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A Rapid Densitometric Comparison Method for The Quantification of Corosolic Acid in *Lagerstroemia Speciosa* Leaves Using HPTLC.

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Abstract:

Background:

Banaba (*Lagerstroemia speciosa*, Lythraceae, Pride of India) is a medicinal plant that grows in the Philippines, Japan, India, Southeast Asia, etc. Traditionally, the leaves are used to treat diabetes and hyperglycemia. This effect is attributed to its chemical constituents belonging to the groups of terpenoids, tannins, and flavanoids. The objectives of this paper are to present a new method of identification and quantification of corosolic acid, a terpenoid, using HPTLC.

Material and methods:

The hydroalcoholic extract of leaves was chromatographed on aluminum plates coated with silica gel $60F_{254}$, with Toluene: Acetone: Formic acid (5:2:1 v/v) as the mobile phase. Densitometric quantification of corosolic acid was performed after derivatization with 10 % methanolic sulphuric acid in fluorescence mode at 366 nm.

Results:

Amounts of corosolic acid (at 366 nm, R_f value = 7.2) in the sample was calculated using regression equations (R^2 = 0.9959 (366 nm) of calibration plots, which showed there was a good polynomial relationship between peak area and amount of corosolic acid in the range 250-3000 ng/band. The limits of detection (LOD) and limits of quantitation (LOQ) were 70 and 245.6 ng/band, respectively. The method was validated for precision and accuracy. Recovery was determined by spiking the extract with corosolic acid standard and found to be in the range 94.4–96.5%.

Conclusion:

The corosolic acid content found in the leaves was 1.5% w/w at 366 nm.

Keywords: *Lagerstroemia speciosa* leaf extract, corosolic acid, HPTLC.

Introduction:

Lagerstroemia speciosa (Lythraceae, commonly known as Banaba, pride of India) is a medicinal plant that grows in the Philippines, China, India and Southeast Asia. In some countries such as Japan, it is sometimes served as its tea. The leaf is opposite, oblong to ovate, glaborus and with short

petiole; flowers are large, showy and regular in shape, varying from pink to purple in colour, in large terminal panicle; fruit is woody, subglobose and the seeds are winged [1],[2]. Major constituents of *Lagerstroemia speciosa* leaf include lagertannin, corosolic acid, maslinic acid; ellagic acid, lagerstroemin, flosin B and reginin A, flosin A, lutein, phytol, sitosterol and sitosterol acetate, kaempferol, quercetin, and isoquercitrin, Traditionally, the whole plant and specifically leaves are used to treat **diabetes** and **hyperglycemia** (elevated blood sugar). The hypoglycemic (blood sugar lowering) effect of banaba extract is reported to be similar to that of insulin which induces glucose transport from the blood into body cells. This effect is attributed to the various active chemical constituents present like corosolic acid and lagertannins [3]. Banaba extracts are also known to have antiobesity [4], anti-oxidant [5] and anti-gout [6] effects.

Corosolic acid is naturally present in plant species belonging to the genera *Lagerstroemia, Rosa, Hyptis, Eucalyptus, Prunera, Careya, Salvia, Corchorus, and Dipterocarpus* [7]. Among these, Lagerstroemia (Fam: Lythraceae) species, especially *L. speciosa*, are reportedly used in Indian and other traditional systems of medicine to treat diabetes. Corosolic acid is a naturally occurring pentacyclic triterpene which is chemically 2 alpha-hydroxy ursolic acid, 2R, 3β-dihydroxyurs-12-en-28-oic acid. It displays a potential anti-diabetic activity [8], [9], [10], [11], [12], [13], [14], [15], [16] as well as anti-oxidant, anti-inflammation, and antihypertension properties [17].

The purpose of our work was to identify and quantify corosolic acid content in plant *Lagerstroemia speciosa* by HPTLC.

Material and Methods:

Chemicals, Reagents, Solutions, and Plant Material:

Standard corosolic acid was procured from IIIM (Jammu, India). A stock solution (0.5 mg / ml) of corosolic acid was prepared in methanol, and used for application on the HPTLC plate to get a concentration range of 250-3000 ng/band.

Toluene, acetone and formic acid (E. Merck, India) were used as solvents for the preparation of the mobile phase. Concentrated Sulphuric acid (98 %, S. D. Fine chemicals, Mumbai, India) in methanol (10% v/v) was used as the spraying reagent.

Lagerstroemia speciosa leaves were collected from the Dharampur region of Gujarat in the month of August, 2012 and authenticated on the basis of macro and microscopic characteristics. The leaves were washed and dried under the shade, powdered and passed through 60-mesh and stored in appropriately labeled, airtight containers.

Sample Preparation:

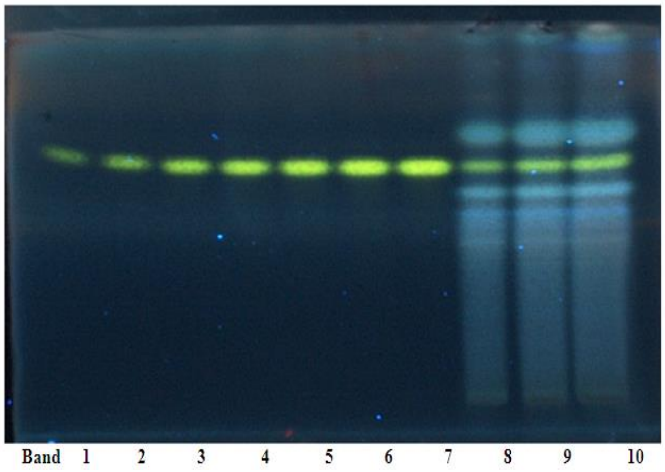
Samples of powdered leaves of *Lagerstroemia speciosa* (100 g; Shimadzu Libror AEG-220 balance) were extracted consecutively with hydroalcoholic mixture (60: 40, 100 ml × 7) under reflux. The 7 extracts were combined and spray dried to get a solid powder (17 gm). The spray-dried powder (2 gm) was extracted with chloroform (25 ml) for 24 hours by maceration and then subjected to filtration. The filtered extract on drying gave a residue (1490 mg), which was subsequently reconstituted in methanol (10 mg/ ml) for analysis.

Chromatography:

Chromatography was performed at 25 ± 2°C on 10 cm × 10 cm aluminum foil-backed HPTLC plates coated with 0.2-mm layers of silica gel 60 F_{254} (E. Merck, Germany). Before use the plates were washed by development with methanol. Samples were applied to the plates as 6 mm bands, 12.8 mm apart, under a stream of nitrogen, by means of a CAMAG (Switzerland) Linomat V semiautomatic sample applicator fitted with a 100-µl Hamilton HPTLC syringe. The spraying rate was 150 nLs^{-1} and the volume applied was 0.5-6 µl for standards and 30 µl for test samples. Ascending development to a distance of 70 mm with Toluene: Acetone: Formic acid (5:2:1 v/v) as mobile phase was performed in a CAMAG twin-trough chamber (20 cm × 20 cm) previously saturated with mobile phase vapor for 30 min. After development, densitometric scanning at 366 nm in fluorescence mode was performed with a CAMAG TLC scanner 3 controlled by CAMAG CATS 4 integration software. The slit dimensions were 6.00 mm × 0.30 mm and the scanning speed was 20 mm s^{-1}.

Identification of corosolic acid in leaves of Lagerstroemia speciosa:

Test solutions and standard solutions were applied to a plate. The plate was developed as described above, dried, sprayed with 10 % methanolic sulfuric acid reagent, then heated at 110°C for 10 min. The plate was observed under UV light at 366 nm. (Figure 1)

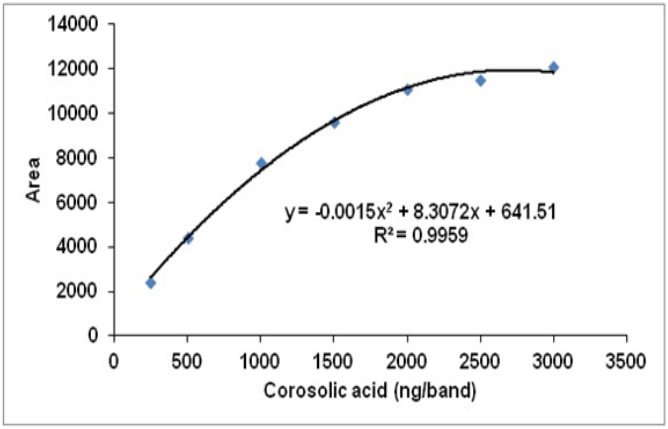


HPTLC plate at 366 nm, Standard corosolic acid (1-7 band, 250-3000 ng / spot) Test extracts (8, 9, 10 bands, 30, 60, 90 nl / spot)

Calibration Plot for corosolic acid:

Different volumes (0.5, 1, 2, 3, 4, 5 and 6 µl equivalent to 250, 500, 1000, 1500, 2000, 2500 and 3000 ng per band, respectively) of corosolic acid standard solution (0.5 mg/ml) were applied to a plate (n = 3 for each amount) and the plate was developed as described above then dried and scanned at 366 nm. Peak area was recorded for each band and a calibration graph was prepared
by plotting amount (ng per band) against peak area (Figure 2, Table 1).

"Figure 2: Calibration curve for standard corosolic acid".



"Table 1: Calibration data for corosolic acid"

Band no.	Concentration (ng / spot)	Mean Area
1	250	2437.8
2	500	4440.5
3	1000	7784.9
4	1500	9647.2
5	2000	11081.5
6	2500	11533.1
7	3000	12073.3

Quantification of corosolic acid in leaves of Legerstroemia speciosa:

Test sample (30, 60 and 90 µl) was applied to a plate and the plate was developed as described above and scanned at 366 nm. Peak area was noted and the amount of corosolic acid present per gram of powder was calculated from the peak areas (Figure 2) using the standard calibration plot of corosolic acid.

Validation of the Method

The method was validated for accuracy, inter-day and intraday precision, specificity, repeatability of measurement of peak area, and repeatability of sample application. Accuracy was measured by determination of recovery of corosolic acid from samples spiked with 50, 100, and 150% of the level originally present in a previously analyzed sample. Intra-day precision was determined for corosolic acid standard (250, 1500 and 3000 ng per band) three times on the same day. Inter-day precision was determined for the same amounts of corosolic acid standard three times over a period of week. The limits of detection and quantification were determined practically by spotting concentration below calibration curve. (Table 2)

"Table 2: Validtion data"

Linearity [ng per band]	250-3000
Coefficient of Determination	0.9959
Precision (CV [%]) Intra day (n = 3) Inter day (n = 3)	 0.16-1.21 % 0.48 -2.35 %
Repeatability (CV [%]) Scanning Spotting	 0.17 % 0.65 %
Limit of detection [ng per band]	70
Limit of quantification [ng per band]	245.6
Recovery [%]	94.4-96.5 %

Results:

Development of the fingerprint profile and analysis of corosolic acid in leaves of *Lagerstroemia speciosa* was performed after use of chloroform for complete extraction of corosolic acid from leaf powder. A distinct fingerprint profile was developed for drug standard and for test extract by using

Toluene: Acetone: Formic acid (5:2:1 % v/v) as mobile phase, which resolved corosolic acid with complete baseline separation at R_f 0.72 when detection was performed under UV light at 366 nm after spraying with 10 % methanolic sulfuric acid reagent and heated for 10 min at 110°C.

Under UV light at 366 nm, yellow fluorescence (Figure 1) was observed for corosolic acid only; this characteristic can be used to identify corosolic acid among all the other constituents separated.

The method was validated for corosolic acid in the range of 250 to 3000 ng per band, with a coefficient of determination being 0.9959 (Figure 2). The average polynomial regression equation was $y = -0.0015x^2 + 8.3072x + 641.51$ (See calibration data in Table 1). The coefficients of variation obtained from measurement of intra-day and inter-day precision were in the ranges 0.16-1.21% and 0.48–2.35%, respectively. These values indicate that the method is precise. The limits of detection (LOD) and quantification (LOQ) were 70 and 245.6 ng, respectively. CV [%] obtained from measurement of repeatability of sample application and area measurement were 0.65% and 0.17%, respectively. Recovery of corosolic acid measured by extraction of spiked sample was 95.5-96.0%. Results from validation of the method are listed in (Table 2).

Conclusion:

The newly developed quantitative HPTLC method for estimating corosolic acid in plant extracts was found to be accurate and precise, and hence was used to determine the quantity of corosolic acid in *Lagerstroemia speciosa*. From the results, the amount of corosolic acid was found to be 1.5 % w/w in leaves of *Lagerstroemia speciosa*.

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