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Research Article

ESTIMATION OF ISOQUERCITRIN BY HPTLC METHOD IN HYGROPHILA SALICIFOLIA, (ACANTHACEAE)

Bhatt, MK^{1*}, Shah, NJ² and Gohel KD³

¹Department of Pharmacognosy, GTU, Gujarat, India

²Department of Pharmaceutical Chemistry, GTU, Gujarat, India

³Department of Quality Assurance, GTU, Gujarat, India

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ABSTRACT

Traditionally *Hygrophila salicifolia* (Acanthaceae) is reported to possess anti-inflammatory and diuretic properties. The objective of the current study was to develop HPTLC method for quantification of Isoquercitrin from the methanolic extract of *Hygrophila salicifolia* which can be used in quality control of the crude drug. Chromatographic analysis of methanolic extract of the whole herb gave typical fingerprints, where, Isoquercitrin, a standard reference marker, resolved at R_f 0.76 under TLC condition. One dark black colour band at R_f 0.76 under the TLC conditions was characteristic of the raw drug. Isoquercitrin was quantified to be 229.801ug/ml using the HPTLC method.

Key Words:

Hygrophila salicifolia, Acanthaceae,
HPTLC, Chromatographic analysis.

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INTRODUCTION

The whole herb of *Hygrophila salicifolia*, has been advocated for the therapy of various diseases including jaundice, urinary infection, gout, hepatic disorder, rheumatism, impotence, anti-bacterial and inflammation (Shah, 1978).

It is an erect or ascending herb belongs to the family Acanthaceae. It is bestowed with many medicinal uses in traditional systems of medicine including ayurveda. Seeds and leaves used as poultice on inflammatory swellings. Leaves are strongly diuretic. The plant is discovered in moist and marshy places throughout the greater part of India. Stems up to 3 ft. long, more or less quadrangular, rooting at the lower nodes, leaves sub sessile, linear-lanceolate; flowers pale purple in dense axillary whorls, capsule oblong, seeds many, ovoid, compressed, mucilaginous, hairy. The leaves are eaten as pot-herb. They contain 8% ash rich in potassium and are strongly diuretic (Shah, 1978).

In Malaya, the leaves are used as poulticing swellings. The seeds swell into a gelatinous shining mass with water and used in java, in poultices for headaches and fevers. They yield 25% of a fatty oil and contain traces of unidentified alkaloid, a bitter

substance and 4% ash consisting chiefly of calcium phosphate and potassium chloride (Chattertee, 1987).

The plant has been reported to contain chlorophyll, pigments, gums, glucose, starch, fat, various minerals, alkaloids, flavanoids, tannins and sterols (Nadkarni, 1978, Basu, 1933, Mukherjee, 2004).

Isoquercitrin has been isolated from the methanolic extract of whole herb of *Hygrophila salicifolia* and has been quantified. Isoquercitrin has been used as an important bioactive marker and quantified in whole herb of *Hygrophila salicifolia*. Isoquercitrin belongs to the class of Flavonoid (6-methoxy-7-hydroxy coumarin) type of compound. Isoquercitrin is reported to possess diuretic action (Arquimedes et al, 2011).

The plant *Hygrophila salicifolia* is well known for its diuretic and anti inflammatory uses in Ayurveda but till date no data available for its standardization, therefore, its quantitative phytochemical evaluation is necessary. The finger printing, HPTLC method development and validation of *Hygrophila salicifolia* will produce scientific data. The extensive survey of literature revealed that *Hygrophila salicifolia*, as an important source of so many pharmacologically and medicinally important phytoconstituents. However, researches are insufficient. The present investigation reports quantification of

*Corresponding author: Bhatt, MK

Department of Pharmacognosy, GTU, Gujarat, India

isoquercitrin content in the methanolic extract of the whole herb of *Hygrophila salicifolia* (Family: Acanthaceae).

MATERIALS AND METHODS

Identification of Isoquercitrin: TLC Identity Test

Test solution

5 gm of dried powder of *Hygrophila salicifolia* was refluxed with 25 ml methanol for 20–25 mins on a water bath. The extract was collected, filtered and dried on a water bath. 25 mg of extract was dissolved in 1ml of methanol (Harbone, 1998).

Standard Solution

Dissolve 10 mg of standard in 10 ml of methanol

Solvent system

Ethyl acetate: Formic acid: glacial acetic acid: water (10:1.1:1.1:2.6, v/v/v/v)

Procedure

Apply two μ l each of the methanolic extract of *Hygrophila salicifolia* and standard & isolated compound solution on a precoated silica gel 60 F₂₅₄ TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in the solvent system in twin trough chamber to a height of 8 cm (Nayana *et al*, 2006).

Visualization

Observed the developed plate under UV light at 254 nm and 366 nm and derivatised with Poly Ethylene Glycol and observed again Under UV light at 254 nm and 366 nm. Record the R_f values and colour of the resolved bands (Anonymous, 2007). (Figure 1 & 2 & Table 1)

Evaluation

A band (0.76) corresponding was visible in methanolic extract of *Hygrophila salicifolia*, standard & isolated compound solution tracks (Mukherjee, 2007).

Estimation of Isoquercitrin: Assay/ Analytical Method

Selection of Chromatographic Condition

Proper selection of HPTLC method depends upon the nature of sample (ionic, ionizable or neutral molecule), molecular weight and solubility. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase composition and solvent ratio were studied. The resulting chromatograms were recorded and chromatographic retention factor and resolution were calculated. The conditions that gave best resolution and retention factor were selected for estimation (ICH, 2005).

Selection of Detecting Wavelength

The sensitivity of HPTLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected.

In the present study standard solution of 400-1200 ng/spot of pure isoquercitrin was prepared in methanol. Both standard and sample solution were then scanned in the UV region of 200-400 nm and the overlain spectrums were recorded.

Reagents and materials

Stationary phase: Precoated HPTLC aluminum sheet (MERCK) silica gel 60F₂₅₄ (10cm x10cm)

Mobile phase: Ethyl acetate: Formic acid: glacial acetic acid: water (10:1.1:1.1:2.6, v/v/v/v)

Reference standard: Isoquercitrin (Sigma Aldrich, USA, Dutta enterprise, Anand)

Test sample: Methanolic extract of *Hygrophila salicifolia*

Instrument

A Camag TLC system comprising of

- Camag Linomat V, a sample applicator as bands using the spray on technique, Camag Switzerland
- Camag Hamilton 100 μ l HPTLC syringe
- Camag twin through chamber (20 x 10 cm²)
- UV cabinet (Chromatogram inspection under short wave and long wave UV light)
- Camag TLC scanner III (chromatogram evaluation by classical densitometry)
- Camag WINCATS software (version 4.06)
- Camag reprostar (image acquisition, documentation by conventional photography)
- Analytical Balance

Model: CP124S

Make: Sartorius GOTTINGEN AG, Germany ISO 9001 certified

Maximum: 120g

Sonicator:

Model: Fast Clean Ultrasonic Cleaner

Make: Enertech electronics Pvt. Ltd.

Chromatographic Conditions

Stationary phase: Pre coated HPTLC aluminum sheet (MERCK) Silica gel 60 F₂₅₄ (10 cm X 10 cm)

Mobile phase: Ethyl acetate: Formic acid: glacial acetic acid: water (10:1.1:1.1:2.6, v/v/v/v)

Spotting volume for standard: 4 μ l, 6 μ l, 8 μ l, 10 μ l, 12 μ l

Spotting volume for test: 2 μ l

Chamber saturation time: 20 mins.

Chromatographic technique: Ascending

Temperature: 37°C

Wavelength: 360 nm

Preparation of Standard Solution

10 mg of isoquercitrin was dissolved in 10 ml of methanol (1mg/ml=1000ng/ μ l). Aliquots of the stock solution were appropriately diluted with methanol to obtain a working standard of 100 μ g/ml. From this stock solution standard solutions of 400-1200 μ g/ml was prepared by transferring aliquots (4 to 12ml) of stock solution to 10 ml volumetric flask and adjusting the volume to 10 ml with methanol. The concentrations of isoquercitrin were 400, 600, 800, 1000 and 1200 ng/spot (Anonymous, 1992).

Calibration curve for Isoquercitrin

4 μ l to 12 μ l of each of the standard solution containing 400 to 1200 ng/spot isoquercitrin respectively were applied on a HPTLC plate. The plate was developed in a mobile phase, Ethyl acetate: Formic acid: glacial acetic acid: water

(10:1.1:1.1:2.6, v/v/v/v) and scanned at 254 nm and 366 nm. Calibration curve of isoquercitrin was prepared by plotting peak area vs. concentration of isoquercitrin applied.

Estimation of Isoquercitrin in the drug

4 µl to 12 µl of each of the standard solution containing 400 to 1200 ng/spot isoquercitrin, respectively and 8 µl test solution were applied on a HPTLC plate. Develop the plate in the solvent system and record the peak area of isoquercitrin as described above and record the peak area of isoquercitrin as described above for the calibration curve (Figure 3, 4, 5 & 6). Calculate the amount of isoquercitrin present in the sample from calibration curve of isoquercitrin.

Table 1 TLC Details of Test extract, isolated & standard compound.

Rf value	Extract	Isolated	Standard
	0.76	0.75	0.76
	0.64	----	----
	0.60	—	—
UV-254	0.44	—	—
nm&	0.38	—	—
V-366 nm	0.31	—	—
	0.25	—	—
	0.08	—	—
	0.01	—	—

RESULTS

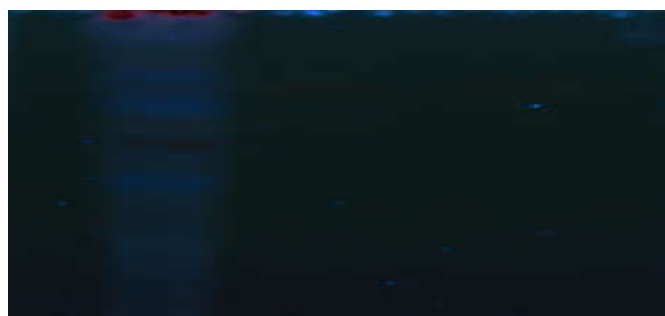


Figure 1 TLC of Methanolic extract of whole herb of *Ipomoea reniformis*, isolated compound & standard at UV-366 nm

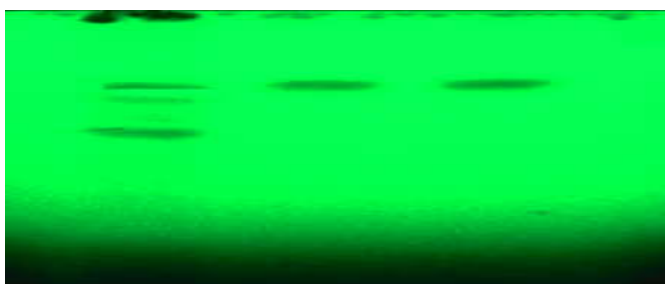


Figure 2 TLC of Methanolic extract of whole herb of *Ipomoea reniformis*, isolated compound & standard at UV-254 nm

T= Test (Methanolic extract of *Hygrophila salicifolia*)

I= Isolated compound

S, = Standard (isoquercitrin)

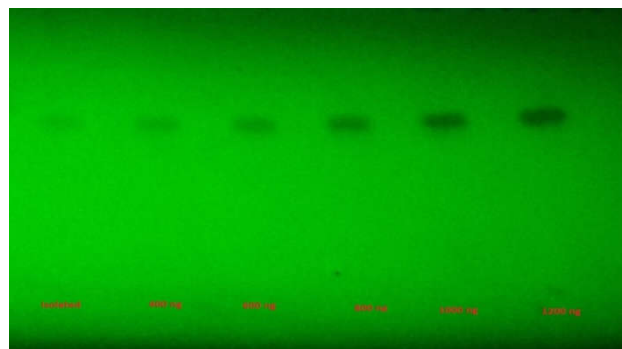


Figure 3 Developed precoated TLC plate using solvent system Ethyl acetate: Formic acid: glacial acetic acid: water (10:1.1:1.1:2.6, v/v/v/v)(400-1200ng/band) of standard and isolated compound in methanol under UV 254 nm



Figure 4 Developed precoated TLC plate using solvent system Ethyl acetate: Formic acid: glacial acetic acid: water (10:1.1:1.1:2.6, v/v/v/v)(400-1200ng/band) of standard and isolated compound in methanol Under UV366 nm.

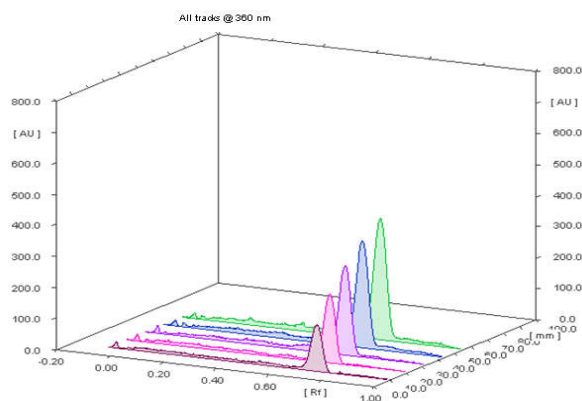


Figure 5 Calibration curve for standard isoquercitrin (400-1200 ng/spot)

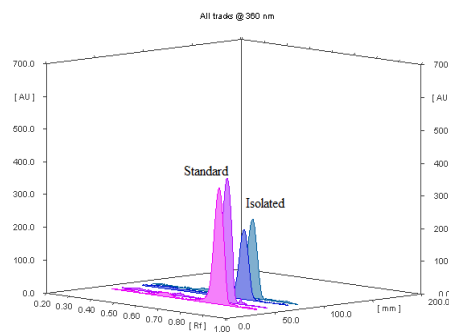


Figure 6 3D Densitogram of isolated compound and standard showing a peak at same R_f

Method validation and Quantitation of isolated compound in methanolic extract of the whole herb of *Hygrophila salicifolia* were done by HPTLC. Amount of isoquercitrin was found to be 229.801ug/ml.

DISCUSSION

TLC identity test results shows that R_f value (0.76) of Standard isoquercitrin match with the methanolic whole herb extract of *Hygrophila salicifolia* (Figure 1 & 2, Table-1). This confirms isoquercitrin present in the methanolic extract of whole herb of *Hygrophila salicifolia*.

Further quantitative estimation of scopoletin was done by HPTLC analysis using. Ethyl acetate: Formic acid: glacial acetic acid: water (10:1.1:1.1:2.6, v/v/v/v) as mobile Phase which was found to be the most suitable mobile phase.

One of the major components in the TLC fingerprint of methanolic extract of *Hygrophila salicifolia* was isoquercitrin with an R_f 0.76.

Isoquercitrin showed black spot under UV 366 nm & UV 254 nm (Figure 1 & 2). An excellent linear relationship ($R^2=0.9957$) was obtained within the range of 400-1200 ng/spot for isoquercitrin content at 360 nm.

Isoquercitrin content in the whole herb of methanolic extract of *Hygrophila salicifolia* was found to be 229.801ug/ml, as quantified by the HPTLC method.

CONCLUSION

Hygrophila salicifolia (Acanthaceae) is a traditionally valuable raw drug and whole herb can be used in Ayurvedic formulations by the industries. It is important to check the authenticity and quality of the drug to ensure batch to batch consistency in performance of the medicine. The current study has developed HPTLC method for quantification of isoquercitrin in whole herb of *Hygrophila salicifolia* (family: Acanthaceae).

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