Research Article

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QUANTITATIVE DETERMINATION OF QUERCETIN A BIOMARKER IN METHANOLIC EXTRACT OF *LAGERSTROEMIA LANCEOLATA* AND *LAGERSTROEMIA PARVIFLORA* LEAVES BY HPTLC METHOD

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ABSTRACT

In Indian Ayurvedic system, *Lagerstroemia lanceolata* (*L. lanceolata*) and *Lagerstroemia parviflora* (*L. parviflora*) are well-known plants used for major and minor ailments. Quercetin identified from the vast plethora of plant extracts has proved to possess ethno pharmacological relevance. The present investigation is to estimate biologically active flavonoid compound, quercetin in methanolic leaves extract of *L. lanceolata* and *L. parviflora* by using high-performance thin-layer chromatography (HPTLC). After extraction and phytochemical screening, the extracts were subjected to quantification for the presence of quercetin by HPTLC. Pre coated silica gel 60 F254 is used as a stationary phase and toluene: ethyl acetate: formic acid in ratio of 7: 5: 1 is used as a mobile phase. Densitometric estimation and quantification of quercetin was carried out at 254 nm. The standard R_f value of quercetin is 0.64. The total peak area of the standard, quercetin was compared and the corresponding peak areas of *L. lanceolata* and *L. parviflora* extracts were estimated to be 390.6 and 5442.8 respectively. A good linear relationship 0.988 was obtained between the concentration ranges of 0.2-1.0 µg. This HPTLC method was found to be simple and convenient for rapid screening of active compounds and quantification of the investigated flavonoids in *L. lanceolata* and *L. parviflora*.

Keywords: *Lagerstroemia lanceolata*, *Lagerstroemia parviflora*, HPTLC, Flavonoid compounds, Quercetin.

INTRODUCTION

Nature still obliges as the man's primary source for the cure of his ailments. Research in preventive medicine showed the importance of functional nutrition in reducing the risk factor of certain chronic diseases. Innate defense system of the human body may be insufficient for the damage caused by continued oxidative stress¹. Flavonoids are a group of polyphenolic compounds, which are extensively dispersed throughout the plant kingdom. Till date about 300 varieties of flavonoids are known². Herbal medicines have situated the test of time for their efficacy, safety, cultural suitability and smaller side effects. Flavonoids are classified as flavonones, flavones, flavonols, flavanols, flavan-3-ols and isoflavones according to the locations of the substitutes present on the parent molecule. Quercetin and other flavonoids have the structure to act as powerful antioxidants and have often proven so *in vitro*. Quercetin, being a major constituent of the flavonoid intake, could be a key in fighting several chronic degenerative diseases³. Growing scientific evidence has shown adverse side effects, like liver damage and mutagenesis, of synthetic antioxidant⁴. Therefore, recently there has been an upsurge of interest in natural products as antioxidants, as they inhibit the free radical reactions and protect human body from various diseases, such as cancer and diabetes. Recent studies showed that a number of plant products including poly phenolic substances (e.g., gallic acid, ellagic acid, pelargonidin and sitosterol) and various plants or herbal extracts exert potent antioxidant actions, which are very well known for their healing powers⁵. Quercetin 5, 7, 3', 4', - tetrahydroxy flavanol exhibit anti-inflammatory, antihepatotoxic⁶, antiulcer⁷, anti-allergic and antiviral actions and some of them provides protection against cardiovascular

mortality^{8,9}. Quercetin in combination with other flavonoids, inhibits a number of enzymes like bradykinin¹⁰, tyrosine kinase¹¹ and 5'- nucleotidase activity¹². *L. lanceolata* Wall (Lythraceae) is a moderate to large deciduous tree, sometimes attaining 30 meters in height and 2.4 to 3.0 meters in girth with a clean cylindrical bole of 12 to 15 meters. It is found from Bombay to Kerala and in the hills of Deccan Peninsula up to an altitude of 1,200 meters. Bark is smooth, greenish or yellowish white, exfoliating in papery strips; leaves elliptic- lanceolate or broadly ovate, 6.2 to 10.0 cm x 1.8 to 5.0 cm, coriaceous, glabrous, shining above, usually white or grayish blue; flowers small, white, in large panicles; capsules ellipsoid; seeds winged¹³. *L. lanceolata* has been used in the treatment of asthma, diabetes mellitus, chronic bronchitis, cold and cough. Seeds have been documented for its multiple pharmacological activities including narcotic principle. Steroid, terpenoids, phenols, flavonoids, alkaloids, ellagic acid and tannins are the major components present in the plant¹⁴. *L. parviflora* Roxb (Lythraceae) is a medium-sized deciduous plant indigenous to India and available even up to a height of 900 m in the Himalayas. The plant is used for the treatment of syphilis, sores and carbuncles¹⁵. Mazumder *et al.* (2003)¹⁶ reported the antibacterial activities of the leaves of the plant and Bhakuni *et al.* (1969)¹⁷ reported the anti-asthmatic activity of the flowers of *L. parviflora*. The leaf juice of this plant is used in traditional medicine to treat fever in Jharkhand, India¹⁵. *L. lanceolata* and *L. parviflora* contain quercetin as an important active constituent and is predictable by HPLC method. Phytochemical assessment is one of the tools for the quality evaluation, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using current analytical techniques. In the last two decades HPTLC method has appeared as a significant tool for the qualitative and quantitative

phytochemical analysis of herbal drugs and formulations. This includes TLC fingerprint profiles and estimation of chemical markers and biomarkers. The chief advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase.

MATERIAL AND METHODS

Plant Material

The leaves of *L. lanceolata* (Voucher no. 550/ACF/TNWS/TAXON/14-15) and *L. parviflora* (Voucher no. 1240) were collected from Tansa Wildlife Sanctuary, Asangaon, Shahapur, Maharashtra and Tirupati, Andhra Pradesh respectively in March 2014. The leaves were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf was air dried under room temperature. The dried plant samples were grinded to make it in powder form. The powdered samples were stored in clean, dry and sterile container for further use.

Reagents and Materials

All chemicals and solvents used were of analytical grade and obtained from SD Fine-Chem. Ltd. (Mumbai, India). The standard Quercetin was purchased from Lobo Chem, Mumbai, India (purity >97%). Stock solutions (1 mg/ml) of the standards were prepared daily in methanol immediately before use. TLC aluminum plates pre-coated with silica gel 60 F254 (100 x 100 mm, 0.2 mm thick) used were obtained from E. Merck Ltd (Mumbai, India).

Extraction of Plant Material for HPTLC Analysis

The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place. 200 gm of dried plant material (leaves of *L. lanceolata* and *L. parviflora*) was exhaustively extracted with methanol as solvent using maceration method. The extract was evaporated above their boiling points and stored in an airtight container, free from any contamination until it was used.

Preparation of Standard and Sample Solutions

Quercetin standard stock solutions were prepared by dissolving 10 mg of it in 10 ml of methanol. From this 2 µl solution was applied using sample applicator. 100 mg of methanolic extract of *L. lanceolata* and *L. parviflora* was dissolved in 10 ml of methanol and filtered. The filtrate (10 mg/ml) was used for the HPTLC chemo profiling.

TLC Study

Thin layer chromatography was conducted to study the number of compounds present in the extract. The adsorbent used for thin layer chromatography was silica gel 60 F 254. The pre-coated TLC plate (Merck, Germany) was heated in an oven for activation. 1 ml of the standard flavonoid quercetin and the methanol extract were applied, dried and then set aside in the developing chamber. The chamber was drenched with the solvents for 20 min at room temperature. Numerous solvent systems were tried to recognize an appropriate developing solvent system for the separation of compounds. After the progress of the plate, it was air-dried, then the numbers of spots were noted and Rf values were calculated.

HPTLC Analysis

Instrument: CAMAG Automatic TLC Sampler, with win CATS software.

Stationary phase: TLC plates silica gel 60 F 254 pre coated layer (20 cm X 10 cm), thickness 0.2 mm.

No. of tracks: 8, band length: 8 mm.

Mobile phase: Toluene: Ethyl acetate: Formic acid (7:5:1)

Standard: Light yellow powder

Sample: Brown powder

Solubility: Methanol

Standard concentration: 50 µg/ml

Standard Injection volumes (µl): 2, 4, 6, 8, 10 µl

Sample concentration: 1 mg/ml

Sample application volumes (µl): 2 µl

Development chamber: Twin trough chamber (20 X 10)

Development mode: Ascending mode

Distance run: 75 mm

Scanning wavelength: 254 nm

Preparation of the Plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA). 100 µg/ml of the standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 2, 4, 6, 8 and 10 µl and the sample concentration was 10 mg/ml. The mobile phase used was toluene: ethyl acetate: formic acid (7:5:1). The chromatograph was developed, dried at room temperature and scanned at 254 nm. The average peak area of the standard was calculated. Regression equation was obtained, via the Win Cats software using the calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis).

Estimation of Quercetin in Herbal Extracts

Estimation of quercetin in extracts of *L. lanceolata* and *L. parviflora*. Using the regression equation obtained from the standard curve, the content of quercetin was quantified, and the mean peak height/area of the sample was calculated.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of methanol extracts of the leaves of *L. lanceolata* and *L. parviflora* revealed the occurrence of amino acids, phenols, sterols, alkaloids, terpenoids, carbohydrates, flavonoids and tannins. The TLC procedure was optimized with a sight to separate the compounds and to recognize one of the phytochemical flavonoids (quercetin) in the extract. At first toluene: ethyl acetate: formic acid in unreliable ratios was tried along with numerous combinations of other solvents. The final system consists of toluene: ethyl acetate: formic acid (7:5:1v/v/v) which gave a sharp and well-defined band with Rf = 0.64 for quercetin. The identity of the quercetin

bands in sample chromatograms was established by the chromatogram obtained from the sample and reference standard solution¹⁸. HPTLC analysis of the methanol leaf extract of *L. lanceolata* and *L. parviflora* was carried out along with the standard quercetin and toluene: ethyl acetate: formic acid (7:5:1) as the mobile phase. The number of bands obtained were three to four (Figure 1-3). The use of standard makes sure the concentration and ratio of the quercetin compound in the leaf (Figure 4-6). This result matches with the study of Sachin *et al.*,¹⁹ who finds a good correlation of quercetin in the dried flowers of *Nymphaea stellata*. HPTLC studies revealed well resolved peaks of extracts containing quercetin. The spots of the entire chromatogram were visualized under UV 254 nm and the percentage of quercetin (Rf 0.64) in *L. lanceolata* and *L. parviflora* extracts was found to be 0.05751 and 0.8377% Table 1. HPTLC fingerprinting is established to be an accurate, liner, precise method for herbal identification and can be used further in authentication and characterization of the medicinally important plant²⁰.

Figure 1: Thin layer chromatography in normal light

Figure 2: Thin layer chromatography in short U.V

Figure 3: Thin layer chromatography in long U.V

Figure 4: Chromatogram of standard quercetin

Figure 5: Chromatogram of *L. lanceolata* extract Figure 6: Chromatogram of *L. parviflora* extract

Table 1: Results of HPTLC estimation

Estimation	Area	<i>L. lanceolata</i> (%)
Quercetin	390.6	0.0039
	5442.8	<i>L. parviflora</i> 0.054482

CONCLUSION

The phytochemical studies showed the occurrence of the majority of the biologically active constituents in the plant. It is usually realized that for monitoring superiority, HPTLC fingerprinting is perfect which involves assessment between a standard and a sample. The chromatographic studies conducted with the methanol leaf extract of *L. lanceolata* and *L. parviflora* revealed an appreciable amount of flavonoid quercetin, which confirms its medicinal value.

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