respectively. The low value of standard deviation showed that the method is precise. From the data, it is obvious that the present method of UV spectrophotometric determination of tannins is simple, precise, accurate, and suitable for routine analysis of *Pueraria tuberosa* root.

Table 1: Addition of reagents for preparation of standard curve of tannic acid.

Tube	Tannic acid Solution(0.1mg/mL) (mL)	Distilled Water (mL)	Folin-Denis' Reagent (mL)	Sodium Carbonate Solution (mL)	Tannic Acid (µg)
Blank	0.00	1	0.5	2.5	0
T1	0.02	0.96	0.5	2.5	2
T2	0.04	0.92	0.5	2.5	4
T3	0.06	0.88	0.5	2.5	6
T4	0.08	0.84	0.5	2.5	8
T5	0.10	0.80	0.5	2.5	10
T6	0.12	0.76	0.5	2.5	12
T7	0.14	0.72	0.5	2.5	14
T8	0.16	0.68	0.5	2.5	16
T9	0.18	0.64	0.5	2.5	18

Table 2: Addition of reagents for recovery studies.

Extract used	Drug	Amount of drug taken (µg/ml)	Amount of drug added (%)
Aqueous	Tannic acid	1	50
			100
			150

The results for all the above mentioned tests were obtained as mentioned below in Table 3.

Table 3: Summary of results for linearity of standard curve of tannic acid dilutions, accuracy, precision, LOD, and LOQ.

Parameter		Method
Drugs used		Tannic acid
Wavelength range (nm)		725
Beer's law limit (µg/ml)		1 to 9
Regression equation		y = 0.101x + 0.037
y = mx+b, where (m=slop, b= intercept)		
Slope (m)		0.101
Intercept (b)		0.037
Correlation Coefficient (R^2)		0.998
Accuracy (% Recovery) (n = 3)	I	98.04±0.44
	II	98.14±0.42
	III	98.56±0.54
Precision	Inter-day	1.145 ±0.0015
(% RSD, n=3)	Intra-day	0.111 ±0.1353
LOD (µg/ml)		0.5853
LOQ (µg/ml)		1.951

CONCLUSION

Development and validation of spectrophotometric method for the estimation of tannins in *Pueraria tuberosa* could be used as a valuable analytical tool in routine analysis to check the batch-to-batch variations. After the drug is approved, pharmaceutical validation and development is necessary to ensure that the drug product meets pharmaceutical standards for identity, strength, purity, stability, evaluation of safety and efficacy. It provides strength and certain assurance of quality of products. Estimation of tannins by UV spectrophotometry can be used as one of the appropriate analytical methods for *Pueraria tuberosa*. UV detection of such compound is primary screening for further analysis of the same by chromatographic technique.

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