# Estimation of Gallic Acid, Rutin and Quercetin in *Terminalia chebula* by HPTLC

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## ABSTRACT

An HPTLC method was developed for the estimation of gallic acid rutin and quercetin from aqueous extract of *Terminalia chebula*, precoated silica gel $GF_{254}$ as stationary phase and mobile phase for tannins toluene: acetone: glacial acetic acid (3:1:2 v/v/v/v/v) and mobile phase for rutin and quercetin, ethyl acetate: dichloromethane: formic acid: glacial acetic acid: water (10:2.5:1:1:0.1, v/v/v/v/v). Detection and quantification were performed densitometrically at $\lambda = 254$ for gallic acid and 366 nm for rutin and quercetin. The $R_f$ values of gallic acid, rutin and quercetin are 0.30, 0.13 and 0.93 respectively. The total peak areas of the standards (gallic acid, rutin and quercetin) and the corresponding peak areas of extracts were compared and the Gallic acid, rutin and quercetin content was estimated to be 8.380, 0.170 and 0.10331 % w/w.

**Keywords:** *Terminalia chebula*, Gallic acid, Rutin, Quercetin, HPTLC, Standardization.

## INTRODUCTION

*Terminalia chebula* is one of the ingredients present in the many Ayurvedic and other traditional medicine system. *T. chebula* is traditionally used in formulations as anti-diabetic, anti-inflammatory, laxative, antibacterial, antifungal, cardiotonic, diuretic, hyperlipidemic activity, jaundice[1-6]. Gallic acid is phenylpropanoid, chemically it is 3, 4, 5,-Trihydroxybenzoic acid, and possess astringent activity. Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. To date about 300 varieties of flavonoids are known[7]. Many have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity[8]. Rutin, 5,7,3', 4',tetrahydroxy flavonol -3-rhamnoglucoside and quercetin 5,7,3', 4',- tetrahydroxy flavonol exhibit anti-inflammatory, antihepatotoxic[9], antiulcer[10], antiallergic and antiviral actions and some of them provides protection against cardiovascular mortality[11,12]. Both possess antioxidant activity and reduce low density lipoproteins (LDL) oxidation[13]. Quercetin in combination with other flavonoids, inhibits a number of enzymes like bradykinin[14], tyrosine kinase[15], and 5'-nucleotidase activity[16]. Rutin and quercetin have shown regulatory activity of hormones, such as transport, metabolism and action of thyroid hormones. High performance thin layer chromatography (HPLC) method is the suitable method for estimation of chemical constituents present in plant materials. Hence *Amaranthus caudatus* contains rutin and quercetin are important active constituents and is estimated by HPLC method.

Phytochemical evaluation is one of the tool for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques. In the last two decades high performance thin layer chromatography (HPTLC) method has emerged as an important toll for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations.

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This includes TLC fingerprint profiles and estimation of chemical markers and biomarkers[17]. The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase. Gallic acid; rutin and quercetin which are important active constituents of *T.chebula* were estimated by HPTLC method[18].

## EXPERIMENTAL

### Preparation of extract

The fruits of *T. chebula* were purchased from Yucca enterprises, Mumbai. It was authenticated by Dr. B.V. K. Naidu, Department of Botany, First Grade College, Chickballapur, Karnataka (India). A voucher specimen was retained in our laboratory. From dried fruits, seeds were separated and the remaining part was made into coarse powder. The coarse powder was then extracted with water by soxhlet apparatus. The extract was filtered using Whatman filter paper and then concentrated in vacuum and dried.

### Reagents and other materials

Rutin, quercetin and gallic acid (Natural remedies, Bangaluru),toluene, acetone, ethyl acetate, dichloromethane, formic acid, glacial acetic acid and methanol (all Reagents of analytical grade, E-Merck) and silica gel $F_{254}$ precoated TLC aluminium plates (E-Merck).

### Preparation of standard and sample solutions

Gallic acid, rutin and quercetin 10 mg were accurately weighed into a 10 mL volumetric flask, dissolved in 5 mL methanol and the solution was made up to 10 mL with the same solvent (1 mg/mL). *T. Chebula* fruit extract was accurately weighed (100 mg) into a 10 mL volumetric flask, dissolved in methanol and then solution was filtered through Whatman filter paper No. 42 and the filtrate was made up to the mark with methanol.

### Development of HPTLC Technique

The samples were spotted in the form of bands with Camag microlitre syringe on a precoated silica gel plates $F_{254}$ (10 cm X 10 cm with 0.2 mm thickness, E.Merck) using Camag linomat V. Automatic sample spotter of band width 7 mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. the distance was 8 cm. subsequent to the scanning, TLC plates were air dried and scanning was performed on a Camag TLC Scanner in absorbance at 254 nm and operated by wincats software 4.03 version (Sethi 1996).

**Gallic acid estimation in *T. chebula***

| | |
|---|---|
| Stationary Phase | :Silica gel $F_{254}$ plates |
| Mobile phase | Toluene: Acetone: Glacial acetic acid (3:1:2) |
| Standard | :Gallic acid 1 mg/ml (5 µL) |
| Sample | : *T. chebula* extract 10 mg/ml (10 µL) |
| Migration distance | : 80 mm |
| Scanning wavelength | : 254 nm |
| Mode of scanning | : Absorption (Deuterium) |

**Rutin and Quercetin estimation in *T. chebula***

| | |
|---|---|
| Stationary Phase | : Silica gel $F_{254}$ plates |
| Mobile phase | :Ethyl acetate: Dichloromethane: Formic acid: Glacial acetic acid: Water (10: 2.5: 1: 1: 0.1) |
| Standard | : Rutin 1 mg/ml (5 µL) |
| Standard | : Quarcetin 1 mg/ml (5 µL) |
| Sample I | : *T. chebula* 10 mg/ml (10 µL) |
| Migration distance | : 80 mm |
| Detection wavelength | : 254 nm |
| Mode of scanning | :Absorption (Deuterium) |

## RESULTS AND DISCUSSION

The $R_f$ value of standard gallic acid was found to be 0.29 and peak area 21802 (Fig.1). Aqueous extract of *T. chebula* showed eight peaks, the fourth peak $R_f$ value (0.29) was coinciding with standard $R_f$ value and its peak area was 18272 (Fig.2). The amount of gallic acid was found to be 8.38 % w/w. The $R_f$ value of standards; rutin and quercetin was found to be 0.13 and 0.93 and its peak area was 36448 and 57858 (Fig. 3 & 4). The aqueous extract of *T. chebula* showed seven peaks in the mobile phase (ethyl acetate:

dichloromethane: formic acid: glacial acetic acid: water). The second and sixth peaks showed $R_f$ values of 0.12 and 0.91 which are almost coinciding with standard $R_f$ values and its peak area was 1239 and 1432.5 (Fig.5). The amount of rutin and quercetin was found to be 0.170 and 0.10331 % w/w respectively.

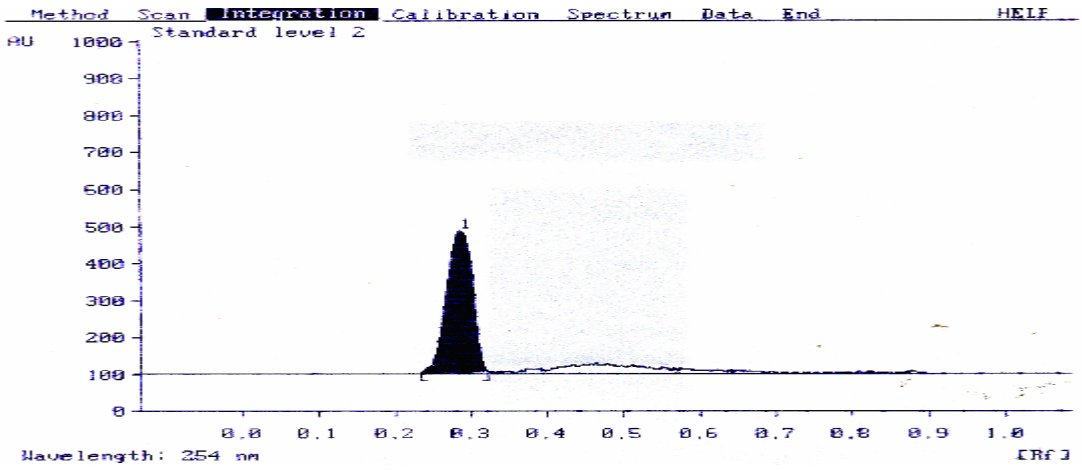


**Figure 1. Gallic acid**

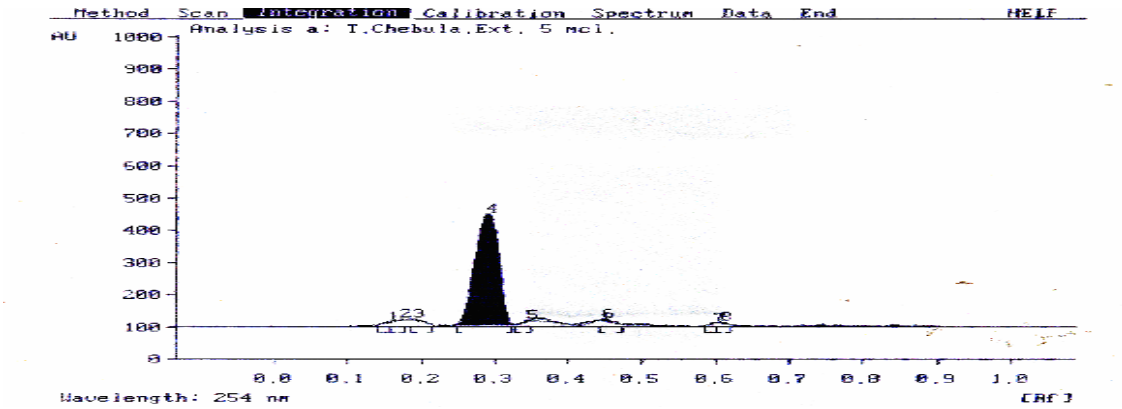


**Figure 2.*Terminalia chebula* aqueous extract**

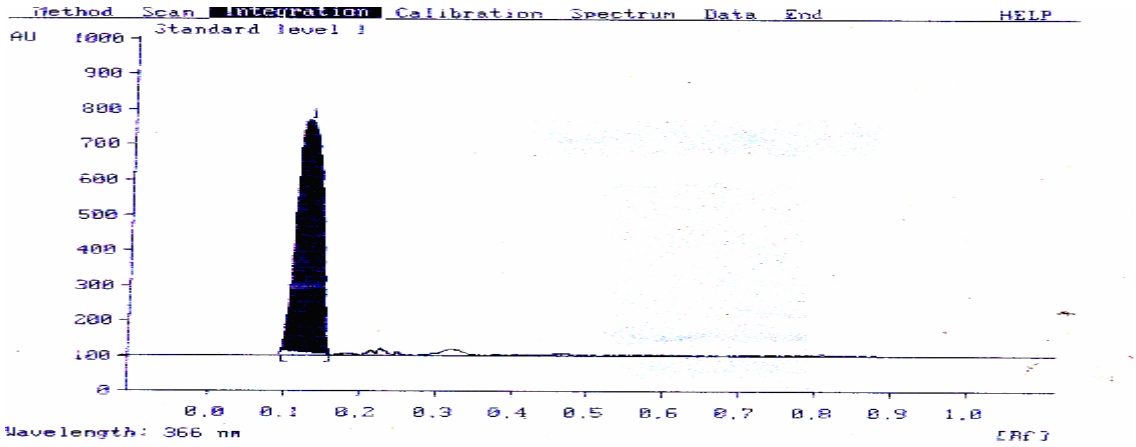


**Figure 3. Rutin**

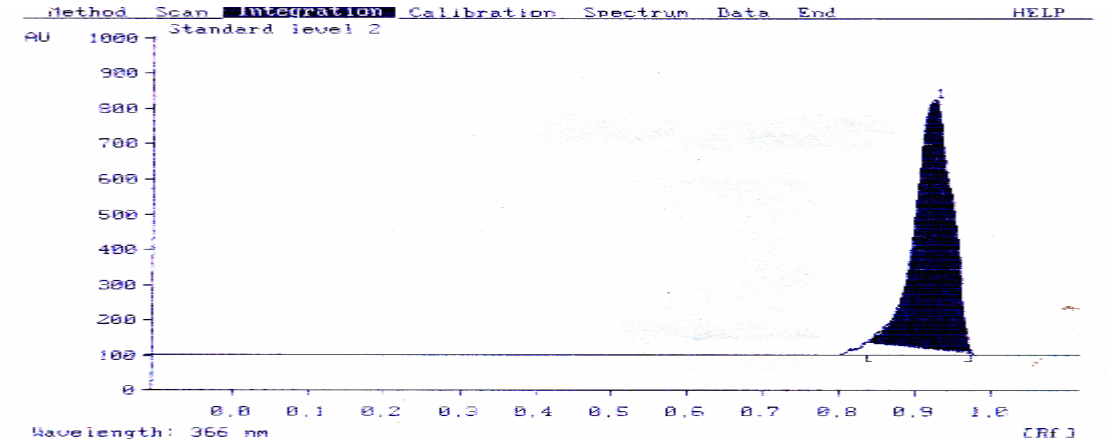


**Figure 4. Quercetin**

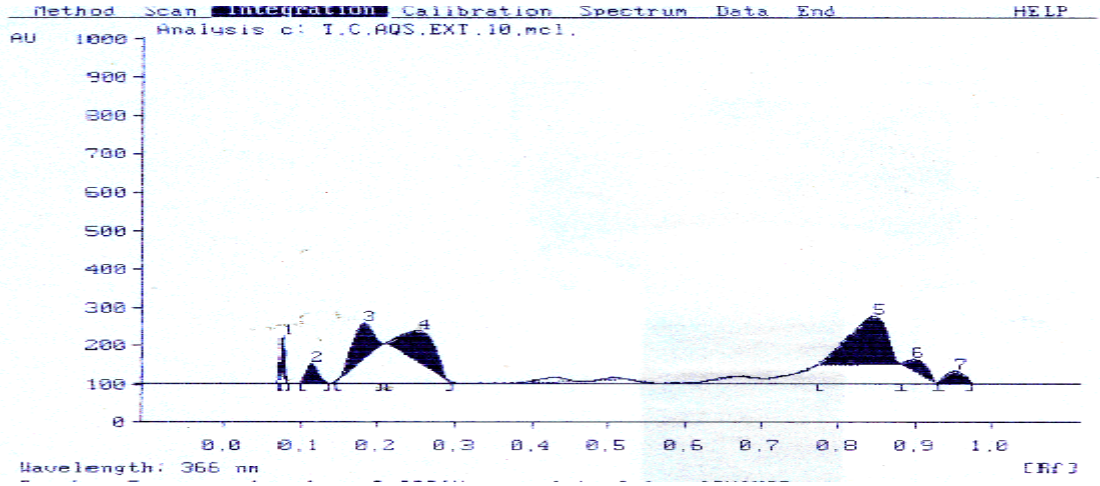


**Figure 5.** ***Terminalia chebula*** **(Aqueous Extract)**

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## REFERENCES

(1) Anonymous. *Indian Herbal Pharmacopoeia*. Jammutwai and Indian Drug Manufacturers Association Mumbai, 2, 1999, 51.

(2) Kirtikar KR and Basu BD. Indian Materia Medica, Dehra Dun, *India*, 1987, 3: 333-335.

(3) Inamdar MC, Khorana ML and Rao MRR, *Indian Journal of Pharmacy*; 1959, 21(12): 333-335.

(4) Sabu MC, and Ramadasan Kuttan, *Journal of Ethnopharmacology*. 2002; 81(2): 155-160.

(5) Miglani B D, Sen P and Sanyal R K, *Indian Journal of Medical Research,* 1971; 59 (2): 281-283.

(6) Khanna A K, Chander R, Kapoor N K, Singh C and Srivastava A K, *Fitoterapia,*1993; 64 (4): 351-356.

(7) Anonymous, *Indian Pharmacopoeia*, Vol II. Controller of Publications, New Delhi, 1996, 53 - 54.

(8) Kuhnau J, *World Res Nut Diet*; 1976, 24: 117-191.

(9) Cesarone M R, Laurora G, Ricci A, Belcaco G and Pomante P, *J Vas Disease*, 1992, 21: 76-80.

(10) Clack W, Heller W, Michel C and Saran M, *J Allergy*, 1950, 21,133-147.

(11) Colergie Smith P O, Thomas P, Scurr J H and Dormandy J A, *Br Med J*, 1980, 296, 1726-176.

(12) Hertog M G L, Hollman P C H, Katan M B and Klohout M, *Nutr Cancer,* 1993; 20, 21-29.

(13) De-whalley C, Rankin S M, Houct J R S, Jessup W, and Leake D S, *Biochem Pharmacol,* 1990; 39, 1743-1750.

(14) Bamard D L, Smee D F, Huffman J H, Meyerson C R and Sidwell R W, *Chemotherapy*, 1993; 39, 203-211.

(15) Hur C Q, Chen K, Shi Q, Kikushkie RE, Cheng YC and Lee KH, *J Nat Pro,* 1994; 57, 42-50.

(16) Beladi, I. Musci, R. Pusztai, M. Bakay, I. Rosztoczy, M. Gabor. 1987; 57: 42-50.

(17) Ravishankar M N, Shrivastava N, Jayathirthaa M G, Padh H and Rajani M, J. Chromatography, 2000; 744: 257-262.

(18) P.D. Sethi. HPTLC, CBS Publishers and distributors 1st edition New Delhi, 1996, 39.

# تقدير حمض الفاليك، الروتين والكويرتيسين في نبتة *Terminalia chebula* باعتماد HPTLC

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## ملخـص

تم تطوير طريقة تقدير حمض الفاليك، الروتين والكويرتيسين من الخلاصة المائية بواسطة HPTLC في نبتة *Terminalia chebula*، مغطاة بطبقة سيليكا $GF_{254}$ كمادة ثابتة وكمادة مشتركة تم استعمال تنين توليين: استون: حمض الخليك (3:1:2) والمادة المشتركة للبورتين والكويرتيسين، اثيل اسيتات: واي كلوروميثان حمض الخليك: حمض الخليك: ماء (10:2.5:1:1:0.1). تحري وكمية لكنت على موجة $\lambda = 254$ لحمض الفاليك و366 ن م للروتين والكويرتيسين كانت 0.30، 0.13، 0.93 على التوالي. مجموع مساحة قمة المعايير (حمض الفاليك الروتين والكويرتيسين) والمشابه لمساحة قيمة الخلاصة مقارنة بحمض الفاليك الروتين والكويرتيسين تم تقديره 0.380، 0.170، و0.1033 w/w%).

**الكلمـات الدالـة:** حمض الفاليك، الروتين، الكويرتيسين، *Terminalia chebula*.

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