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Quantitative determination of Ferulic Acid in *Ricinus communis* Linn. Leaves and its Geographical Variation using HPTLC Fingerprint

Subash Chandra Verma[*†], Rachana Rani[*], Pramila Pant[*], M. M. Padhi[*], C. L. Jain[†] and Ramesh Babu[*]

[*]*Central Council for Research in Ayurveda and Siddha, Janakpuri, New Delhi, India*
[†]*Chemistry Department, M. M. H. College, Ghaziabad, U.P., India*

ABSTRACT

*Simple, economical and conventional extraction of Ricinus communis Linn. leaves and quantitative determination of ferulic acid have been carried out by High Performance Thin Layer Chromatography. Geographical variations are found in TLC fingerprints of the leaves analyzed through HPTLC techniques. Resolutions of bands as well as number of bands are found better in the plant collected from Delhi location than the other locations. Ethanolic extract of the leaves was used for the analysis and HPTLC experiments were performed on pre-coated silica gel 60 F_{254} aluminum sheets. For achieving good separation, mobile phase consisting of chloroform: methanol (95:5, v/v) was used. The densitometric determination of ferulic acid was carried out at 366 nm in remission/fluorescence mode. Method was validated in term of linearity, accuracy and precision. The calibration curve was linear in the range of 0.3 to 0.9 µg of ferulic acid, regression equation (Y=11.41+0.7283*X ±0.82) revealed a linear relationship (r = 0.9997) between the mass of ferulic acid applied and the peak areas. The limit of detection and limit of quantitation were found 3.72 ng and 11.26 ng, respectively. Leaves collected from Delhi location showed better yield of ferulic acid content (2.87 µg/g leaves) while leaves collected from Guwahati and Jhansi showed lower yield of ferulic acid content 1.15 µg/g and 0.24 µg/g, respectively than Delhi location. This study will be useful for rapid quantitative determination of bioactive ferulic acid in R. communis Linn. containing formulations for purpose of QA and QC and to help in selection of location for collection of plant material for manufacturers to prepare high quality formulations.*

Keywords: *Ricinus communis* Linn., Geographical variation, Ferulic acid, HPTLC Fingerprint analysis.

INTRODUCTION

Ricinus communis Linn. is an important plant of Ayurvedic system of medicine, is in continuous use for health care in India and other parts of globe. It is used as a single plant remedy or in polyherbal formulation in organized system of medicine such as Ayurveda and Homeopathy. The species of the genus *Ricinus* is known for fixed oil [1, 2] and several amino acids. Fixed oil (45 to 55%) contains a mixture of triglycerides, triricinolein- 75% which on hydrolysis yields ricinoleic acid responsible for the cathartic effect. Leaves contain besides ricinine, N-demethylricinine, 3-O-β-D- rutinosides of Kaempferol and quercetin [3].

Ricinus communis is a species of flowering plant in the spurge family, *Euphorbiaceae*. Probably native to Africa, Castor bean has been introduced and is cultivated in many tropical and subtropical areas of the world, frequently appearing spontaneously. It is found throughout India, cultivated and found wild up to 2400 m [4]. It is a monoecious evergreen shrub growing up to 4m. It is a glabrous and glaucous, branched shrub. Leaves are alternate, palmatifid, 6-10 lobed, each 1-nerved with many lateral nerves and peltate. Microscopic description shows that leaves contains paracytic type of stomata are present on lower and upper epidermii, more abundant on the lower side [5].

Castor is cultivated both in the plains and the hills. As it has deep root system it is hardy and capable of resisting drought. It does not withstand water logging and frost. It requires hard dry climate for proper development of plant.

Therapeutic uses of the plant contains stimulant cathartic, lactagogue and antirheumatic[5]. Plants part is also used in hepatoprotective [6-8], antitympanitic [9], antitumour [10-12], antimicrobial [13], contraceptive [14], nematicidal [15-17], infertility [18], antidote for poisonous bites [19], nitric oxide synthase [20]. The leaves are useful in burns, nyctalopia, strangury and for bathing and fermentation and vitiated conditions of *vata*, especially in rheumatoid arthritis, urodynia and arthralgia. Fresh leaves protected against liver injury induced by carbon tetra chloride in rats while cold aqueous extract provided partial protection [21]. Fresh leaves are also used by nursing mothers in the Canary Island as an external application to increase the flow of milk.

For nutraceutical point of view, per 100 g of the leaves are reported to contain on a zero-moisture basis, 24.8 g protein, 5.4 g fat, 57.4 g total carbohydrate, 10.3 g fiber, 12.4 g ash, 2,670 mg Calcium, and 460 mg Phosphorous [22].

For the purpose of therapeutic uses, three parts viz. roots, leaves and seeds of plants are used in Ayurveda. A lot of work has been reported on seed and roots. Best of my knowledge, there is no work reported till date on the quantification of ferulic acid in the leaves of the plant. Therefore, we have chosen leaves for study and High Performance Thin Layered Chromatography (HPTLC) for the quantitative determination of ferulic acid.

MATERIALS AND METHODS

Chemicals:
All the solvents were used of analytical grade from Merck (India). The TLC Aluminum sheet, 60 F_{254} (20×10 cm) (Cat. No. 1.05554.0007) was purchased from E. Merck (Mumbai).

Plant Material:
Leaves of *R. Communis* Linn. were collected from three different locations of India viz. Delhi, Jhansi and Guwahati. The material was brought to the laboratory and dried at room temperature (25-30^{o}C). The dried samples were powdered using grinder mill and stored in desiccators.

Stock Solution:
The stock solutions containing (50 µg/ mL) of ferulic acid was prepared in methanol. Appropriate quantities of theses stock solutions were spotted to obtain ferulic acid in the range of 14, 12, 10, 8, 6 and 4 µg/ spot.

Preparation of Crude Extract:
Air dried leaves (powdered) of three different locations of *R. communis* Linn. (2 g each) was separately placed in to a three different conical flask and was extracted with 25 ml ethanol (99.9%) for 24 h. Each extract was filtered through Whatmann No. 1 filter paper. The filtrate was concentrated under reduced pressure at 50°C using rotary vacuum evaporator. Final weight of crude extract was weight and calculated for the yield. The extraction of each sample was done in triplicate. Final extract of each location was dissolved in ethanol and made up to 25 mL accurately and used for HPTLC analysis.

Apparatus:
A Camag HPTLC system equipped with Camag Linomat V automated TLC applicator, Scanner 4, and integrated software Win CATS version 1.4.5 was used for the analysis. HPTLC was performed on a pre-coated silica gel 60 F_{254} aluminum sheets plates of 20×10 cm. Samples were applied on TLC plates using a spray on technique

Chromatographic Condition:
Chromatographic studies were performed using the following conditions. Stationary phase: HPTLC aluminum sheet silica 60 F_{254} precoated (20×10 cm); Mobile phase: *Chloroform: methanol (95:5, v/v)*; Volume of mobile phase: 20 mL; Chamber saturation time: 30 min; Temperature: 25-27 ^{o}C; Relative humidity: 35-40%; Migration distance: 80 mm; Migration time: 25 min; Wavelength of detection: 366 nm; Scanning speed: 20 mm/s; Data resolution: 100 µm/step; Band width: 6 mm; Space between two bands: 6 mm.

A Camag Video Documentation system in conjunction with the Reprostar 3 was used for imaging and archiving the thin layer chromatograms. The object was captured by means of a high sensitive digital camera with 5.0 M pixel CCD sensor and 3× optical zoom. A special digitizing board (frame grabber) assisted in rapid processing via the computer. Image acquisition processing and archiving were controlled via Win CATS software.

Chromatographic Separation:

Ethanolic extract of *R. Communis* (Leaves) solution was spotted on the HPTLC aluminum sheet plate, 10 mm from the bottom and 10 mm from the side, using a Camag Linomat V automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nL sec^{-1} from the syringe. The TLC plate was developed in ascending mode in a twin trough chamber pre-saturated for 30 min with mobile phase, *chloroform: methanol (95:5, v/v; 20 mL)*. The plate was removed from the chamber, dried in air, and scanned in remission/fluorescence mode of a Camag TLC scanner 4 at 366 nm .Peak area data were recorded using Camag Win CATS software (Figure 1).

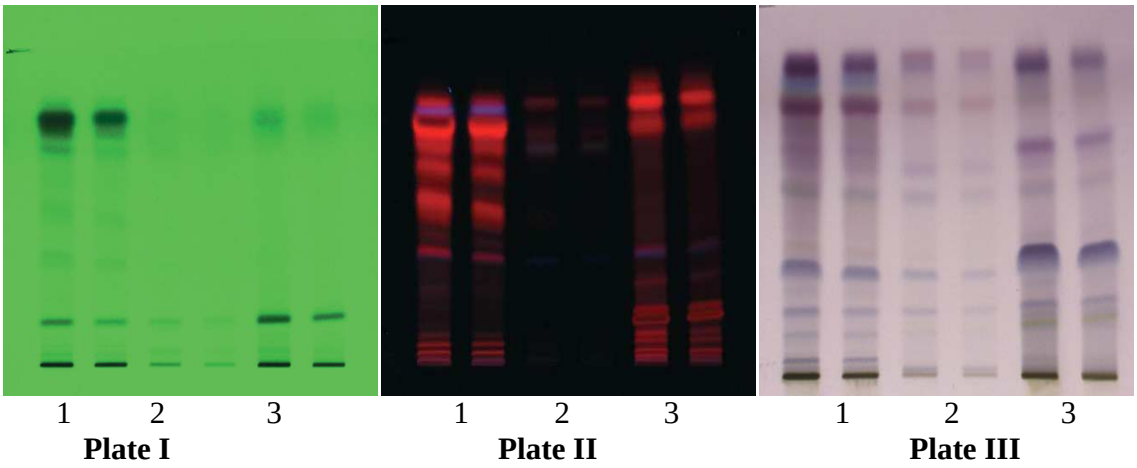

Figure 1: HPTLC fingerprint of ethanolic extract of three different locations at 254 nm (Plate I); 366 nm (Plate II); after derivatized with Anisaldehyde – sulphuric acid reagent (Plate III)
1 Leaves collected from Delhi; 2 Leaves collected from Jhansi; 3 Leaves collected from Guwahati.

VALIDATION OF HPTLC METHOD

Specificity:

The specificity of the method was ascertained by analyzing samples along with standard ferulic acid. The band for ferulic acid in ethanolic extract samples were confirmed by comparing the R_f and spectra of the band with that of the standard. The peak purity of ferulic acid was assessed by comparing the spectra at three different levels, i.e., peak start, peak apex, and peak end positions of the spot.

Calibration Curve:

Standard solution concentration of 50, 100, 150, 200, 250, 300 and 350 ng/ spot of ferulic acid were applied to the plate corresponding to a concentration of 300 ng – 900 ng for the preparation of a calibration curve. The linear regression is Y=11.41+0.7283*X ±0.82.

Accuracy:

The result summarized in Table1, showed the accuracy of the method according to mean values and the %CV value calculated from the analysis for ferulic acid.

Precision:
Six bands of 10 µL of ferulic acid were applied from a single stock solution (666 ng) on aluminum sheet of Si 60 F_{254} plates and analyzed by the proposed method for system precision studies. To determine variations due to the instrument, six different samples of the same concentration (666 ng each) were spotted on aluminum sheet of Si 60 F_{254} plates and analyzed by the proposed method to determine variations arising due to method itself.

Limit of detection and Limit of quantification (LOD and LOQ):
Ferulic acid in the sample extract was identified and confirmed on the basis of matching R_f and UV spectra with that of the standard. The limit of detection (LOD) is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The limit of quantitation (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantitated (signal to noise ratio of 10). LOD and LOQ of the developed method were determined by using linear regression equation of the calibration curve. The LOD and LOQ were calculated based on the standard deviation (SD) of the y-intercept and the slop (S) as 0.8200SD/S and 0.7283SD/S, respectively Table 1.

TABLE 1: Summary of Validation Parameters

Parameters	Range
Linear range[ng/ spot]	300 - 900
Correlation coefficient [*r*]	0.9997
SD of y intercept	0.8200
Slop	0.7283
Limit of detection (LOD) [ng/spot]	3.72
Limit of quantitation (LOQ) [ng/spot]	11.26
Instrumental precision (RSD [%] n = 6) Migration time (R_t) %RSD Peak Area %RSD	 1.3968 1.7199
Accuracy % CV	0.9812

RESULTS AND DISCUSSION

Chromatography:
Various compositions and combinations of the mobile phase were tried for the desired resolution of ferulic acid. A solvent combination of chloroform and methanol (95:05, v/v) gave good resolution. The identification of ferulic acid was carried out by matching the R_f values of ferulic acid in samples with the standard track, and further, they were confirmed by matched UV – VIS spectra. The sample bands at R_f 0.42 corresponded to ferulic acid (Figure 2A & 2B).

The calibration plot shown in Figure 3 indicates the response is linear function of concentration *versus* peak area in of the range of 300 ng- 900 ng /spot ferulic acid.

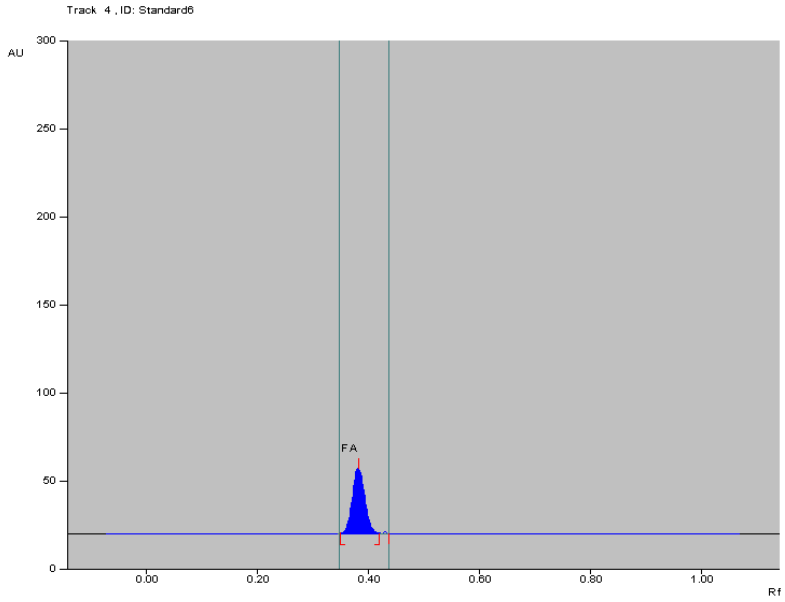

Figure 2A. HPTLC Chromatogram of standard ferulic acid.

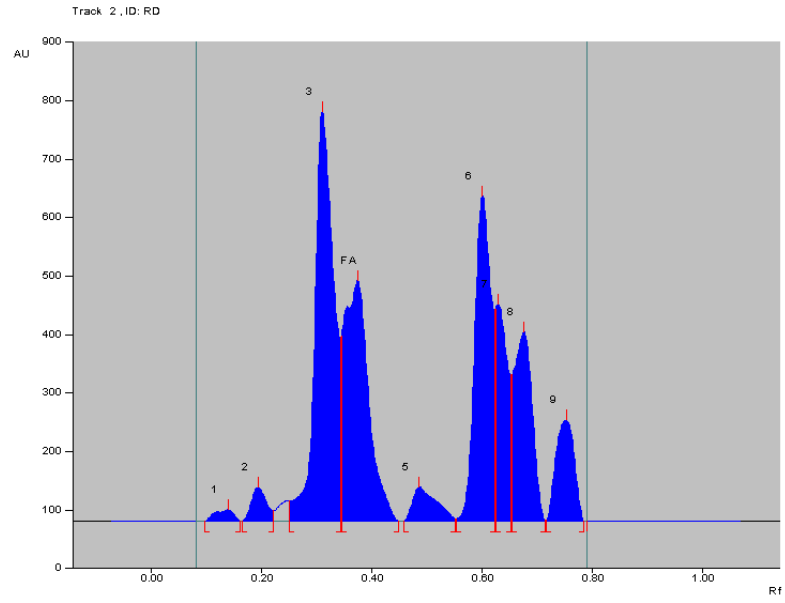

Figure 2B: HPTLC Chromatogram of ethanolic extract of leaves collected from Delhi.

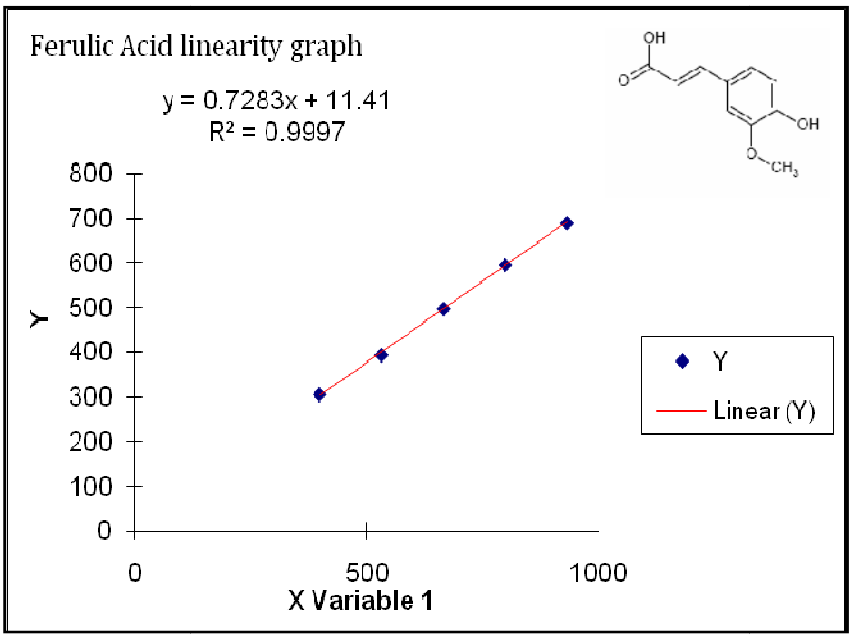

Figure 3: Linear Curve of Ferulic Acid.

Method validation:

The method was validated for its linearity, precision, accuracy, LOD and LOQ. Good correlation (r = 0.9997) was obtained between sample and standard of ferulic acid, Table1. The method showed acceptable precision (R_f and peak Area) and accuracy as evident in Table1. The limit of detection (LOD) and quantitation (LOQ) were found 3.72 ng and 11.26 ng respectively, which indicated the adequate sensitivity of the method, Table1.

The Instrumental precision RSD was calculated and found to be Migration time and Peak Area were 1.3968 and 1.7199, respectively (Table 1).

Geographical Variation:

Geographical variation is found in the plant due to changes in the active constituents. The environmental factors such as light, temperature, carbon dioxide availability, soil conditions etc. have a prominent effect on the secondary metabolism resulting in the extreme variability in the phytochemicals contents of wild/cultivated plants and the products derived from them [23]. The main function of plant secondary metabolites is thought to be the adaptation of plants to their environment [24]. Quantitatively and qualitatively variations in secondary metabolites in the plant of the same species grown in different geographical location is well understood [25].The densitometric determination of the fingerprinting of leaves of different locations was carried out at in 254 nm, 366 nm and after derivatization with anisaldehyde-sulfuric acid regent in remission/fluorescence mode (Figure 1). Resolutions of bands as well as number of bands are found better in the plant collected from Delhi location than the other locations. Ferulic acid is present in *R. communis* leaves of the three different locations. Leaves collected from Delhi location showed better yield of ferulic acid content (2.87 µg/g leaves) while leaves collected from Guwahati and Jhansi showed lower yield of ferulic acid content 1.15 µg/g and 0.24 µg/g, respectively than Delhi location. Leaves collected from Delhi location may be preferred for

research and preparation of its formulations due to better yield of ferulic acid than the other location.

CONCLUSION

A simple and reliable HPTLC method for the determination and geographical variation of ferulic acid in leaves of *R. communis* was developed. The method enabled good resolution for ferulic acid. The developed method has been satisfactorily verified for its accuracy, precision and selectivity. In addition, the HPTLC study of geographical variation allowed us to conclude that the percentage variation of ferulic acid in all the three locations (Delhi, Jhansi and Guwahati) remain in the order Delhi > Guwahati >Jhansi**.** The method can be used for quality control of herbal formulations containing *R. communis* leaves and it can also be used for pharmacokinetic studies of related extracts and drugs.

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