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Original Article

High Performance Thin Layer Chromatographic Analysis for the Simultaneous Quantification of Two Polyphenolic Biomarkers in Methanolic Fraction of *Bauhinia tomentosa* L. Floral Buds

Abhishek Gupta[1,2], Harinath Dwivedi[2], AKS Rawat[1*]

ABSTRACT

Objectives: A high-performance thin layer chromatography (HPTLC) method for the simultaneous quantitative determination of caffeic acid and quercetin in methanolic fraction of *Bauhinia tomentosa* L. floral buds was developed for the first time. **Method:** For achieving good separation, a mobile phase of toluene: ethyl acetate: formic acid (7:3:0.5, v/v/v) was used. The densitometric determination was carried out at 366 nm in reflection/absorption mode. The calibration curves were linear in the range of 100-600 ng per spot for caffeic acid and quercetin. **Results:** During the analysis methanolic fraction of *Bauhinia tomentosa* L. floral buds showed the pres¬ence of caffeic acid (0.02%) and quercetin (0.018%). **Conclusion:** The proposed method is simple, precise, specific, accurate, less time consuming and cost effective. The statistical analysis of data obtained proves that the method is reproducible and selective and can be used for routine analysis of reported phenolic compounds in crude drug and extracts. The simultaneous quan¬tification of these compounds has not yet been reported in floral buds of *Bauhinia tomentosa* which may be utilized for the proper standardization of the plant.

Key words: *Bauhinia tomentosa,* Caffeic acid, HPTLC, Quercetin, Validation.

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INTRODUCTION

Bauhinia tomentosa Linn., belonging to family Caesalpiniaceae, is an erect shrub.[1] It is known as Phalgu or Pita-kanchana in Sanskrit. It has been reported to contain amino acids, proteins,[2] fatty acids,[3] minerals,[4] lectins,[5] protocatechuic acid,[6] rutin, quercetin[7] and isoquercetin.[8] *B. tomentosa* L. has been valued in Ayurveda for possessing variety of therapeutic properties. In Ayurveda, the plant parts are recommended for the treatment of snake bite and scorpion-sting.[1] Leaves, buds and flowers are edible. In folklore medicine, they are used to treat headache, malaria, dysentery and diarrhea. The bruised bark is applied externally for tumors and wounds. In India, decoction of the root bark is used as a vermifuge and an infusion of the stem bark as an astringent gargle. The root bark is administered internally for conditions of the large intestine, and inflammation of the liver. The fruit is diuretic, while seeds are edible and are used as tonic with aphrodisiac action and yield fatty oil called Ebony Oil.[1,9] Bruised bark ground with rice-water into a paste is externally applied to tumors and wounds.[10] Leaves are externally applied to the forehead for fever.[11] *B. tomentosa* L. showed anti-diabetic activity,[12] anti-bacterial activity,[13,14] anti-microbial activity [15] and protective effect on acetic acid induced ulcerative colitis.[16] Some researchers have performed the qualitative HPTLC analysis of *B. tomentosa* L. leaves and flowers and confirmed the presence of various phytochemicals including phenolic compounds and tannins but the identification and quantitative estimation of specific phenols and flavonoids was not performed.[17,18] We have earlier performed the simultaneous estimation of four phenolic compounds in *B. purpurea* L., *B. variegata* L. and *B. acuminata* L. flowers and floral buds.[19] However, there has been no study on the quantitative HPTLC analysis of specific phenolic compounds from the floral buds of *Bauhinia tomentosa* L. The simultaneous quantification of caffeic acid and quercetin with validation has not yet been reported in floral buds of *B. tomentosa* L., which may be utilized for the proper standardization of the drug.

MATERIALS AND METHODS

Chemicals and Reagents

HPTLC analyses were performed on Merck 20 cm × 10 cm HPTLC silica gel 60F254 (0.25 mm) plates. Caffeic acid and quercetin were supplied by Sigma, Aldrich, Germany. All the reagents used in the experiment were of analytical grade and were supplied by Merck, Darmstadt, Germany.

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Preparation of Standard Solutions

Stock solutions caffeic acid and quercetin were prepared separately by dissolving those 0.1 mg/mL in methanol.

Plant material

The plant material i.e. floral buds of *Bauhinia tomentosa* L. were collected from Lucknow, U.P, India in the month of August. The plant was identified and authenticated by Dr. Tariq Hussain, CSIR-NBRI. A voucher specimen has been submitted in LWG herbarium.

Sample Preparation

The fresh floral buds of *Bauhinia tomentosa* L. were collected, thoroughly washed with water to remove all debris. The plant material was shade dried and powdered by using electric grinder at 60 mesh size. Extraction was performed by soxhlation method. Firstly the powdered plant material was defatted under soxhlet assembly using 250 mL of 98% petroleum ether for 6 h. This is followed by 9 h soxhlation of defatted powder by using 250 mL chloroform, followed by methanol. The final methanolic fraction obtained was passed through Whatman No. 1 filter paper. The filtrate obtained was concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The dried extracts were dissolved in 98% methanol to obtain a stock solution of 10 mg/mL, which is used for application of spots on HPTLC plates.

HPTLC Instrumentation and Chromatographic Conditions

The following were the instruments and chromatographic conditions used. Spotting device: Linomat V automatic sample applicator; CAMAG (Muttenz, Switzerland), Syringe: 100 µL Hamilton (Bonaduz, Switzerland). TLC chamber: glass twin trough chamber (20 × 10 × 4 cm); CAMAG. Densitometer: TLC Scanner 3 linked to winCATS software V.4.06; CAMAG. HPTLC plates: 20 × 10 cm, 0.2 mm thickness precoated with silica gel 60 F254; E. Merck (Darmstadt, Germany). Experimental conditions: temperature, 25±2°C; relative humidity, 40%. Solvent system: toluene–ethyl acetate–formic acid (7:3:0.1). Detection wavelength: 254 nm for caffeic acid and quercetin. Slit dimension: 5.00 × 0.45 mm. Scanning speed: 10 mm s^{-1} and source of radiation: deuterium lamp.

Calibration Curve of Caffeic acid and Quercetin

Stock solutions of caffeic acid and quercetin (100 µg mL^{-1}) were prepared in HPLC grade methanol. Different volumes of stock solution were spotted on the TLC plate to obtain concentrations of 100–600 ng per band of caffeic acid and quercetin respectively. The data of peak areas plotted against the corresponding concentrations were treated by least square regression analysis method validation.

Method Validation

The method was validated according to the ICH guidelines [20,21] and the statistical analysis was done using Excel 2000 (MS Office®).

Precision

Repeatability of the sample application and measurement of peak area were carried out using nine determinants (3 concentrations/ 3 replicates) covering the specified range for the procedure (200, 400, and 600 ng per band of caffeic acid and quercetin) and was expressed in terms of relative standard deviation (RSD). The intra and inter-day variation for the determination of caffeic acid and quercetin was carried out in three different concentration levels of 200, 400, and 600 ng per band. Acceptance criteria for a procedure's repeatability or intermediate precision are based on the intended use of the analytical method.

Robustness of the Method

By introducing small changes in the mobile phase composition, mobile phase volume, duration of mobile phase saturation, and activation of prewashed TLC plates with methanol, the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 200 ng per band for caffeic acid and quercetin and the RSD and SD of peak areas were calculated.

Limit of Detection and Limit of Quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times and the signal-to-noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting the known concentrations of caffeic acid and quercetin until the average responses were approximately 3 or 10 times of the responses for six replicate determinations.

Recovery

The pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard caffeic acid and quercetin and the mixtures were reanalyzed by the proposed method. The experiment was conducted six times. This was done to check the recovery of the targeted analytes at different levels in the formulations.

Ruggedness

Caffeic acid and quercetin solutions of concentration 200 ng per band were prepared and analyzed on day 0 and after 6, 12, 24, 48, and 72 h. Data were treated for % RSD to assess the ruggedness of the method.

Specificity

The specificity of the method was confirmed by analyzing the standard drugs and the extract. The band for caffeic acid and quercetin in the sample was confirmed by comparing the Rf values and spectra of the band with that of the standard. The peak purity of the caffeic acid and quercetin was assessed by comparing the spectra at three different levels, viz., peak start (S), peak apex (M), and peak end (E) positions of the band.

RESULTS AND DISCUSSION

In this study, several solvent systems used for individual estimation of these phenolics and flavonoid were investigated to evaluate the combinatorial separation of these compounds in a single solvent system. Among the different solvents systems investigated, mobile phase consisting of toluene: ethyl acetate: formic acid in the ratio of 7: 3: 1 *v*/*v*/*v* demonstrated compact spots with typical Gaussian shaped peaks with good resolution between other peaks of the extract.

The procedure for the separation and determination of different compounds in methanolic fraction of *Bauhinia tomentosa* L. using HPTLC-densitometry is reported at six point calibration curve in which caffeic acid and quercetin were observed and quantified with method validation **(Table 1).** HPTLC chromatogram and densitograms were obtained from standard compounds and methanolic fractions (**Figure 1**) and both targeted compounds were identified by retention factor (Rf), peak purity and overlay UV-spectrum **(Figure 2).** Precision studies have been performed by analyzing intra and inter-day variation for the determination of these phenolics compounds which was carried out at three different concentration levels of 200, 400, and 600 ng per band; mean percentage relative standard deviation values were found to be 0.31, and 0.53 for caffeic acid and quercetin respectively, in intra-day analysis while inter-day analysis showed mean percentage relative standard deviation values of 1.42 and 1.45 for caffeic acid and quercetin respectively, which shows good precision **(Table 3).** For recovery studies, preanalyzed sample of *Bauhinia tomentosa* L. was spiked with extra 50, 100, and 150% of the standard compounds and the mixtures were reanalyzed which shows a good recovery ranging from 97.14% to 100.05% for caffeic acid and 97.96% to 99.20% for quercetin. The experimental

Table 1: Summary of validation parameters.

Parameters	Caffeic acid	Quercetin
Rf	0.43±0.01	0.49±0.01
Linearity range	100-600	100-600
Regression via area	y=10.790*x-513.036	y=8.685*x-324.228
r	0.999	0.998
Slope	10.790	8.685
Intercept	-513.036	-324.228
LOD (ng)	35	35
LOQ (ng)	100	100
Scanning (nm)	330	330

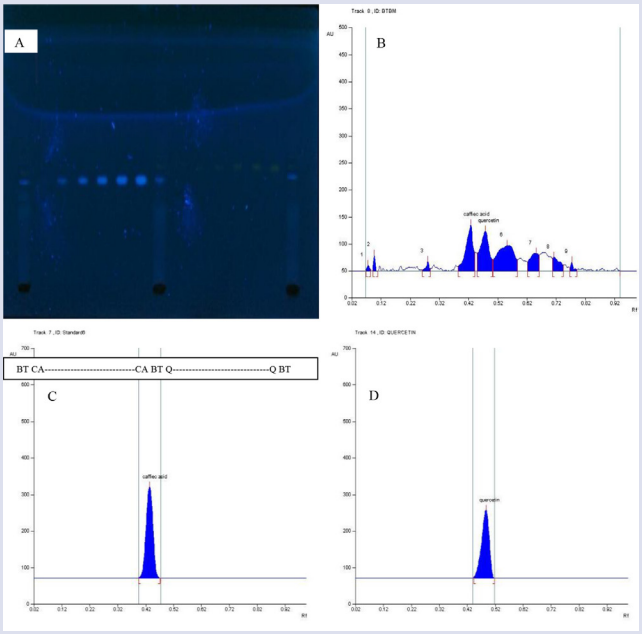

Figure 1: A. HPTLC Plate showing BT: *Bauhinia tomentosa*, CA: Caffeic acid and Q: Quercetin B. Peaks of *Bauhinia tomentosa* showing Caffeic acid and Quercetin. C. Peak of standard Caffeic acid. D. Peak of standard Quercetin

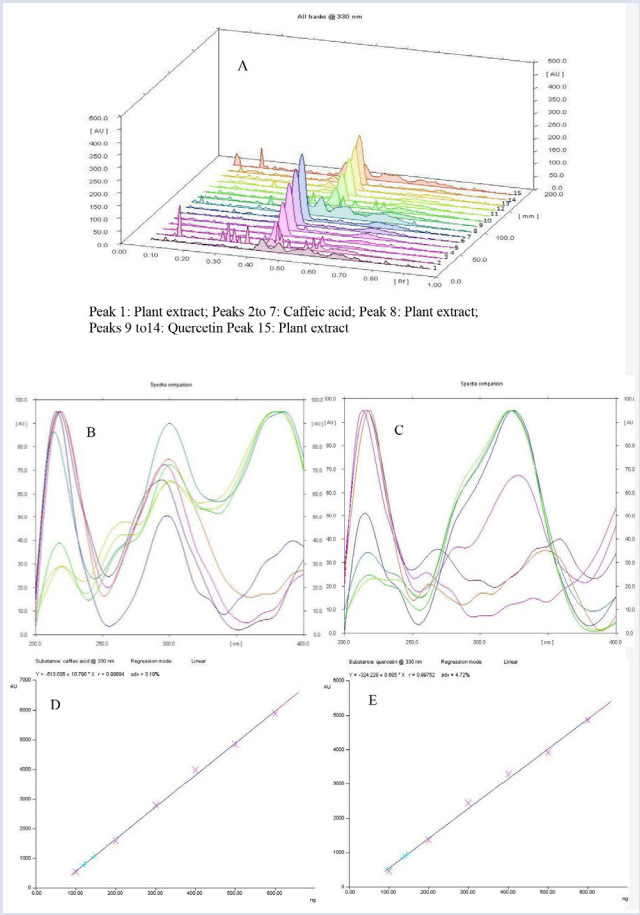

Figure 2: A. HPTLC Chromatogram showing peaks of sample and standards 1. *Bauhinia tomentosa* L., 2-7: Caffeic acid, 8: *Bauhinia tomentosa* L., 9-14: Quercetin and 15: *Bauhinia tomentosa* L. B. UV overlay spectra of Caffeic acid with sample. C. UV overlay spectra of Quercetin with sample. D. Calibration curve of caffeic acid. E.

Table 2: Quantification of caffeic and quercetin in methanolic fraction of *Bauhinia tomentosa* L.

Plant Sample	Caffeic acid (%)	Quercetin (%)
Bauhinia tomentosa L.	0.11	0.13

Table 3: Intra-day and inter day precisions.

Standard Markers	Conc.(ng/band)	Intraday		Interday	
		%RSD	Mean RSD	%RSD	Mean RSD
Caffeic acid	200	0.53		1.81	
	400	0.66		1.46	
	600	0.28	0.49	0.90	1.39
Quercetin	200	0.49		1.18	
	400	0.59	0.48	1.67	1.25
	600	0.36		0.89	

data are expressed as mean percentages of recovered analytes, standard deviation and relative standard deviation is also presented (Table 4). The methanolic fractions of *Bauhinia tomentosa* L. floral buds showed the presence of caffeic acid (0.11%) and quercetin (0.13%), Table 2.

CONCLUSION

A validated HPTLC analytical method has been developed for the simultaneous determination of caffeic acid and quercetin in *Bauhinia tomentosa* L. floral buds. The proposed method is simple, precise, specific, accurate, less time consuming and cost effective. The statistical analysis of data obtained proves that the method is reproducible and selective and can be used for routine analysis of reported compounds in crude drug and extracts. The method can be used to determine the purity of the *Bauhinia tomentosa* L. available from various sources by detecting the related impurities as well as for quality control of herbal formulations containing *Bauhinia tomentosa* L. floral buds as an ingredient. HPTLC analysis has indicated the presence of optimum amount of caffeic acid and quercetin in the samples. This can be used in the pharmaceutical industry as a pharmacognostical tool to identify this medicinally important plant. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic. In an earlier paper published by our team,[19] we have quantified syringic, vanillic, caffiec acid and kaempferol in *Bauhinia purpurea*, *B. variegata* and *B. acuminata* flowers and floral buds, but these two phenolic compounds from the floral buds of *B. tomentosa* were quantified for the first time. Further, identification of other phenolic compounds and their separation and characterization from the plants is to be evaluated and reported in near future.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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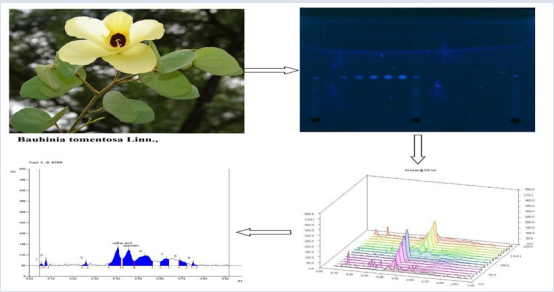
REFERENCES

1. Anonymous. The Wealth of India. A dictionary of Indian raw materials and industrial products. Raw materials Vol. 2, Publications and Information Directorate, CSIR, New Delhi, India. 1988;53-8.
2. Agbede JO. Chemical analysis of leaf meal and processed seed flours of an aesthetic plant: *Bauhinia tomentosa*. Journal of Food Agriculture and Environment. 2007;5(2):233.
3. Daulatabad CD, Mulla GM, Mirajkar AM. Vernolic acid from *Lagerstoroemia thomsonil* and *Bauhinia tomentosa* seed oils. Journal of Oil Technologist's association of Inida. 1991;23:53-4.
4. Chowdhury AR, Banerji R, Misra G, Nigam SK. Fatty acid composition and mineral composition of the seeds of some species of *Bauhinia*. Feete Seifen Anstrichmittel. 1984;86(6):237-9.
5. Rao CK, Satyanand N, Rama G. Pollen lectins. Journal of Plant Science Research. 1991;6:15.
6. Nageshwar G, Anuradha SM, Radhakrishnaiah M, Narayana LL. Distribution pattern of phenolic constituents in species of Bauhinia Linn., and its taxonomic significance. Proceedings Indian academy of Sciences Plant Sci. 1986;96(1):1-7.
7. Row LR, Viswanadham N. Coloring matter of the flower petals of *Bauhinia tomentosa*. Proceedings Indian Academy of Sciences, Section A. 1954; 39(5):240-2.
8. Subramanian SS, Nair AGR. Isolation of isoquercitrin from flowers of *Bauhinia tomentosa*. Indian Journal of Chemistry. 1963;1(10):450.
9. Singh MP, Panda H. Medicinal herbs with their formulations. Vol. 1, Daya Publishing House, New Delhi, India. 2005;157-60.
10. Nadkarni AK. Indian Materia Medica. Popular Prakashan Pvt Ltd., Tardeo, Mumbai. 1976;1:183-4.
11. Gupta SK. Phytopharmacognostic investigation of *Bauhinia tomentosa* Linn. Journal of Advanced Scientific Research. 2011;2:1-4.
12. Devaki K, Beulah U, Akila G, Narmadha R, Gopalakrishnan VK. Glucose lowering effect of aqueous extract of *Bauhinia tomentosa* L. On alloxan induced type 2 diabetes mellitus in wistar albino rats. Journal of Basic and Clinical Pharmacy. 2011;2(4):167-74.
13. Sathya V, Bharathidasan R, Tamil SS, Sophia RN, Ilakkiya R, Prabakaran M. Quantitative, qualitative phytochemical analysis and *in vitro* antibacterial activity of *Bauhinia tomentosa* L. Journal of Natural Product and Plant Resources. 2013;3(2):31-6.
14. Tiwari V, Singh A, Tiwari A. Pharmacognostical, Phytochemical, Antimicrobial Evaluation of *Bauhinia tomentosa* Stem. Journal of Pharmacy Research. 2011;4(4):1173-5.
15. Dugasani S, Balijepalli MK, Tandra S, Pichika MR. Antimicrobial activity of *Bauhinia tomentosa* and *Bauhinia vahlii* roots. Pharmacognosy Magazine. 2010;6(23):204-7.
16. Kannan NC. Guruvayoorappan. Protective effect of *Bauhinia tomentosa* on acetic acid induced ulcerative colitis by regulating antioxidant and inflammatory mediators. International Immuno pharmacology. 2013;16(1):57-66.
17. Devaki K, Beulah U, Gopalakrishnan VK. Gopalakrishnan. Finger Print Analysis of *P. Edulis* and *Bauhinia tomentosa* using HPTLC Technique. Asian J Pharm Clin Res. 2012;5(3):51-9.
18. Kumar VK, Kumar PS, Rajan M, Kumar AV, Boppana R, Reddy PS, Alzeber HF. Alzeber. Qualitative Phytochemical analysis of *Bauhinia tomentosa* Linn flower by HPTLC. Journal of Pharmacy Research. 2011;4(9):2868-80.
19. Gupta A, Verma S, Dwivedi H, Rawat AK. High-performance thin-layer chromatographic analysis for the simultaneous quantification of four phenolics in flowers and flower buds of *Bauhinia purpurea* L., *Bauhinia variegata* L., and *Bauhinia acuminata* L. Journal of planar chromatography. 2015;29(6):423-8.
20. ICH-Q2A, Text on Validation of Analytical Procedures, Harmonized Tripartite Guideline prepared within the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Geneva. 1994.
21. ICH-Q2B, Validation of Analytical Procedures: Methodology, Harmonized Tripartite Guideline prepared within the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Geneva. 1996.

GRAPHICAL ABSTRACT



SUMMARY

- In current research work a high-performance thin layer chromatography (HPTLC) method for the simul¬taneous quantitative determination of caffeic acid and quercetin in methanolic fraction of *Bauhinia tomentosa* L. floral buds was developed for the first time.
- During the analysis methanolic fraction of *Bauhinia tomentosa* L. floral buds showed the presence of caffeic acid (0.02%) and quercetin (0.018%).
- The proposed method is simple, precise, specific, accurate, less time consuming and cost effective. The statistical analysis of data obtained proves that the method is reproducible and selective and can be used for routine analysis of reported phenolic compounds in crude drug and extracts.

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