ORIGINAL ARTICLE

# Standardization of quercetin in *Hibiscus rosa-sinensis* flower by high-performance thin-layer chromatography

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## Abstract

**Aim:** This is done from regulatory perspective to ensure the efficacy, quality, as well safety of the herbal drugs present in a plant. **Materials and Methods:** Standardization of plants material by high-performance thin-layer chromatography (HPTLC): HPTLC is a method to standardize and identify the chemical component which is expected to be present in a medicinal plant. This is done from regulatory perspective to ensure the quality, efficacy, and safety of the herbal drugs present in a plant. **Results and Discussion:** The flower of *Hibiscus rosa-sinensis* was dried and pulverized. The phytoconstituents of the pulverized plant material were extracted with methanol. Quercetin, reported to have anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic, and antiatherosclerotic, etc., activities, was selected as the active biomarker for quantification of the aforementioned plant material. HPTLC was carried out for quantification. The percentage content of quercetin in *H. rosa-sinensis* methanolic extract was found to be 0.30% w/w by HPTLC. **Conclusion:** The percentage content of quercetin in methanolic extract of *H. rosa-sinensis* flower was found to be 0.30% w/w. This was determined by a calibration curve with the equation of Y = 3303.952*X + 2991.844.

**Key words:** *Hibiscus rosa-sinensis,* high-performance thin-layer chromatography, quantification, standardization

## INTRODUCTION

The plant *Hibiscus rosa-sinensis* is a perennial shrub with taproot. The leaves are 3.5–12 cm in length and 2–5.5 cm wide. Leaves are simple ovate or ovate-lanceolate. Leaves are entire at the base and coarsely toothed at the apex. Taste is mucilaginous. Flowers are pedicellate, actinomorphic, pentamerous, and complete. Corolla consists of five petals, red in color, and about 3 inches in diameter, generally available in many areas within its hardiness range. *H. rosa-sinensis* is native to tropical Asia. A native of Southeastern Asia (China), the plant is commonly found throughout the tropics and as a houseplant throughout the world. Most ornamental varieties are hybrids. The present wide range of cultivars is considered to be a complex of interspecific hybrids, among eight or more different species originating from the African East coast and islands in the Indian and Pacific ocean.[1] The herb is a source of antioxidants such as taraxeryl acetate, sterculic and malvalic acids, quercetin and its glycosides, cyanin and cyanidin chlorides, kaemperol-3-xylosylglucoside, thiamine, riboflavin, niacin, and ascorbic acid.[2]

They have many pharmacological properties including antipyretic, antispasmodic, hypotensive, antifungal, anti-inflammatory, and many more.[3] The aqueous-ethanolic extract of aerial parts of *H. rosa-sinensis* was reported for its use in constipation and diarrhea. The alcoholic extract of flowers of *H. rosa-sinensis* has been proved to possess anticonvulsant property. In traditional medicine, the leaves of the plant are used in fatigue and skin disease. Fresh root juice of the plant is given for gonorrhea and powder root for menorrhagia. Flowers of the plant are used in epilepsy,

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leprosy, bronchial catarrh, and diabetes. An infusion of the petal is widely used in ayurvedic medicine in India as a demulcent refrigerant drink in fever and decoction is given in bronchial catarrh. It has been reported that the plant flower possesses antispermatogenic and androgenic, antitumor, and anticonvulsant activities. The use of flower to treat heart disorders has also been described and has demonstrated the antidiabetic activity of *H. rosa-sinensis* in diabetic rural population. Infusion of the petals is given as refrigerant and demulcent. Leaves are used as laxative while root is used in cough.[4]

For the establishment of a consistent biological activity of any herbal item, standardization is an important step. It provides a consistent chemical profile or simply a quality assurance program for production and manufacturing of an herbal drug.[5] Standardization, thus, is a tool in the quality control process. High-performance thin-layer chromatography (HPTLC) is a modern adaptation of thin-layer chromatography with better and advanced separation efficiency and detection limits. HPTLC provides a number of advantages including easy separation process for with colored compounds. HPTLC can be used for different modes of evaluation, allowing identification of compounds having different light absorption. HPTLC[6] is a method to standardize and identify the chemical ingredients which are expected to be present in a medicinal plant. This is done from regulatory perspective to ensure the efficacy, quality, as well safety of the herbal drugs present in a plant. Thus, it provides a very reliable way of determining the purity and percentage content of the active biomarker in the plant extracts.[7] Standardization of herbal products is a current issue of interest. For quality control of these herbal materials or their extracts, one needs to proceed by selecting one of the different phytoconstituents of the product, preferably, the one showing maximum desired bioactivity and subsequent method of quantification of that specific constituent is required to be developed. The method so accepted should be simple and cost-effective.[1]

## MATERIALS AND METHODS

Standardization of plants material by HPTLC: HPTLC is a method to standardize and identify the chemical component which is expected to be present in a medicinal plant. This is done from regulatory perspective to ensure the quality, efficacy, and safety of the herbal drugs present in a plant. Thus, it provides a very reliable way of determining the purity and percentage content of the active biomarker in the plant extracts.

### Equipment and Reagents

The CAMAG HPTLC system consisting of WINCATS software, LINOMAT V automatic sample applicator, and automatic development chamber, scanning densitometer

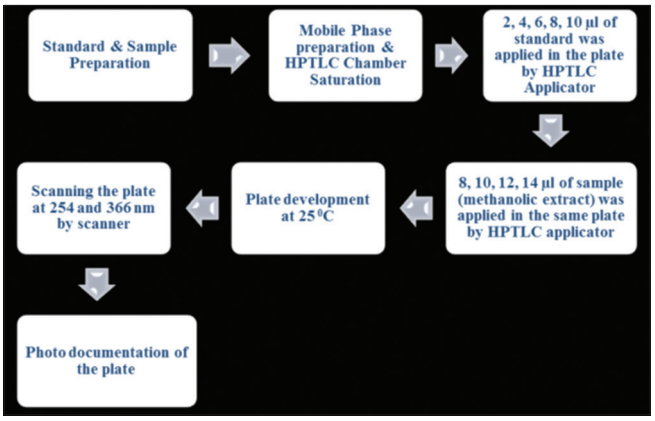


**Figure 1:** Standardization of quercetin in *Hibiscus rosa-sinensis* flower by high-performance thin-layer chromatography

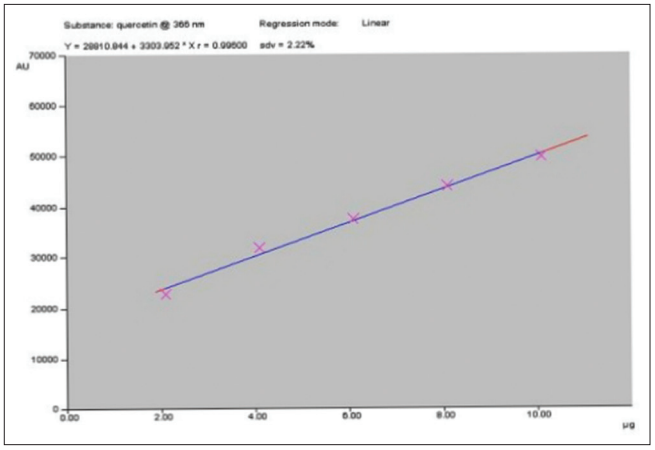


**Figure 2:** Calibration curve of standard quercetin

CAMAG scanner 3, and photo documentation apparatus CAMAG reprostar-3 were used. Stationary phase was used as aluminum-based silica gel plate 60 F254 (Merck, Mumbai) with 10 cm × 10 cm in a particle size of 5–10 µm. All the solvents were used of analytical grade. 100 µl syringe (HAMILTON, Switzerland) was used for sample application on HPTLC plates. Quercetin was present in laboratory. Methanol, toluene, and ethyl acetate (analytical grade) were procured from Merck (Mumbai, India). All the samples were filtered through Whitman's syringe filter (NYL 0.45 µ).

### Preparation of Standard Solution

About 1 mg of quercetin standard was weighed and put into 1 mL Eppendorf tube. 1.0 mL of methanol was added with it and mixed in vortex mixture till the material completely dissolved. It was then filtered through 0.45 µ syringe filter and kept for further study.

### Preparation of Sample Solution

About 10 mg of *H. rosa-sinensis* flower methanolic extract was dissolved in 1 mL methanol in Eppendorf tube. It was

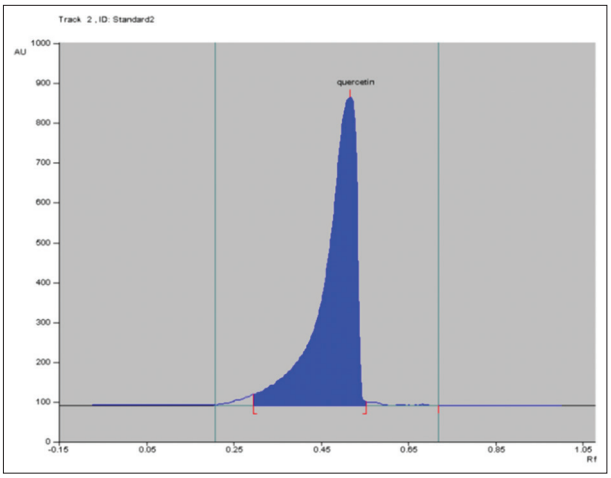


**Figure 3:** High-performance thin-layer chromatography chromatogram of standard quercetin

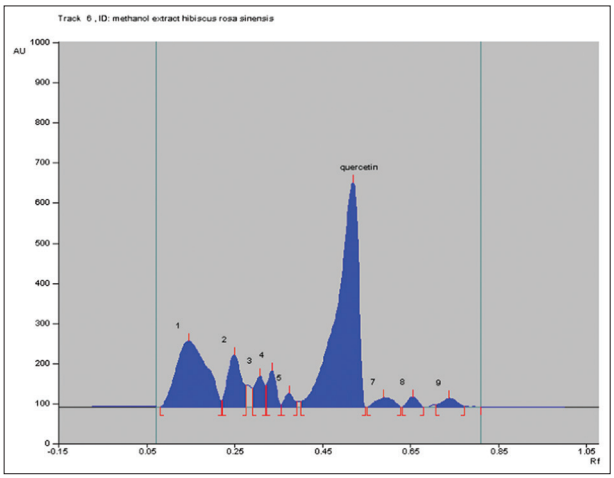


**Figure 4:** High-performance thin-layer chromatography chromatogram of methanolic extract of *Hibiscus rosa-sinensis* flower

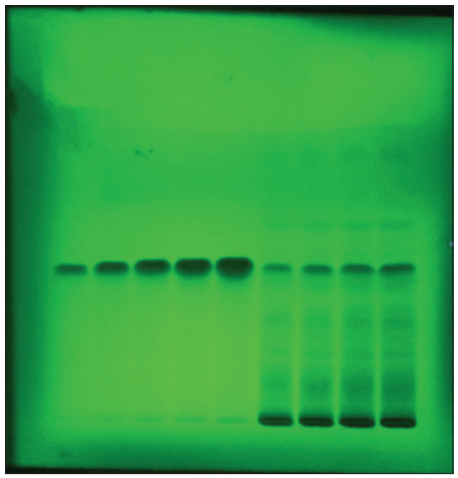

**Figure 5:** Photodocumentation of methanolic extract of *Hibiscus rosa-sinensis* flower at 254 nm

then mixed in vortex mixture and put to ultrasonication bath till the material completely dissolved. Then, it was

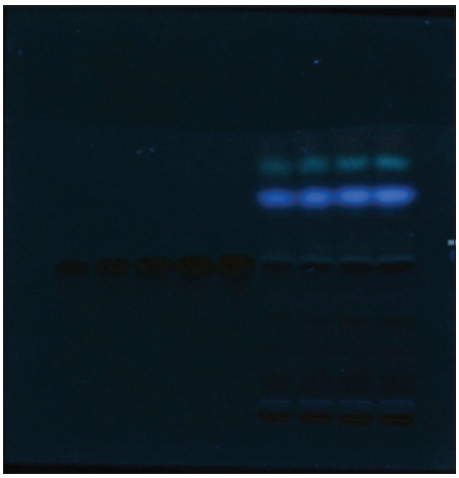

**Figure 6:** Photodocumentation of methanolic extract of *Hibiscus rosa-sinensis* flower at 366 nm

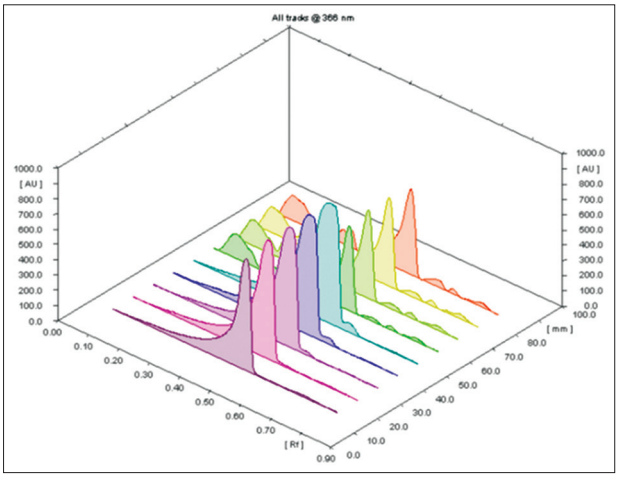


**Figure 7:** 3D chromatogram of *Hibiscus rosa-sinensis* and standard quercetin at 540 nm

filtered through 0.45 µ syringe filter and kept for further study.

### Chromatographic Conditions

HPTLC analysis was performed using isocratic technique by external methods. The mobile phase was optimized with toluene:ethyl acetate:methanol in a ratio of 5:3:2 v/v. The temperature was kept at 25°C and mobile phase was developed in a twin trough glass chamber. The standard solution was applied 2, 4, 6, 8, and 10 µL, and sample solution was applied consequently in the range of 8, 10, 12, and 14 µL. Total nine tracks in HPTLC plate were used for the development of five standard and four sample solution, respectively, in a band wise fashion. After drying, the colored bands were observed at 254–360 nm Figure 1.

## RESULTS AND DISCUSSION

The percentage content of quercetin in methanolic extract of *H. rosa-sinensis* flower was found to be 0.30% w/w. This

was determined by a calibration curve with the equation of Y= 3303.952*X + 2991.844 (correlation coefficient = 0.9960 and standard deviation = ± 2.22%) as shown in Figure 2, where X represents the amount of quercetin and Y represents area under the curve.

Rf value of standard quercetin was found to be 0.52. Specificity was confirmed by comparing the Rf of standard and sample [Figures 3-7].

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