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HPTLC fingerprinting of stem bark extract of *Nyctanthes arbor-tristis* (L.)

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ABSTRACT

Fingerprint profile of bark extract of *Nyctanthes arbor-tristis* using High Performance Thin Layer chromatography (HPTLC) has been established. HPTLC is a valuable tool for the investigation of medicinal plants with reference to the qualitative analysis of the phytoconstituents. Separation of the active constituents from the extracts has been developed using solvent system of Toluene: Ethyl acetate: Formic acid (5:4:1). The HPTLC analysis showed the presence of the flavonoid quercetin in the standard as well as in the sample and the Rf value was 0.73. These images of fingerprinting help in the proper identification and quantification of the marker compounds. On the basis of the marker compounds, new drugs could be formulated to treat various diseases

Keywords: *Nyctanthes arbor- tristis,* HPTLC analysis, quercetin, bark extract

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INTRODUCTION

Nyctanthes arbor-tristis L. is a medicinal plant which is widely distributed in Bangladesh. It is also found widely in India, Nepal and other tropical and sub tropical regions of South East Asia. It is a small flowering shrub that grows well in secluded and semi-shady places. This woody shrub has a maximum life span of 20 years and is used mostly in Indian Systems of medicines such as Ayurveda [1]. It is also used extensively by Unani and Siddha Practitioners. Various parts of this plant have been used by traditional medicine practitioners for various pharmacological actions like anti-leishmaniasis, antifungal, antipyretic, antihistaminic, anti-oxidant, and anti-inflammatory activities [2].

HPTLC is the most powerful advanced form of Thin Layer Chromatography (TLC) and consists of chromatographic layers of utmost separation efficiency and the application of sophisticated instrumentation for all steps in the procedures which include accurate sample application, standardized reproducible chromatogram development and software controlled evaluation. HPTLC is a concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis. HPTLC meets all quality requirements for today's analytical laboratories, to increase the resolution and to allow more accurate quantitative measurements[3].

MATERIALS AND METHODS

Collection of plant materials

Bark of *Nyctanthes arbor- tristis* linn was collected from in and around Mannargudi, Thiruvarur district, Tamilnadu, India. The collected materials were cleaned, shade dried and coarsely powdered. The powder was used for further studies. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematic. St. Joseph's college, Tiruchirappalli.

Preparation of ethanol extract

The powder was exhaustively extracted with solvents in 1:5 ratio using soxhlet apparatus for 6-8 hours and then centrifuged. From the centrifuged extract the supernatant was filtered through Whatman No. 1 filter paper. The filtered extract was then subjected to dryness under reduced pressure at 37 ºC (not exceeding 40 ºC), until usage DMSO was added and stored at -80 ºC. All the extracts were stored in a dessicator for further evaluation.

High Performance Thin Layer Chromatography (HPTLC) analysis

Standard Preparation

About 10mg of Quercetin was dissolved in 10ml of ethanol. One ml of the stock solution was diluted to 10ml with

ethanol. From this 3µl to 8µl was spotted containing concentration in the range of 300ng to 800ng/ml.

Test Solution

About 2.5g of bark powder was macerated with ethanol for 24hrs. It was then filtered and evaporated to dryness. The dried residue was dissolved in ethanol and used for TLC analysis.

3µl to 8µl of standard solution and 20 to 30µl of test solutions were applied on a precoated silica gel 60 F_{254} HPTLC plate (E.Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. The plate was developed in the solvent system to a distance of 8cm. The plate scanned densitometrically at 254nm using TLC Scanner3. The plate was observed under UV light at 254nm & 366nm using CAMAG REPROSTAR[3].

Stationary phase	:	Silica gel 60 F_{254}
Mobile phase	:	Toluene : Ethyl acetate: Acetic acid: ethanol (2.5:7:0.25:.25)
Scanning wavelength	:	254nm
Applied volume	:	20, 22.5, 25, 27.5 and 30µl
Development mode	:	Ascending mode
Evaluation	:	A band (Rf-0.73) corresponding to quercetin is visible in both reference and test solution tracks.

RESULTS AND DISCUSSION

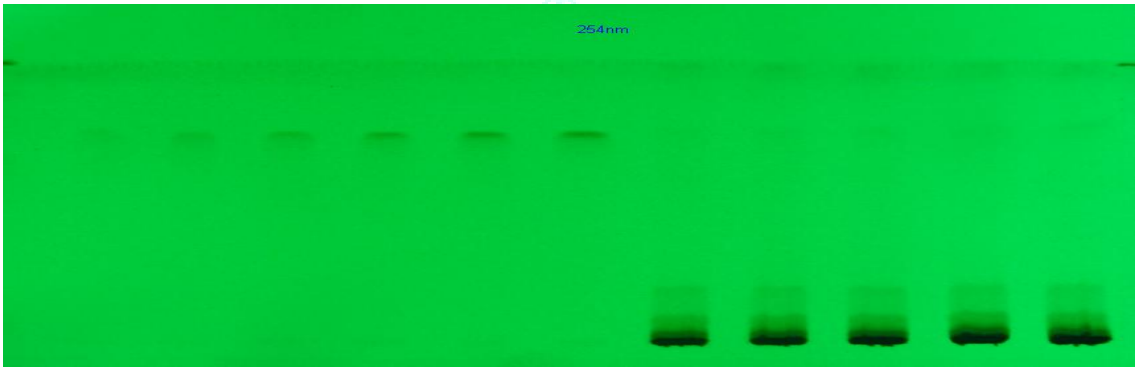
High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC analysis of the ethanol bark extract of *Nyctanthes arbor- tristis* was made along with the standard flavonoid quercetin and the solvent mixture toluene: ethyl acetate: Acetic acid: ethanol (2.5:7:0.25:0.25) was used as the mobile phase. The number of bands developed **(Figure 1)** was observed, identified and the bands of quercetin in the ethanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG REPROSTAR3 **(Figure 6).** The standard quercetin showed the R_f value of 0.73 **(Table 1).** Good linear relationship (r2 = 0.99647 and 0.99998 with reference to height and peak area, respectively) was recorded between the concentration ranges of 300-800 ng/ spot **(Figure 4).**The use of standard ensures the concentration and ratio of the test compound in the stem bark **(Figure 2(A-F)and 3(A-E).** The 3D spectra along all tracks were scanned **(Figure 5).** This result coincides with the study who reported a good correlation (*r* = 0.9998) between the standard and the sample of quercetin in the dried flowers of *Nymphaea stellata*[4].

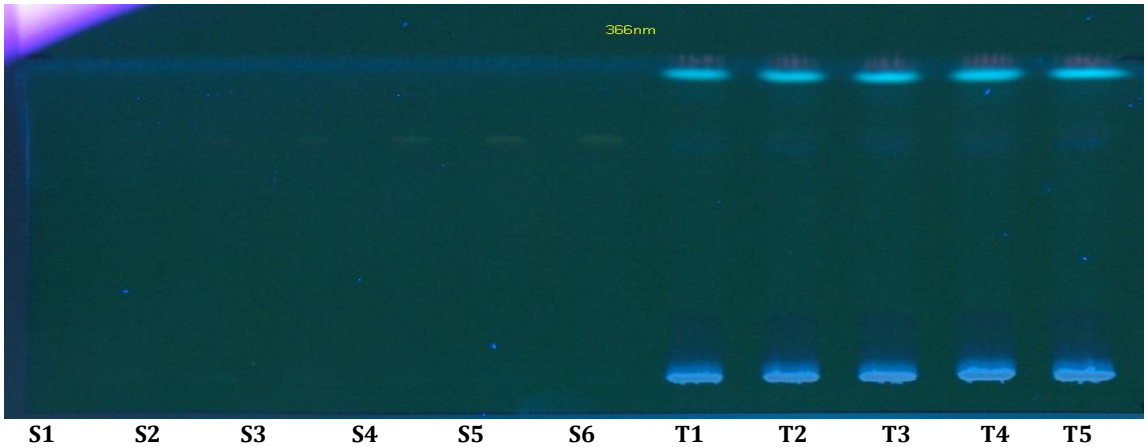
The limit of detection and limit of quantification was found to be 300 ng and 800 ng respectively. The regression equation was Y = -100.939+0.405*X with reference to height and Y=-1707.111+8.643*X with respect to area, where Y is the peak height / area and X is concentration of quercetin. With the help of the above statistical data, the content of quercetin was determined in the ethanol stem bark extract of *Nyctanthes arbor-tristis* which was 198.2 mg/100gm.

Figure 1: HPTLC finger printing of *Nyctanthes arbor – tristis* photo documentation under UV

AT 254nm



AT 366nm



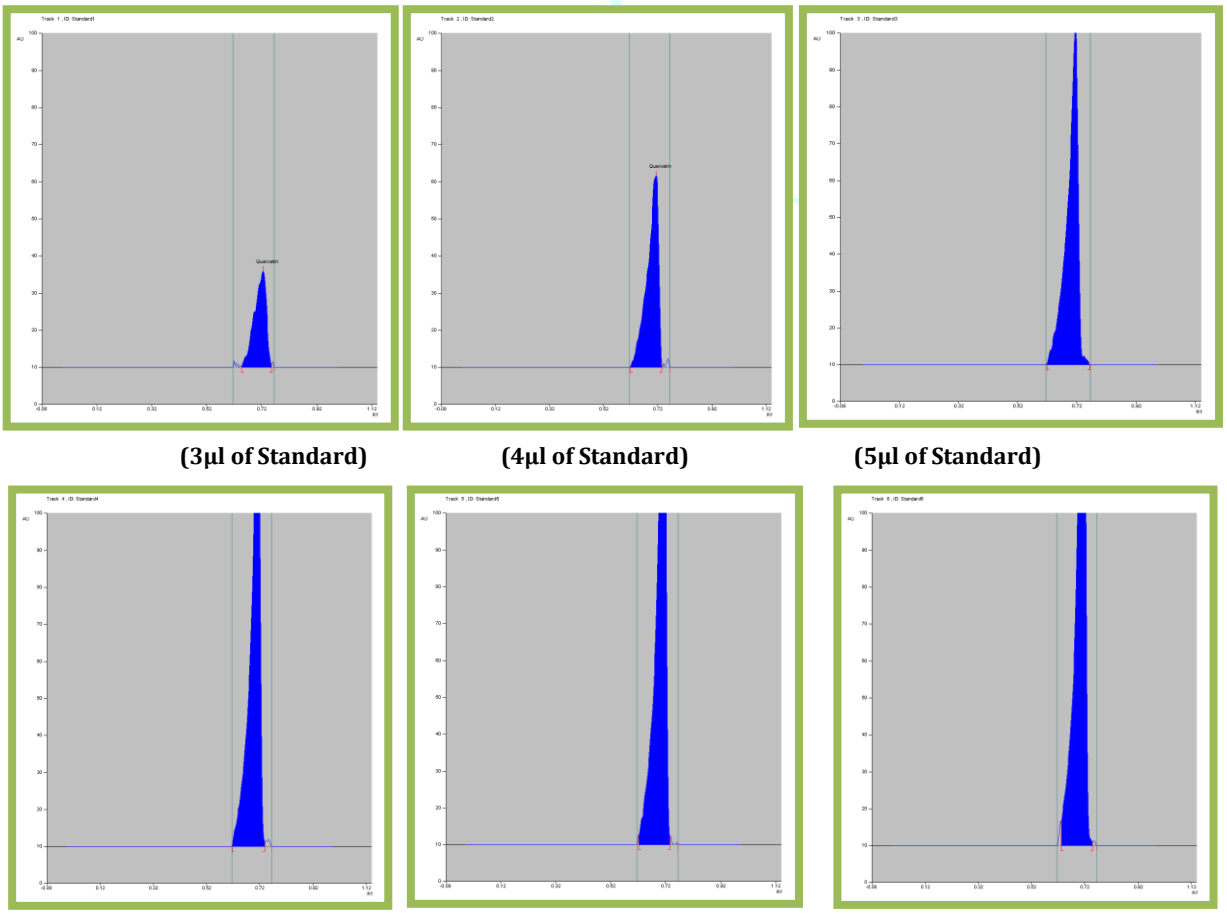
Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion and progression of tumors. Reduction of coronary heart disease has been reported to be associated with intake of flavonoid. *Barteria nigritiana, Moringa oleifera, Combretodendron macrocarpum, Cordia millenii, Afrormosia laxiflora and Sacoglottis gabo- nensis* contain appreciable quantity of flavonoid. Apart from the antioxidant properties of flavonoid, other biological functions it possesses include protection against platelet aggregation, microorganisms, hepatotoxins, viruses, tumors, ulcers, free radicals, inflammation, and allergies[5-7].

Jyothi *et al*., (2013) reported quercetin in *Cassia auriculata* L. using HPTLC fingerprint profile. The present study is first of its kind to report the HPTLC fingerprint of ethyl acetate and methanol extracts of *C. fistula* leaves showing maximum number of components i.e., 16 and 15 respectively at 400nm with solvent system of Toluene: Ethyl acetate: Formic acid in the ratio of 5:4:1. The HPTLC studies showed that ethyl acetate and methanol extracts had a mixture of compounds. This densitometric

HPTLC fingerprint profile could be used as marker for quality evaluation and standardization of the drug. Thus, HPTLC fingerprint profile along with their R_f values could serve as a reference standard for the scientist engaged in research on the medicinal properties of plants[8].

Table 1: R_f values of standard and ethanolic extract of *Nyctanthes arbor - tristis*

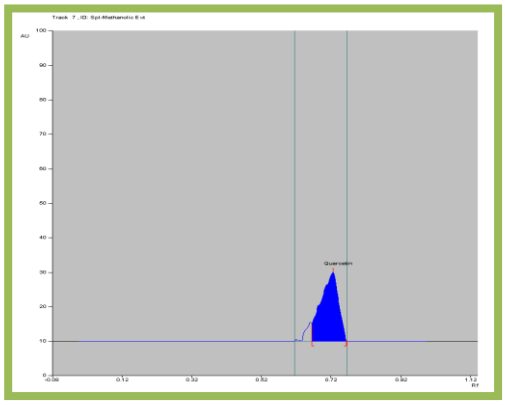
S.No	Start R_f	Start Height	Max R_f	Max Height	Height %	End R_f	End Height	Area%
Standard	-	-	-	-	-	-	-	-
Track1	0.65	0.2	0.73	25.9	100	0.76	0.9	868.3
Track2	0.62	0.2	0.72	51.7	100	0.74	1.1	1648.3
Track3	062	0.2	0.72	91.4	100	0.77	0.0	2527.7
Track4	0.62	0.4	0.72	147.4	100	0.74	1.0	3642.8
Track5	0.63	2.6	0.72	184.3	100	0.74	2.3	4312.7
Track6	0.64	6.6	0.72	220.5	100	0.75	1.0	5051.4
Sample	-	-	-	-	-	-	-	-
Track7	0.67	5.1	0.73	20	100	0.77	0.2	764.2
Track8	0.67	7.2	0.73	25.9	100	0.77	0.0	956.7
Track9	0.67	8.2	0.73	28.9	100	0.77	0.4	1036.5
Track10	0.65	2.3	0.73	32.7	100	0.77	0.7	1228.1
Track11	0.67	4.5	0.74	29.3	100	0.77	3.7	995.5



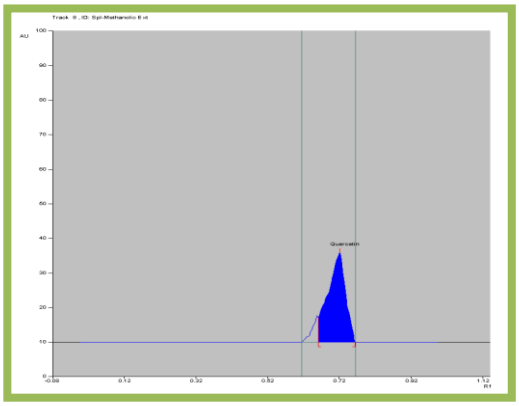
(3µl of Standard) **(4µl of Standard)** **(5µl of Standard)**

(6µl of Standard) **(7µl of Standard)** **(8µl of Standard)**

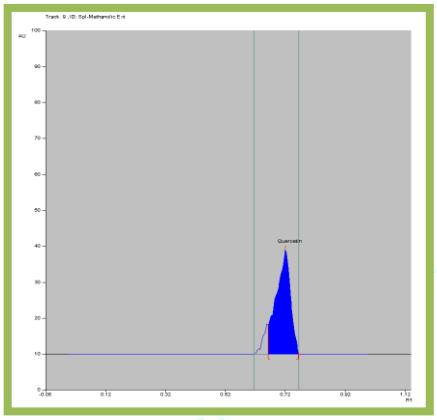
Figure 2: (A-F) HPTLC chromatogram of Quercetin standard



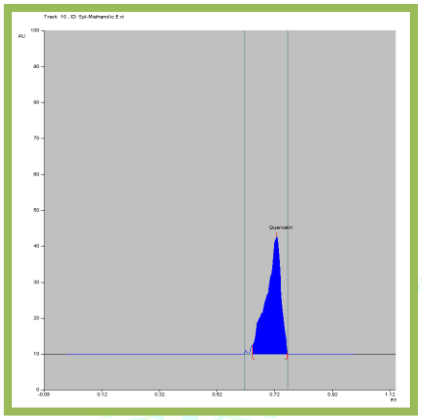
(20µl of Sample)



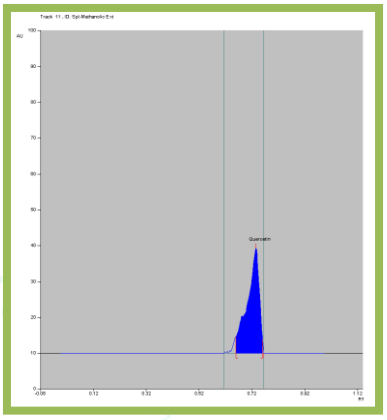
(22.5µl of Sample)



(25µl of Sample)



(27.5µl of Sample)



(30µl of Sample)

Figure 3: (A-E) HPTLC chromatogram of ethanolic extract of *Nyctanthes arbor -tristis*

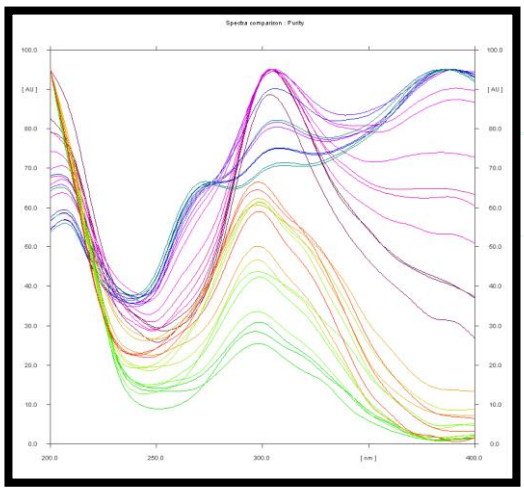


Figure 4: Spectral comparison of purity of sample Tracks with standard at selected wavelength

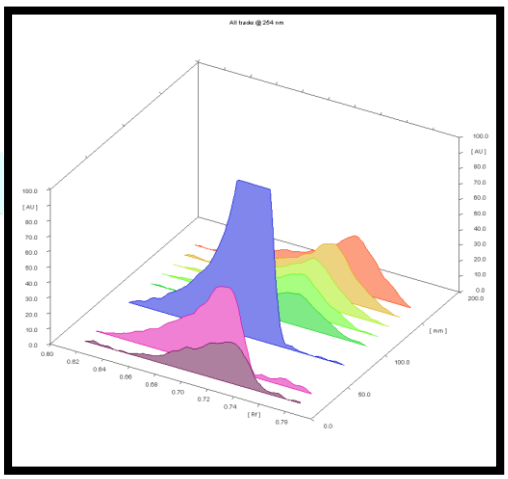


Figure 5: HPTLC – 3D display *Nyctanthes arbor - tristis*

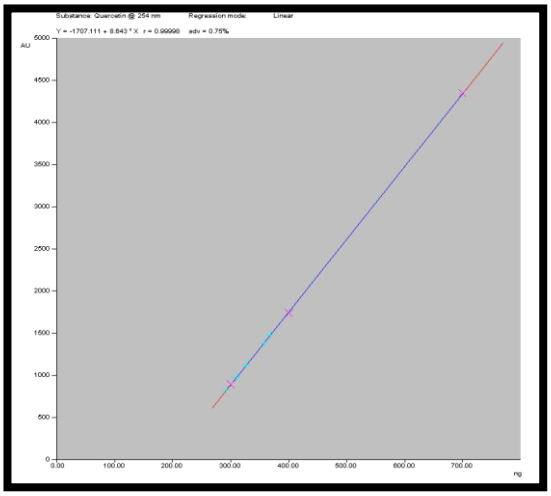


Figure 6: Standard curve of Quercetin

CONCLUSION

A rapid, simple, accurate and specific HPTLC method for quantitative estimation of quercetin present in the dried stem bark of *Nyctanthes arbor- tristis* has been developed and validated. The data could be used as a QC standard. The chromatographic studies conducted with the ethanol stem bark extract of *Nyctanthes arbor- tristis* revealed an appreciable amount of flavonoid quercetin, which confirms its medicinal value. In future, these fingerprinting images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy.

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