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# DEVELOPMENT OF VALIDATED HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY DENSITOMETRIC METHOD FOR QUANTIFICATION OF QUERCETIN IN *CAPPARIS DECIDUA* EDGEW. STEM

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**Keywords:**

*Capparis decidua*, Capparidaceae, Quercetin, High-performance thin-layer chromatography

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**ABSTRACT:** *Capparis decidua* Edgew. (Capparidaceae) is an important medicinal plant widely used in Ayurveda as anti-inflammatory, anti-arthritic, hepatoprotective, diuretic, digestive, laxative, analgesic and anti-asthmatic. In this study, a sensitive and reliable high-performance thin-layer chromatography (HPTLC) method has been developed for the quantification of a flavonol quercetin as an important marker in *C. decidua* stem. Ethyl acetate extract prepared from hydrolyzed alcoholic extract was applied on precoated silica gel G 60F254 plate and the plate was developed using chloroform-methanol-formic acid (9:1:0.6, v/v/v) as the mobile phase. The detection and quantification was carried out by densitometric scanning at 366 nm. The system was found to provide good resolution of quercetin from other constituents present in *C. decidua* stem. The method was validated for linearity, inter-day and intra-day precision, repeatability, accuracy, specificity, limit of detection and limit of quantification. The linear regression analysis data for the calibration plots showed a good linear relationship ($r^2 = 0.995$) in the concentration range of 500-1500 ng. The accuracy of the method was confirmed from recovery studies conducted at different levels using the standard addition method. The developed method was found to be simple, precise, sensitive, accurate and reproducible. The study is the first report for estimation of quercetin in *C. decidua* stem by HPTLC method and would serve as a suitable tool for routine analysis and authentication of *C. decidua*.

**INTRODUCTION:** *Capparis decidua* Edgew. (Capparidaceae), commonly known as 'Caper plant' and 'Karira' [1-3], is a large, 1.5 to 2 m high, straggling, densely branching, glabrous shrub or a small tree and usually leafless or with scanty, small, caducous leaves found only on young shoots [4, 5].

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It grows wildly in dry places, bare grounds, waste lands and is also common in the plains and at the foothills, especially in the Deccan Peninsula, Rajasthan, Punjab, Sind, Balochistan, Arabia, Socotra, Egypt and tropical Africa. It is indigenous to India and distributed throughout the Central India, Gujarat and Tinnevelly [1, 4, 6].

Various parts of the plant are highly valued for its medicinal properties in different traditional systems of medicine. In Ayurveda, it is used as anti-inflammatory in arthritis and other inflammatory diseases and also for treatment of skin diseases and jaundice [7-10]. It is also considered to be useful as diaphoretic, diuretic, anthelmintic, laxative, digestive,

carminative, tonic in general weakness and fairly used in management of 'vata', dry cough and asthma [9, 10]. Traditional healers prescribe it in phthisis, heart diseases, colic pains, loss of appetite and scurvy. Juice of fresh plant is dropped into the ear to kill worms and also used in toothache [3, 8, 11].

In Punjab, the young shoots and leaves are made into powder and used in boils, eruptions, swellings, affections of the joints and as an antidote to poison [1, 4, 12]. The young shoots and leaves are also useful in toothache when chewed [4, 13]. The tips of fresh young twigs are crushed and soaked in water and eaten with butter to give relief from pain after a bruise or fall [1]. Young stems are used in dry cough along with Calotropis leaves [14].

Crushed young shoots are mixed with flour of wheat, which is then fed to animals to relieve pain in their bodies. Paste of tender branches made by adding castor oil is applied externally to the animals having infested maggots, bleeding wounds and acute pain. Stem bark is crushed to make poultice which is prescribed for the treatment of wounds [3, 15].

In Unani medicine also, the plant is used as carminative, tonic, emmenagogue, aphrodisiac and alexipharmic. It is also utilized in improving the appetite and in treatment of rheumatism, lumbago, hiccough, cough and asthma [1]. In Sudan, the plant is widely used in jaundice, swellings and infection of joints [15]. Several phytochemical investigations have revealed the presence of many bioactive compounds in the plant including isothiocyanate glucoside like glucocapparin [16]; phenolic compounds like gallic acid, coumaric acid, apigenin, kaempferol and epicatechin [17]; phenolic acids like p-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, vanillic acid, genetisic acid, 2-hydroxy-6-methoxybenzoic acid, ferulic acid and o- and p-coumaric acids [18]; flavonol glycosides named kaempferol-3-rutinoside, rutin, quercetin-7-rutinoside and quercetin-3-glucoside-7-rhamnoside [19], sterols like β-sitosterol [20-22], 24-β-methylcholest- 7- ene- 22- one- 3β- ol and [24] β-methylcholest- 9(11)- ene-22-one-3α-ol [23], alkaloid stachydrine [21, 22], spermidine alkaloids named isocodonocarpine [24,] 14-N-acetylisocodonocarpine, 15-N-acetylcapparisine [25], capparisinine [26], cappa-ridisine [27]; oxygenated heterocyclic compounds, capparisesterpenolide and decidua terpenolides A-E [28]. Quercetin, a flavonol, is one of the important phytoconstituents responsible for the therapeutic potential of the plant which is required to be quantified. A literature survey revealed that no HPTLC method has been reported for the estimation of quercetin in *C. decidua*. The present study is the first attempt to establish a simple, accurate and precise HPTLC method for the quantification of quercetin in *C. decidua* stem as an important marker compound for its routine analysis.

## MATERIALS AND METHODS:

**Plant Material and Chemicals:** The fresh stems of *C. decidua* were collected from Gujarat University campus area, Ahmedabad during the flowering season and authenticated by a taxonomist at the Department of Botany, Gujarat University, Ahmedabad. The authenticated plant specimen was deposited in the Department of Pharmacognosy of L. M. College of Pharmacy, Ahmedabad for future reference with specimen number LMCP/ PHARMACOGNOSY/2011/29. After collection, the stems were cleaned, dried at room temperature, powdered to 60# and then used for the present study.

Reference standard quercetin (purity 99.9%) was procured from Sigma-Aldrich Chemicals (India). Methanol, ethyl acetate, hydrochloric acid, chloroform and formic acid (E. Merck, Mumbai, India) of analytical grade were used.

**Preparation of Standard Solution:** A stock solution of standard quercetin was prepared by dissolving an accurately weighed 5 mg of quercetin in 5 ml of methanol in a volumetric flask (1 mg/ml). 0.25 ml of the above solution was further diluted up to 5 ml in a volumetric flask with methanol to prepare a working standard solution of 50 µg/ml for chromatographic analysis.

**Preparation of Sample Solution:** Accurately weighed 10 g of powdered drug was exhaustively extracted with 100 ml of methanol for 2 h, filtered and filtrate was further refluxed with 25 ml of 15% methanolic hydrochloric acid for 1 h. The mixture was extracted with 20 ml (× 3) ethyl acetate, the fractions were collected and combined. Solvent was then evaporated under vacuum.

50 mg of dried residue was dissolved in ethyl acetate in a 5 ml volumetric flask and volume was adjusted up to the mark with ethyl acetate to get a concentration of 10 mg/ml. The test solution was applied to the plate for chromatographic analysis.

**Chromatographic Method:** HPTLC analysis was performed on 10 cm × 10 cm precoated HPTLC plates of silica gel G $60F_{254}$ (E. Merck, Darmstadt, Germany). The plates were prewashed with methanol and activated in an oven at 120°C for 20 min prior to analysis. The standard and sample solutions were applied to the plates as bands 8 mm wide, 12 mm apart, 10 mm from bottom edge and 10 mm from side edge by means of CAMAG Linomat-IV semi-automatic spotting device (CAMAG, Muttenz, Switzerland) fitted with a 100 µl Hamilton HPTLC syringe. The linear ascending development was performed to a distance of 80 mm using chloroform-methanol-formic acid (9:1:0.6, v/v/v) as mobile phase in CAMAG twin trough glass chamber (20 cm × 10 cm), presaturated at room temperature (25 °C and 40% RH) with mobile phase vapor for 30 min before development. The plate was dried in air after development and immediately scanned at 366 nm using a CAMAG TLC Scanner operated by winCATS 4 integration software, having slit dimensions 5.0 mm × 0.1 mm and deuterium lamp as the source of radiation. Photodocumentation of the plate was done using a TLC visualizer (CAMAG Reprostar-3).

**Quantification of Quercetin:** A series of standard solutions were prepared from the stock solution and amounts in the range of 500 to 1500 ng for quercetin were applied to the plate. Calibration curve was derived by plotting the area *vs.* concentration of each peak corresponding to the respective spot. 10 µl of test solution was spotted along with standard quercetin solution on the HPTLC plate. The peak areas were noted and quantification of quercetin was performed using linear regression equation of the compound.

**Validation of the HPTLC Method:** The method was validated as per International Conference on Harmonization (ICH) guidelines in terms of linearity, inter-day precision, intra-day precision, repeatability, accuracy, specificity, limit of detection and limit of quantification [29]. From the calibration curve data, the correlation coefficient, slope, intercept and regression equation were calculated to determine the degree of linearity. Prior to method validation process, instrument precision was evaluated in terms of sample application, positioning of TLC scanner stage and repeated scanning of the same spot. A standard solution of quercetin of 50 µg/ml concentration was used. To check precision of sample application, 15 µl of the solution was repeatedly applied on the plate to make seven bands.

To check the reproducibility of scanning, the same spot was scanned seven times without changing the plate position and the results were analyzed. The intra-day precision was determined by analyzing quercetin (500, 1000 and 1500 ng/spot) three times on the same day, while inter-day precision was determined by performing the same analysis daily for three days. The repeatability of the measurement of the peak area was performed by sevenfold measurement of the same spot.

For repeatability of the sample application, the same volume of the standard solution was applied seven times and the area was measured for the peaks. The limit of detection (LOD) and limit of quantification (LOQ) were measured at signal-to-noise ratios of 3:1 and 10:1, respectively. Accuracy of the method was performed by conducting recovery studies using standard addition method. The known concentration of analyte were added at three levels (50, 100 and 150% of working concentration) to the predetermined amount of sample solution and estimating the quantity of the analyte using the proposed method.

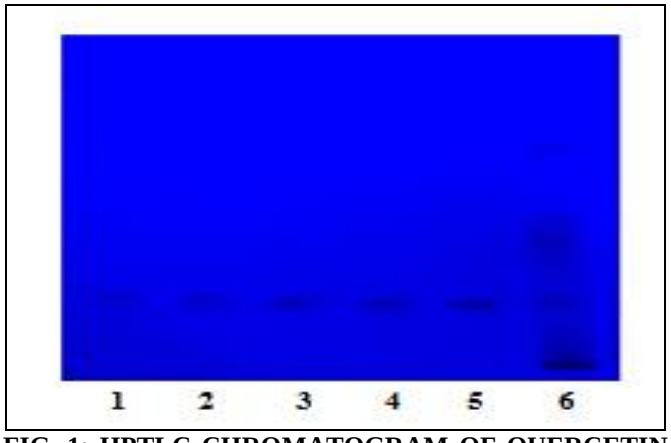


**FIG. 1: HPTLC CHROMATOGRAM OF QUERCETIN WITH TEST EXTRACT OF *C. DECIDUA* SCANNED AT** 366 NM (1-5: Standard quercetin solutions; 6: Test extract of *C. decidua* stem)

## RESULTS AND DISCUSSION:

**Quantification of Quercetin in the Prepared Sample:** The identity of quercetin was confirmed by co-chromatography and overlain absorption spectra with reference standard, when scanned at 366 nm. The pattern of HPTLC chromatogram of the test extract of *C. decidua* stem and the calibration curve are presented in **Fig. 1** and **Fig. 2**, respectively.

The densitometric chromatogram of HPTLC fingerprint of test extract is shown in **Fig. 3**. The peaks resolving at $R_F$ 0.24 in test solution were found to be superimposing with that of quercetin standard. Peak purity was studied by UV overlay spectra obtained from the standard and from the corresponding peak from test **Fig. 3**.

The content of quercetin was found to be 0.494 ± 0.014% (w/w) in the test extract of ***C. decidua*** stem, while its concentration in stem powder was calculated to be 0.012 ± 0.001% (w/w).

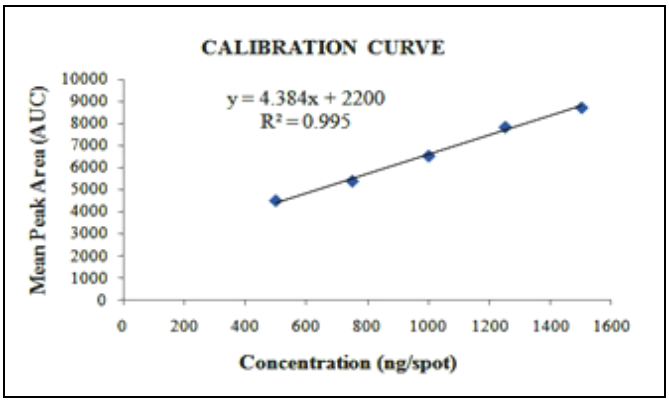


**FIG. 2: CALIBRATION PLOT OF STANDARD QUERCETIN**

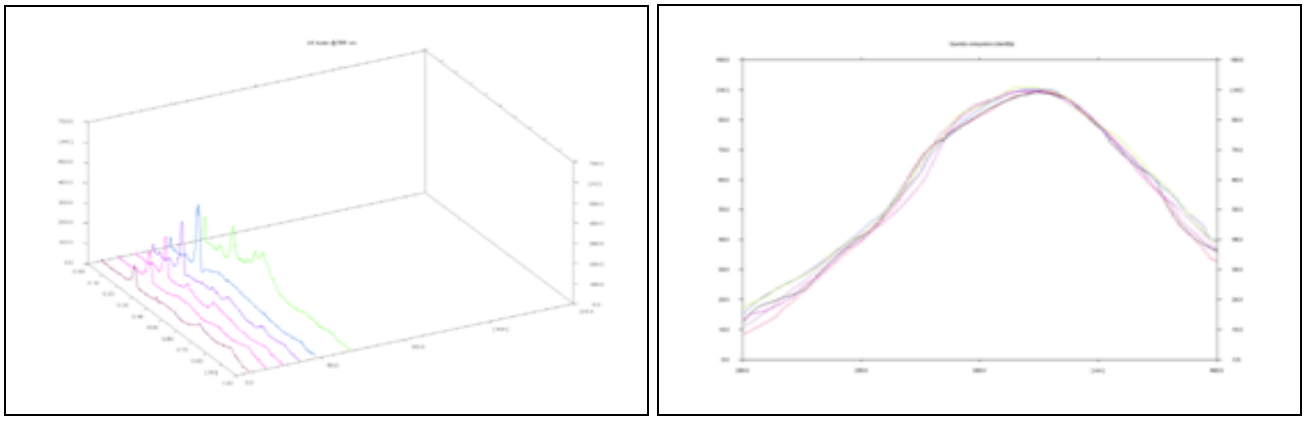

**FIG. 3: DENSITOMETRIC CHROMATOGRAM AND PEAK PURITY SPECTRA OF QUERCETIN WITH TEST EXTRACT OF *C. DECIDUA* SCANNED AT 366 NM**

**TABLE 1: VALIDATION DATA OF DEVELOPED HPTLC METHOD**

| Parameters | Quercetin |
|---|---|
| Linearity range (ng per band) | 500-1500 |
| Correlation coefficient ($r^2$) | 0.995 |
| Intra-day precision (% RSD) | 0.32-1.05 |
| Inter-day precision (% RSD) | 0.35-2.30 |
| Repeatability of measurement (% RSD) | 0.864 |
| Repeatability of application (% RSD) | 1.65 |
| LOD (ng per band) | 48.94 |
| LOQ (ng per band) | 148.29 |

**Validation of Developed HPTLC Method:** The regression data obtained for quercetin showed good linear relationship. The data of intra-day precision, inter-day precision, repeatability of application, repeatability of measurement, LOD and LOQ are shown in **Table 1**. The Relative Standard Deviation (RSD) for intra-day and inter-day precisions varied from 0.32 to 1.05% and 0.35 to 2.30%, respectively.

The low values of Relative Standard Deviation (RSD) indicated that the developed method is quite precise for the estimation of quercetin. The method was also found to be specific for quercetin because it resolved the standard properly in the presence of other phytoconstituents in *C. decidua* stem.

**TABLE 2: RESULTS OF RECOVERY STUDY FOR QUERCETIN MARKER**

| Amount present (ng band$^{-1}$) | Amount spiked (ng band$^{-1}$) | Amount found (ng band$^{-1}$) | % Recovery | Average % recovery |
|---|---|---|---|---|
| 493.955 | 250 | 740.5 ± 119.22 | 98.64 ± 1.41 | |
| 493.955 | 500 | 984.1 ± 81.14 | 98.04 ± 1.27 | 98.65 |
| 493.955 | 750 | 1238.5 ± 129.43 | 99.28 ± 1.16 | |

The study of accuracy revealed negligible influences of other components present in matrix on the quantification parameters and also indicated that the method is accurate.

The results of the recovery study are given in **Table 2**. The results suggested that the developed HPTLC method is precise and can be used for the routine analysis of this marker in *C. decidua* stem.

**CONCLUSION:** The marker compound quercetin were quantified by validated HPTLC densitometric method for the first time in *Capparis decidua* stem. The proposed method provides good resolution of each marker from other constituents present in the test extract of *C. decidua* stem. The peak resolving in test solution was found to be superimposing with that of the standard quercetin. Peak purity was also ascertained by UV overlay spectra obtained from the standard and from the corresponding peak from the test. Thus, the developed method would be useful for both qualitative and quantitative analysis of *C. decidua* stem. Since, the proposed method is simple, precise, reliable, reproducible and economical, it can be applied for the routine analysis of the plant of *Capparis decidua* Edgew.

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**CONFLICTS OF INTEREST:** The authors declare that they have no conflict of interest.

## REFERENCES:

1. Kirtikar KR and Basu BD: Indian Medicinal Plants. M/s Bishen Singh Mahendra Pal, Delhi, Second Edition 1975.
2. Varier PS: Indian Medicinal Plants- A Compendium of 500 species. Orient Longman Ltd., Madras, 1994.
3. Nadkarni KM: Indian Materia Medica- Vegetable Kingdom. Popular Book Depot, Mumbai, Edition 3, 1954; 1: 265.
4. CSIR: The Wealth of India- A dictionary of Indian Raw Materials and Industrial Products. Council of Scientific and Industrial Research, Delhi, 1950; 2: 67.
5. Cooke T: Forest Flora of the Bombay Presidency and Sind. Bishen Singh Mahendra Pal Singh, Dehradun and Periodical Experts, Delhi 1976; 1: 51-64.
6. Patel RI: Forest Flora of Gujarat State. Forest department, Gujarat State, Government Press, Baroda, 1971; 64-65.
7. Khemraj K: Haritakyadinighantu. Steam Press, Bombay, 1938; 206-07.
8. Chaube D: Abhinavanighatu. Bombay Bhushan Press, Mathura 1986; 154.
9. Sharma PV: Dravyaguna Vigyana. Chaukhambha Bharti Academy Publisher, Varanasi, , Second Reprint 2005; 3: 261.
10. Shashtri GM: Bhavaprakash - Purvakhand. Sastu Sahitya Vardhak Karyalaya, Mumbai, First Edition 1958; 368.
11. Vyas HB: Aryabhishek. Sastu Sahitya Vardhak Karyalaya, Mumbai, Eleventh Edition 1951; 176-78.
12. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, 1956; 49-50.
13. Chunekar KC and Pandey GS. Bhavprakash Nighantu. Chaukhambha Bharti Academy Publisher, Varanasi, Eight Edition 1999; 546.
14. Bapalal G: Dravyguna Shashtra. University Granthnirman Board, Ahmedabad, Second Edition 1995; 454-56.
15. Marwat SK, Rehman F, Usman K, Khakwani AA, Ghulam S, Anwar N, Sadiq M and Khan SJ: Medico-ethnobotanical studies of edible wild fruit plant species from the flora of North-Western Pakistan (D.I. Khan District). Journal of Medicinal Plants Research 2011; 5(16): 3679-86.
16. Juneja TR, Gaind KN and Panesar AS: Investigations on Capparis decidua Edgew. study of isothiocyanate glucoside. Res Bull (N.S.), Panjab University 1970; 21: 519-21.
17. Imran I, Muhammad Z, Calani L, Mazzeo T and Pellegrini N: Phenolic profile and antioxidant potential of selected plants of Pakistan. J Appl Bot Food Qual 2013; 87: 30-35.
18. Daniel M and Sabnis SD: Chemotaxonomical studies on Capparidaceae - Cleomaceae. Curr Sci 1977; 46: 472-74.
19. Mohamed S, Mohamed A and Nabiel AMS: Flavonoids of four cleome and three capparis species. Biochemical Systematics and Ecology 1997; 25(2): 161-66.
20. Rathee S, Mogla OP, Rathee P and Rathee D. Quantification of β-sitosterol using HPTLC from Capparis decidua (Forsk.) Edgew. Der Pharm Chemica 2010; 2(4): 86-92.
21. ICMR: Quality Standards of Indian Medicinal Plants. Indian Council for Medicinal Research, New Delhi, Vol. III, 2005: 98-06.
22. Gaind KN and Juneja TR: Investigations on *Capparis decidua*. Planta Medica 1969; 17: 95-97.
23. Gupta J and Ali M: Phytoconstituents of *Capparis decidua* root barks.J Med Aromat Plant Sci 1998; 20: 683-89.
24. Ahmad VU, Ismail N and Amber A: Isocodonocarpine from Capparis decidua. Phytochem 1989; 28(9): 2493-95.
25. Ahmad VU, Ismail N, Arif S and Amber A: Two new N-acetylated spermidine alkaloids from *Capparis decidua*. Journal of Natural Products 1992; 55(10): 1509-12.
26. Ahmad VU, Arif S, Amber AR and Fizza K: Capparisinine, a new alkaloid from *Capparis decidua*. Liebigs Ann Chem 1987; 2: 161-62.
27. Ahmad VU, Arif S and Amber A: A new spermidine alkaloid from *Capparis decidua*. Heterocycl 1985; 23(12): 3015-20.
28. Gupta J and Ali M. Oxygenated heterocyclic constituents from *Capparis decidua* root barks. Indian J Hetero Chem 1997; 6: 295-02.
29. International Conference on Harmonisation, Q2 (R1) Validation of analytical procedures- Text and methodology, Geneva, 2005.

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