



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Document heading doi:

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## Detection and quantification of quercetin in roots, leaves and flowers of *Clerodendrum infortunatum* L.

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### ARTICLE INFO

#### Article history:

Received 15 August 2012

Received in revised form 18 September 2012

Accepted 19 November 2012

Available online 28 December 2012

#### Keywords:

Bhant

*Clerodendrum infortunatum* L.

HPTLC

Quercetin

### ABSTRACT

**Objective:** To detect and quantify the concentrations of Quercetin in the root, leaves and flower test sample of *Clerodendrum infortunatum* L. **Method:** In the present study High Performance Thin Layer Chromatography has been developed for detection and quantification of Quercetin in *Clerodendrum infortunatum* L. Increasing serial dilutions of reference standard Quercetin (20 to 100  $\mu$ g/ml) were scanned at 366 nm to detect and quantify the concentrations of Quercetin in the test sample. **Result:** The estimated values obtained from the same were 0.05mg/g, 0.111mg/g and 0.199mg/g of Quercetin in powdered root, leaves and flower sample respectively. **Conclusion:** The method provided a rapid and easy approach for detection and the quantitation of the bio-marker Quercetin. In the present study we established the HPTLC profile for the vegetative and reproductive parts of *Clerodendrum infortunatum* L. to detect and quantify the Quercetin.

## 1. Introduction

*Clerodendrum infortunatum* Gaertn. is known in Ayurveda by the Sanskrit names “Bhargi”, “Bhrigubhava”, “Padma”, “Fanji” and “Brahman yastika”, as “peruvelam” in Kerala, and in Hindi as “Bhant” or “Bharangi”<sup>[1]</sup>. The first description of the genus was given by Linnaeus in 1753, with identification of *C. infortunatum*. *Clerodendrum* is a very large and diverse genus and are widely distributed in Asia, Australia, Africa and America<sup>[2]</sup>. The plant is useful as an excellent laxative cholagogue, anthelmintic, ascarides, antiperiodic, febrifuge, in malarial fever, in torpidity of the liver, in dysentery etc<sup>[3]</sup>. Various parts of the plant have been used by tribes in colic, scorpion sting, snake bite, tumour and certain skin diseases, also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy<sup>[4]</sup>. The roots have been reported to possess laxative, diuretic, analgesic, anti inflammatory, anti tumour

and antibacterial activities<sup>[5]</sup>. Leaves of the plant are used as bitter tonic, antiperiodic, vermifuge, pain killer, laxative and cholagogue. The leaves and roots are externally used for tumors and in certain other skin diseases as paste. The medical practitioners of Bhadra Wild Life Sanctuary (Karnataka, India), are using the tender leaf paste to cure cut wounds and leprosy since long time<sup>[6]</sup>. The tribal of Chotanagpur region uses the leaves of the plant in preparing traditional expectorant pills<sup>[7]</sup>. The aerial part of the plant contains sterols; the root contains  $\beta$  sitosterol, lupeol and steroidal glycosides; the leaf contain a diterpene clerodin and the flower contains  $\beta$ –sitosterol, lupeol, cleridine, hentriacontane and Fumaric acid esters of Caffeic acid<sup>[8]</sup>. Quercetin (3, 3', 4', 5', 7–pentahydroxyflavone) is a flavonoid that forms the chemical backbone for other flavonoids. Quercetin offers several potential therapeutic uses in the prevention of CVD, cancer, cataract, schizophrenia and prostatitis<sup>[9]</sup>. Flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity, anti-cancer, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic anti-tumour activity<sup>[10]</sup>.

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## 2. Materials and methods

### 2.1. Collection and Authentication

The plant *Clerodendrum infortunatum* L. were collected from local areas of Lucknow in the month of August 2011 and authenticated by National Botanical Research Institute {N.B.R.I., (C.S.I.R.)} Lucknow, also a voucher specimen was submitted for future reference (Ref No. NBRI/CIF/293/2012). The air dried plant material was first washed with running tap water, then again washed twice with double distilled water and the washed specimens (root, leaves, flower) was air-dried and size comminuted to a moderately fine powder and stored in an air-tight container at 25°C for future/further studies.

### 2.2. Extraction of plant material

The powdered drug (root, leaves, and flower) after defatting with petroleum ether (60–80°C) for 48h was successively extracted with methanol and water for 48 hrs in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

### 2.3. TLC profile

TLC fingerprint profiles were carried out by preparing the extract with 10ml methanol and 0.01g powdered drugs. TLC of this alcoholic extract on Silica gel “G” plate using solvent system, Toluene: Ethyl acetate: Formic acid (5: 4: 1) shows the spots.

### 2.4. High performance thin layer chromatography (HPTLC) fingerprinting

The preliminary phytochemical investigation of the Methanol extract of Root, Leaves and Flower of *Clerodendrum infortunatum* L. showed the presence of Flavonoid. Hence the Methanol fraction was used for HPTLC studies to detect and quantify the Quercetin in the above mentioned extracts. Solvents: All the solvents used were of AR grade from Sigma Aldrich and SD Fine Chem.

Reference standard: The reference standard (Quercetin) was obtained from Sigma Aldrich, USA, through an authorized institutional supplier M/S Sohan Lal & Sons.

HPTLC plate: Silica gel GF254 (Merck) 10 X 10 cm

Mobile phase: Toluene: Ethyl Acetate: Formic acid (5:4:1)[2].

Wavelength: 366 nm

Standard preparation: A stock solution of Quercetin (100  $\mu\text{g mL}^{-1}$ ) was prepared by dissolving 1.0 mg of accurately weighed Quercetin in methanol and diluting it to 10.0mL with methanol. Further dilutions were made with methanol to obtain working standards 20, 40, 60, 80 and 100  $\mu\text{g mL}^{-1}$ .

Sample preparation: A 10 mg of Methanol extract of Root,

Leaves and Flower was dried and was redissolved to a final volume of 10mL with methanol to obtain test samples (1000  $\mu\text{g mL}^{-1}$ ).

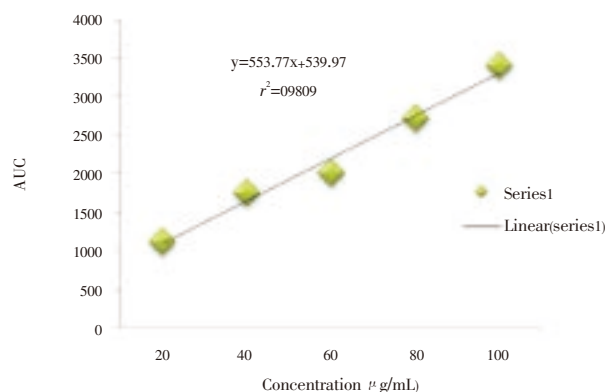
Procedure: The TLC plate was activated by placing in an oven at the temperature of 110 °C for 20 min. the plate was spotted with test and standard preparation maintaining a distance of 15mm from the edge of TLC plate. It was developed up to 75mm in the twin trough chamber using mobile phase, dried in an oven and subjected for TLC scanning at 366nm[11].

## 3. Results

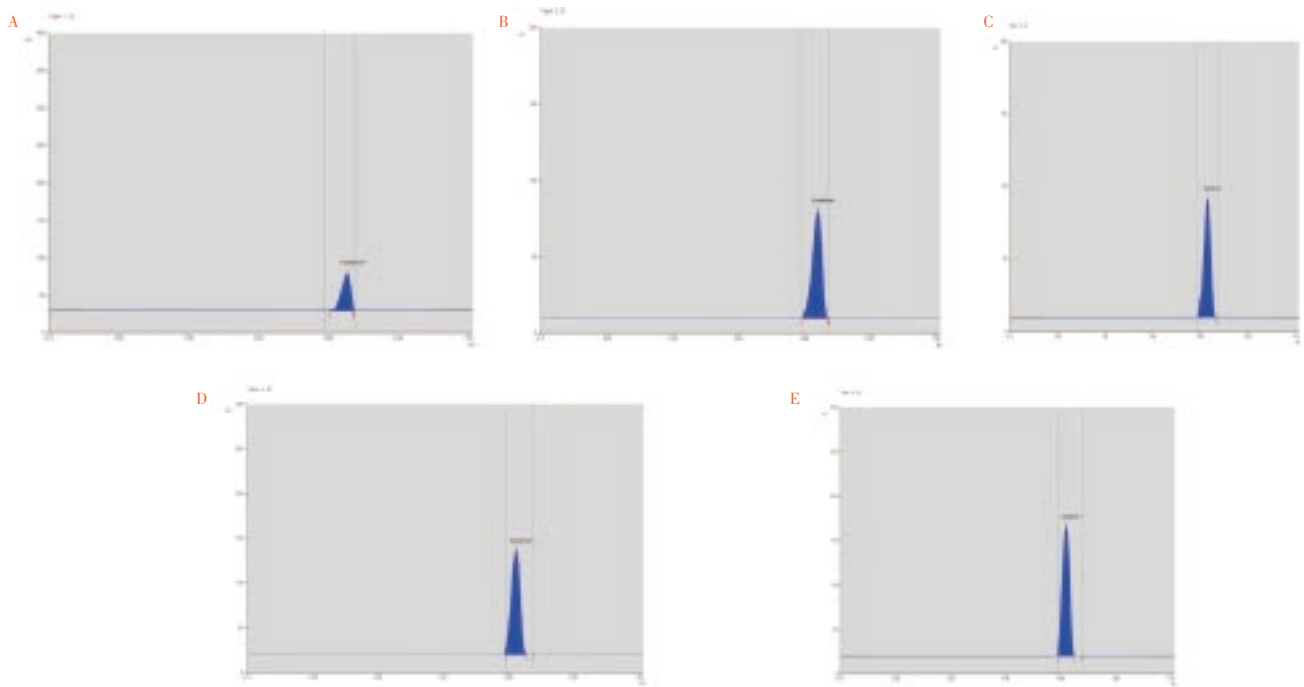
Plant materials were extracted in methanol using hot continuous extraction process (Soxhlet) method. The extracts obtained were dried under vacuum and the percentage yield was found to be maximum in methanolic extract of flower (2.82%)> methanolic extract of leaves (2.22%)> methanolic extract of roots (0.356%). Then these dried extracts were subjected to chromatographic studies. Under the chromatographic conditions described above, the Rf value of Quercetin was determined to be approximately 0.73 for *Clerodendrum infortunatum* L. The chromatograms of standard Quercetin i.e. track peaks were shown in Figure 1 (a–e) and that of Quercetin in different part of *Clerodendrum infortunatum* L. methanol extract were shown in Figure 2 (a–c). The respective Rf's obtained for each track shown in Table 1. The 3D spectra of all tracks scanned at 366 nm were shown in Figure 3 (a–b). The calibration curve was linear in the range of 20 to 100  $\mu\text{g mL}^{-1}$  illustrated in Figure 4. From the regression equation,  $y = 553.77x + 539.97$ , the amount of Quercetin on per gram basis of powdered drug was found to be maximum in flower followed by the leaves and root, as shown in Table 2.

**Table 1.** Maximum Rf and AUC of Standard Quercetin (Track 1–5)

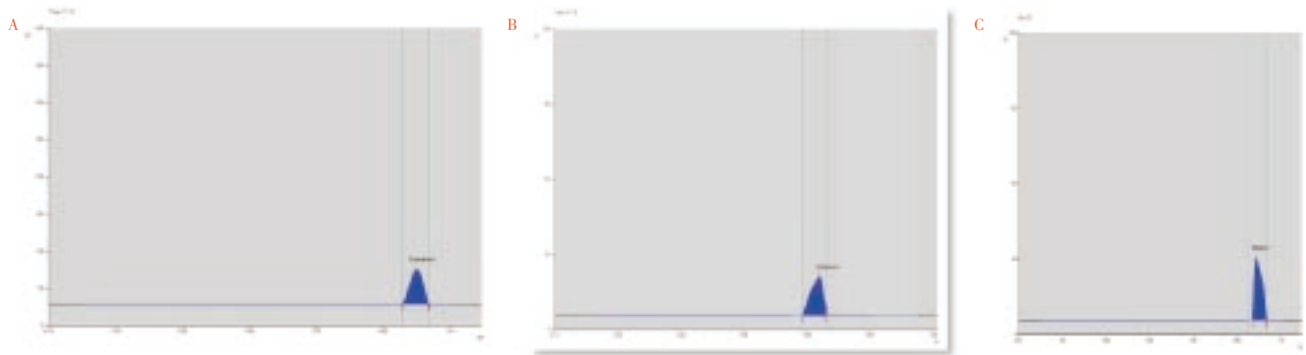
Tracks of Standard	Concentration ( $\mu\text{g/ml}$ )	Rf	AUC
Track 1	20	0.75	1118.7
Track 2	40	0.74	1754.6
Track 3	60	0.73	2010.7
Track 4	80	0.72	2715.1
Track 5	100	0.71	3407.3



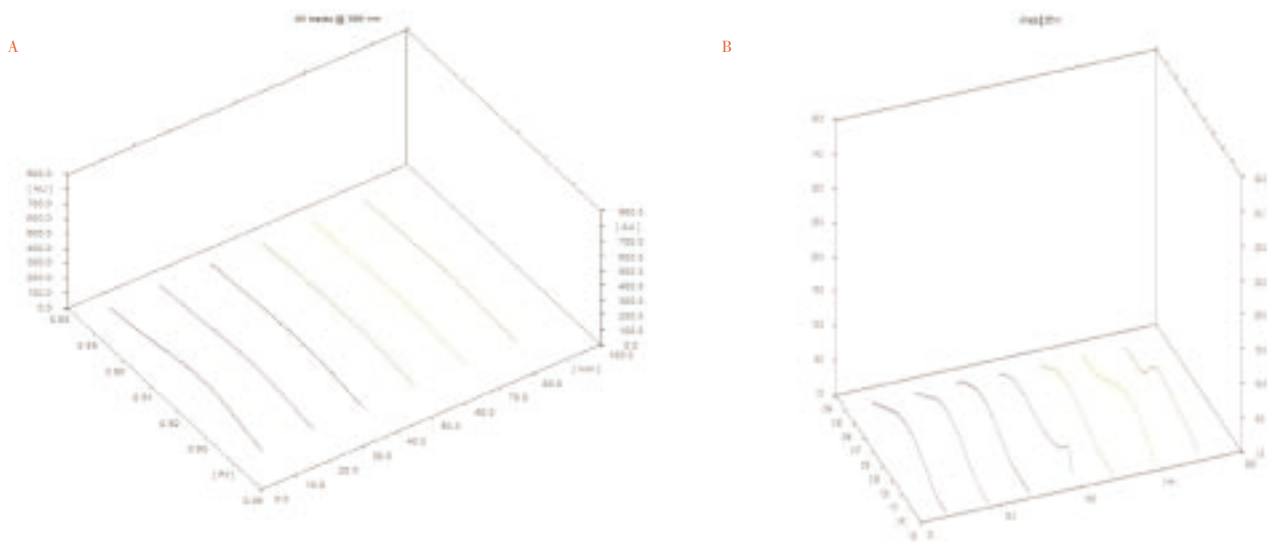
**Figure 4.** Calibration Curve for Standard Quercetin.



**Figure 1.** A: HPTLC chromatogram of Quercetin working standard Track 1; B: HPTLC chromatogram of Quercetin working standard Track 2; C: : HPTLC chromatogram of Quercetin working standard Track 3; D: : HPTLC chromatogram of Quercetin working standard Track 4; E: : HPTLC chromatogram of Quercetin working standard Track 5.



**Figure 2.** A: HPTLC chromatogram of Quercetin in *C. infortunatum* L. Root (Track 6–1000  $\mu$ g/ml); B: HPTLC chromatogram of Quercetin in *C. infortunatum* L. Leaves (Track 7–1000  $\mu$ g/ml); C: HPTLC chromatogram of Quercetin in *C. infortunatum* L. Flower (Track 8–1000  $\mu$ g/ml).



**Figure 3.** A: 3D Spectra of Tracks 1–5 (Standard) and Track 7 (Leaves) scanned at 366 nm; B: 3D Spectra of Tracks 1–5 (Standard), Track 6 (Root) and Track 8 (Flower) scanned at 366 nm

#### 4. Discussion

Polyphenols like quercetin have recently gained considerable attention by plant researchers due to its prophylactic as well as therapeutic properties in the prevention of Cardio vascular diseases (CVD), cancer, cataract etc. Quercetin has recently been proven to have ameliorating effect on C.N.S. functions particularly learning and Memory. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation and quantification of botanical materials. In the present study High Performance Thin Layer Chromatography has been used for detection and quantification of Quercetin in different parts of the plant *Clerodendrum infortunatum* L. The 3D spectra obtained from the present study bring out the spectra's for all tracks viewed together and are suggestive of similarities between the test tracks and the standard tracks also elucidating strong presence of the biomarker in the plant extracts. From the HPTLC it was found that Quercetin was found to be highest in flowers of *Clerodendrum infortunatum* L. followed by leaves and root.

#### Conflict of interest statement

We declare that we have no conflict of interest.

#### Acknowledgement

The authors are thankful to Prof. (Dr.) Shubhini A. Saraf for providing the necessary laboratory facilities and also to AICTE–MODROBS grant (F. No. 8024 / RID / BOR / MOD–458 /2009–10) for making the research work possible on selected plants.

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