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DETERMINATION OF NARINGENIN IN HYDROALCOHOLIC EXTRACT OF *COMMIPHORA MUKUL* (BURSERACEAE) BY HPTLC METHOD

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ABSTRACT

Naringenin is a naturally occurring flavanone (flavonoid) identified to have a bioactive effect on human health and is mainly found *Commiphora mukul* as one of the active constituent. Naringenin possesses various biological activities such as antidiabetic, antiatherogenic, antidepressant, immunomodulatory, antitumor, anti-inflammatory, DNA protective, hypolipidaemic, antioxidant, peroxisome proliferator-activated receptors (PPARs) activator, and memory improving. In the present study, an attempt was made to quantify the flavonoid Naringenin in the *Commiphora mukul* hydroalcoholic extract. TLC was performed to confirm the presence of Naringenin and HPTLC procedure has been developed for quantification of Naringenin in the hydroalcoholic extract. HPTLC separation was achieved on Merck TLC aluminium sheets precoated with silica gel $60F_{254}$ using Toluene: Ethyl acetate: Formic acid (6: 4: 1, v/v/v) as mobile phase. Quantitative analysis was carried out at 289 nm. A good linear relationship was obtained for Naringenin in the concentration range of 200-1200 ng $band^{-1}$.

KEYWORDS: *Commiphora mukul*, Flavonoid, Naringenin, HPTLC.

INTRODUCTION

The preference to the herbal drug remedy is due to various side effects associated with the conventional drugs. As per WHO (World health organisation) about 80% of the world population uses the herbal medicines for prevention and treatment of variety of diseases and disorders.[1] *Commiphora mukul* (Burseraceae) is commonly known as Guggul, Indian bdellium-tree, gugal or mukul myrrh tree. It is a small shrub with maximum height of 4 mts. The branches are thorny; leaves are simple or trifoliate, leaflets are ovate 1-5 cm long, 0.5-2.5 cm broad.[2] *Commiphora mukul* is traditionally used as, anti-inflammatory, antispasmodic, carminative, emmenagogue, hypoglycaemic, alterative, antiseptic, aperitif, astringent, sedative, stomachic, diaphoretic, diuretic, expectorant, aperient, thyroid stimulant, anthelmintic, deprative, vulneray, demulcent, aphrodisiac, lithonotropic, hyperlipidaemia and ant diabetic and also helpful in decreasing obesity and osteoarthritis.[3] The C. mukul comprises of many phytoconstituents as volatile oil, steroids, flavonoids, guggultetrols, amino acids, sugars, lignans etc. Some of the major of these found in guggul are Dimyrcene, camphorene, linoleic, oleic, stearic, palmetic acids, sitosterol, quercetin, cembranoid, myrrhanol, pinene, Ellagic acid, arabinose, muscanon, diayangambin, naringenin etc.[4] Naringenin is a naturally occurring flavanone (flavonoid) identified to have a bioactive effect on human health and is mainly found *Commiphora mukul* as one of the active constituent which acts as hepatoprotective.[5] Chemically, it is 2, 3-dihydro-5, 7-dihydroxy-2-(4- hydroxyphenyl) - 4H-1-benzopyran-4-one and it has a molecular weight of 272.26.[6]

Quantitative estimation of active constituents in plant extract is important for current research and a variety of methods are required for this. TLC and HPTLC are the methods primarily used for separation, qualitative identification and semi-quantitative visual analysis of the samples.[7] High Performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identity of crude drug and also for quality control of finished product.[8]

Reports related to the quantitative determination of naringenin in *Commiphora mukul* plant extract by HPTLC has been not reported in literature. Consequently, the present study was focused on the quantitative estimation of the flavonoid naringenin by high performance thin-layer chromatography in the *Commiphora mukul*. Hydroalcoholic extract of *Commiphora mukul* was subjected to thin layer chromatography and high performance thin-layer chromatography to find out the likely number of compounds present in them.

MATERIALS AND METHODS

Plant material

The plant was identified and collected, the plant sample (resin) was washed thoroughly with tap water, dried at room temperature away from sun light, cut into small pieces, and then powdered. Hydroalcoholic extract was prepared by cold maceration of gum resin powder in ethanol and water for 7 days. The extract was filtered, concentrated under reduced pressure.

Selection of detection wavelength

Working standard solution was prepared by dissolving 10 mg of naringenin in 10 mL of methanol to get concentration of 1000 µg mL^{-1}. The resulting solution was diluted further to get solution having final concentration 20 µg mL^{-1} and absorbance was recorded in range of 200-400 nm using UV visible spectrophotometer. Naringenin showed maximum absorbance at 289 nm and was chosen as detection wavelength.

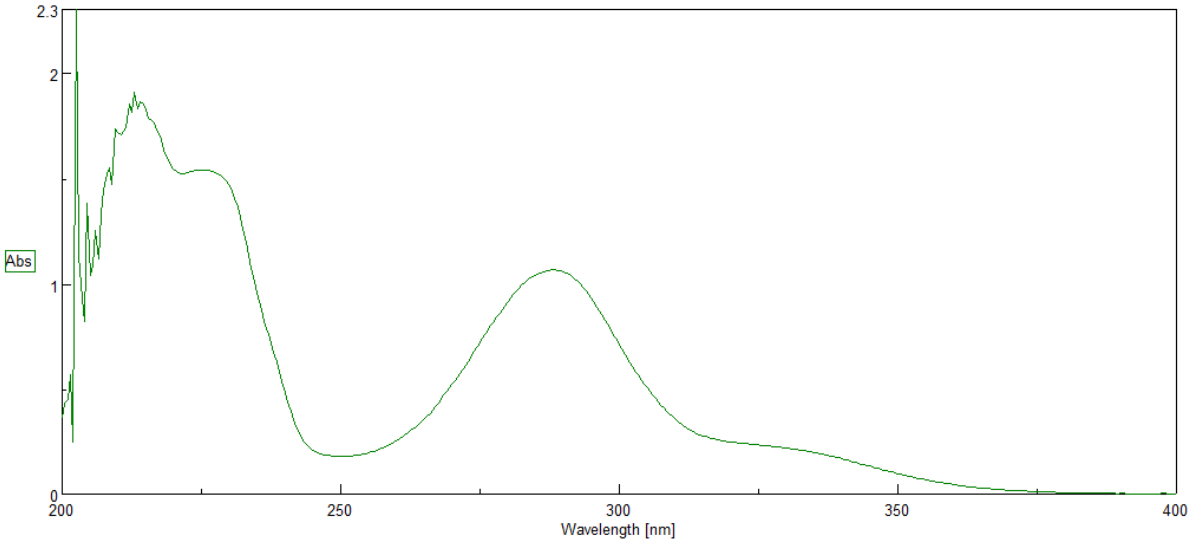

Fig: 1. UV spectrum of Naringenin (20 µg mL^{-1}).

Preparation of standard solution

Standard solution of naringenin was prepared by dissolving 10 mg in 10 mL of methanol to get concentration of 1000 µg mL^{-1} which was further diluted appropriately to obtain final concentration 100 ng μL^{-1}.

Preparation of the sample solution

Sample solution was prepared by dissolving 10 mg of *C. mukul* extract in 10 mL of methanol to get concentration of 1000 µg mL^{-1} which was further diluted appropriately to obtain final concentration 100 ng μL^{-1}.

HPTLC analysis

The chromatographic separation was achieved on precoated silica gel $60F_{254}$ aluminium plates (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate with band width of 8 mm using Camag 100 µL sample syringe (Hamilton, Switzerland) equipped with Linomat V sample applicator (Camag, Switzerland). The mobile phase optimised consisted of Toluene: Ethyl acetate: Formic acid (6: 4: 1, v/v/v). Linear ascending development of TLC plate was carried out in CAMAG twin trough glass chamber (10 cm × 10 cm) followed by 15 min saturation time for mobile phase. 10 mL mobile phase was used per run and migration distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner III operated by win CATS software version 1.4.2. A solution of 100 ng μL^{-1} of naringenin in methanol and extract was prepared and was spotted using Camag Linomat V sample applicator and detection was carried out at 289 nm.

Preparation of calibration curve of naringenin

From standard solution (100 ng μL^{-1}), volumes 2, 4, 6, 8, 10 and 12 µL were applied on the TLC plates with sample applicator in nitrogen stream. The plate was prewashed by running in methanol and then dried at room temperature for 10 min and then was developed in a mobile phase previously saturated for 15 min. The mobile phase utilized consisted of Toluene: Ethyl acetate: Formic acid (6: 4: 1, v/v/v) and separated spots were analyzed densitometrically at 289 nm using Camag TLC Scanner III operated by the winCATS software version 1.4.2. The calibration curve of the standard drug concentration (X-axis) against the average peak area (Y-axis) was prepared to get a regression equation.

Estimation of naringenin in extract

The mean peak area of the sample was calculated and the content of naringenin was determined using the regression equation obtained from the standard calibration curve.

Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as signal-to-noise ratio of 3:1 and 10:1.[9]

RESULTS AND DISCUSSION

Preliminary phytochemical investigation of hydroalcoholic *Commiphora mukul* extract demonstrated the presence of alkaloids, phenols, terpenoids, carbohydrates, amino acids, sterols, tannins and flavonoids. TLC procedure was optimized with the objective of separation of compounds and to identify one of the phytochemical flavonoid i.e. naringenin in the extract. The solvent system comprising of Toluene: Ethyl acetate: Formic acid (6: 4: 1, v/v/v) was proficient to give a dense, compact and well-defined peak for naringenin with Rf 0.60 as well as for the extract (Fig. 2 and 3). This confirmed the presence of the bioactive compound flavonoid. The identification of the naringenin in sample densitogram was confirmed by compairing the Rf value with that obtained from pure marker and also by comparing the UV absorption spectra with that of standard naringenin (Fig. 4).

Regression data obtained from calibration curve demonstrated excellent linear relationship over 200-1200 ng band^{-1} concentration range. The linear regression equation was found to be y = 5.359x + 1077 having correlation coefficient 0.990 (Fig. 5 and 6). The limit of detection and limit of quantitation was found to be 9.00 ng band^{-1} and 27.30 ng band^{-1}, respectively. With the help of above statistical data, the content of naringenin was determined in the hydroalcoholic plant extract which was found to be 94%. The HPTLC data obtained for the quantification of naringenin is summarized in Table 1. HPTLC analysis was carried out to determine the content and to quantify the naringenin present in C. mukul plant extract.

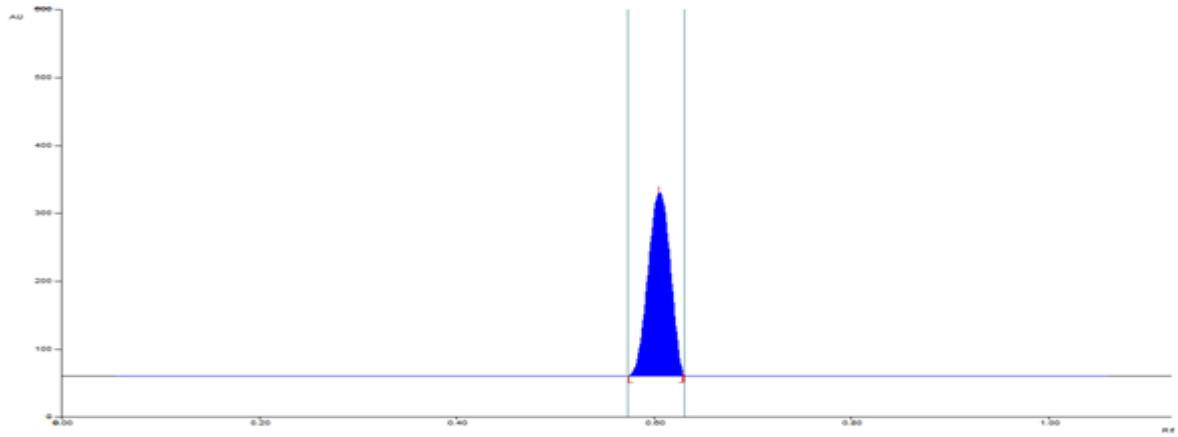

Fig: 2. HPTLC densitogram showing the presence of Naringenin (Rf =0.60).

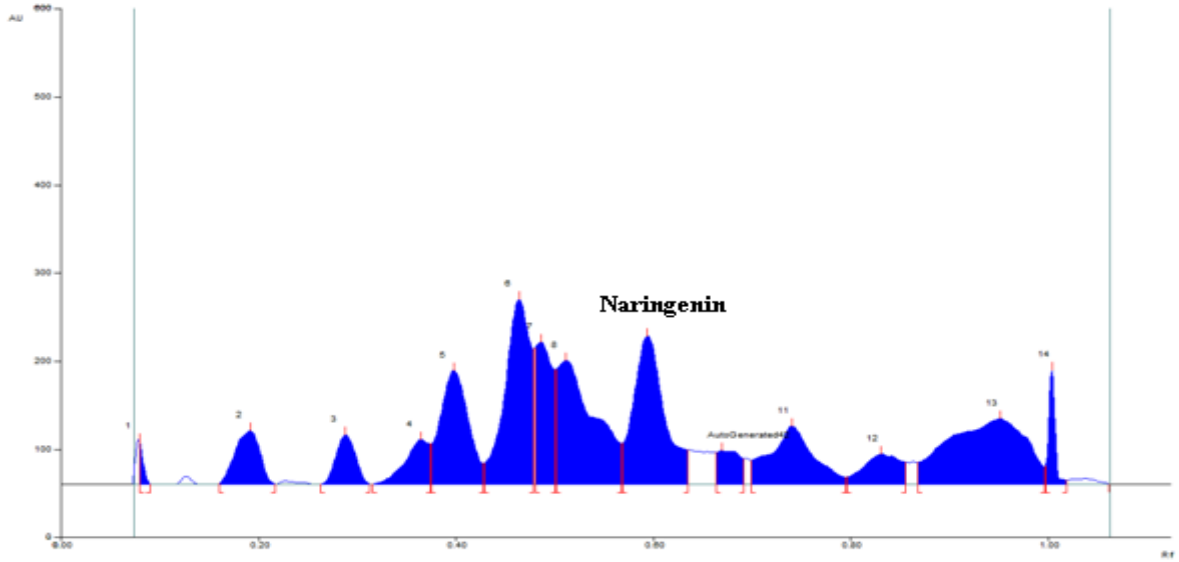

Fig: 3. Densitogram of C. *mukul* extract showing the presence of Naringenin (Rf =0.60).

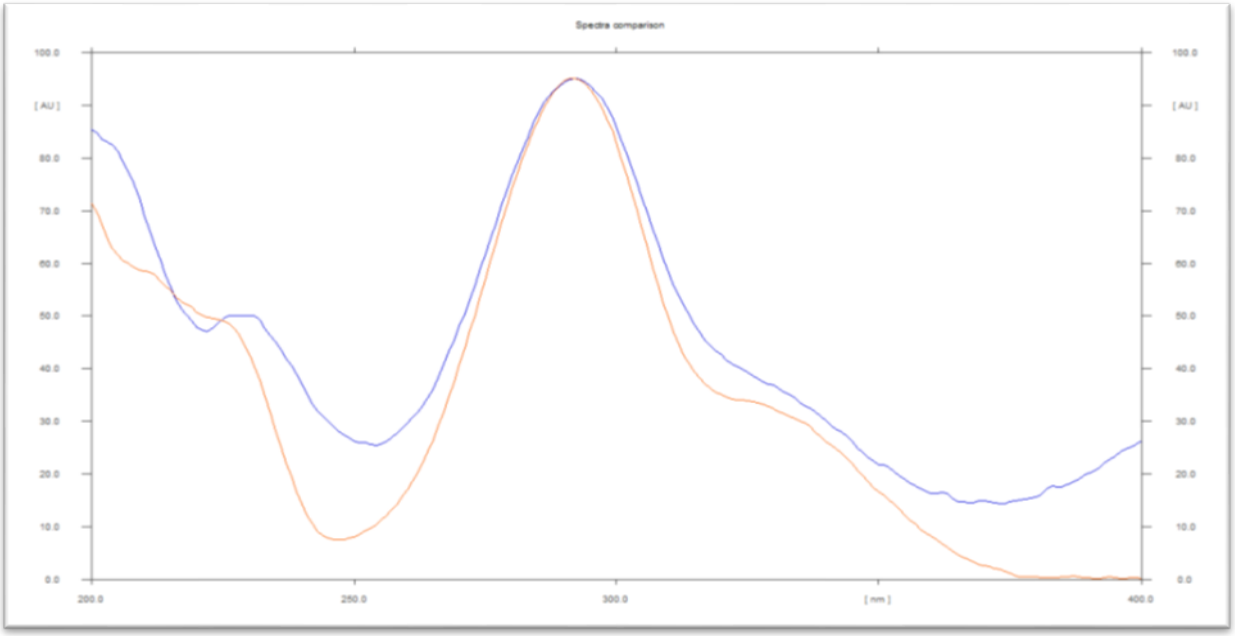

Fig: 4. Spectra of *C. mukul* extract with naringenin at 289 nm.

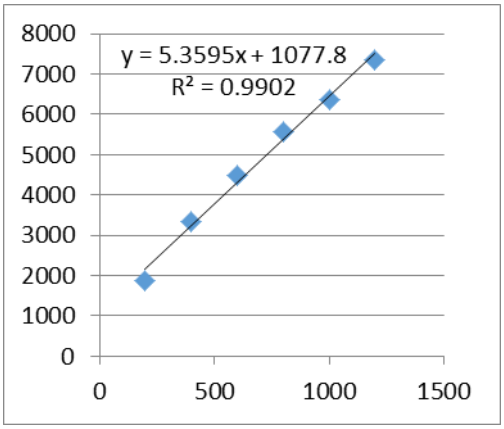

Fig: 5. Calibration curve for naringenin.

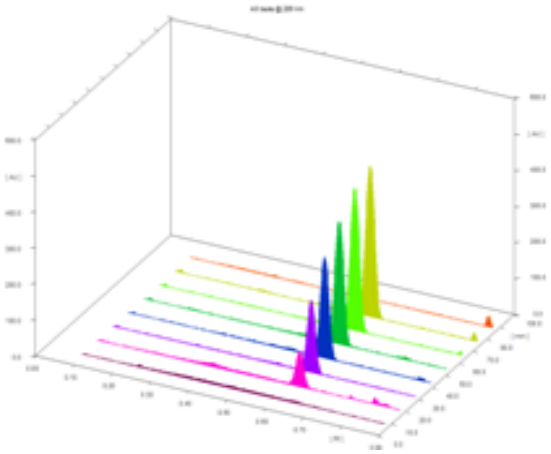

Fig: 6. 3D Densitogram of naringenin.

Table 1: HPTLC data for naringenin.

Sr. No.	Parameter	Naringenin standard
1	Linearity	200-1200 ng band^{-1} R^2 =0.990
2	Regression equation	y = 5.359x+1077
3	Correlation coefficient	0.990
5	Limit of detection	9.00 ng band^{-1}
6	Limit of quantitation	27.30 ng band^{-1}

CONCLUSIONS

The presence of most of the biologically active compounds in the plant was identified by phytochemical analysis. The chromatographic studies conducted with the hydroalcoholic plant extract of *Commiphora mukul* revealed a significant amount of flavonoid naringenin, which possess its distinct medicinal value.

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