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High-Performance Thin-Layer Chromatographic (HPTLC) Analysis of Roots of *Euphorbia hirta* Linn.

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Abstract
India is well known for its Ayurveda medicines worldwide. In India it is estimated that about 8000 plants are used in traditional and herbal medicine. There are many species of Euphorbia from family Euphorbiaceae which are used in traditional medicines. The aim of the present study was to check the presence of phytochemical constituent flavanoid, using HPTLC analysis. The root extract of *Euphorbia hirta* was prepared using solvent alcohol. The extract has been subjected to HPTLC analysis for flavanoid profile. Quercetin was used as standard. The presence of flavanoid in alcoholic extract was confirmed based on the colour zones obtained. Quercetin was isolated from the diethyl ether fraction of alcoholic extract by solvent - solvent extraction technique and was identified and characterized by chemical tests, melting point and by a simple, sensitive, and validated HPTLC method. So, the present study explores the presence of flavonoid (quercetin) in the roots of *Euphorbia hirta* and future work aimed to isolate the flavonoid in the plant.

Keywords
Euphorbia hirta, flavonoid, HPTLC assay, quercetin,

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INTRODUCTION
Euphorbia hirta belongs to family *Euphorbiaceae* commonly known as Dudhi. The roots are fibrous, rough, and dark brown in colour. [1] It is found abundantly along the roadsides and in open grasslands areas. [2, 3] It has been reported to contain alkaloids, saponins, tannins and flavonoids which are responsible for the main pharmacological actions like antioxidant, anti-inflammatory, anti-dengue, and anti-cancer. Traditionally, it is used in the treatment of bronchial and respiratory diseases, kidney stone, gastrointestinal disorders, and diabetes. [4] It also shows antipyretic, anxiolytic, antifertility, analgesic, and anti-inflammatory activities. [5, 6] Herbal products contains a wide variety of natural chemical constituents known as 'Phytochemicals' like flavonoids, phenolic compounds and tannins. [7] India is one of the richest countries in the world regarding genetic resources of medicinal plants. About 80% of its total population depends directly upon the traditional medicines for primary health service. [8] This phenomenon has been proved by an increasing attention to herbal medicines as a form of alternative therapy by the health professionals. Herbal compounds have been reported scientifically for their biological activities. [9, 10] The therapeutic actions of the plants are due to the various chemical constituents mainly secondary metabolites, present in them. Plant metabolites are the plant chemicals which have

protective and disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. [11]

Chromatographic techniques are generally carried out for the separation and purification of phytoconstituents based on their shape or size. High Performance Thin Layer Chromatography (HPTLC) is becoming a routine analytical technique due to its low operating cost, high sample throughput and need for minimum sample clean-up. The major advantages of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. It is an important technique used for the identification, characterization and purity testing of herbal extracts and formulated products. [12, 13] Due to the growing interest in herbal medications, standardization techniques like phytochemical analysis and chromatographic techniques, e.g. Thin layer chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC) and High-performance liquid chromatography (HPLC), have been developed to analyze phytochemical constituents of plant extracts and prevent adulteration [14, 15, 16, 17]. Thus, the aim of the present study was to standardize the alcoholic extract of *Euphorbia hirta* with HPTLC profiling.

MATERIALS AND METHODS

Collection of Plant material

The roots of *Euphorbia hirta* were collected in the month of June 2019 from Hodal, India. A voucher specimen has been retained at the School of Medical and Allied Sciences, K.R. Mangalam University, Sohna Road, Gurgaon. The roots were cleaned thoroughly with distilled water to remove any type of contamination. Washed roots were air dried in shade.

Extraction by using Soxhlet apparatus

To prepare root extract of *Euphorbia hirta* Linn, the dried roots were powdered by using dry grinder and passed through sieve. This powder was packed into Soxhlet apparatus and extracted successively with alcohol. The process was continued until the solvent in the thimble becomes transparent. The extract was solidified under reduced pressure in a rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10^0 C. [18]

Chemicals

The reagents and solvents used for the extraction and HPTLC profiling were analytical grade reagents from Sigma Aldrich, Bangalore, India. Before use, solvents were filtered through a Whatmann filter paper no.1.

Fractionation and Isolation

About 20 g of alcoholic root extract was suspended in 200 ml of distilled water and extracted several times, by taking 10ml of each of solvents of decreasing polarity (such as chloroform, n-butanol, and methanol), in a separating funnel. All fractions were concentrated under vacuum and placed in a desiccator at reduced pressure for complete drying. Approximately 5g of completely dried n-butanolic fraction (dark brownish mass) was dissolved in 200 ml of distilled water. The insoluble material was filtered out and filtrate was set aside for 48 h at 4°C. The filtrate was then kept at room temperature for 1h and further extracted several times with diethyl ether. The ethereal fractions were pooled in a petri-dish and solvent was evaporated in the air. A brownish-yellow amorphous powder was obtained, which was dissolved in 30 ml of ice-cold water and centrifuged at 1500 rpm for 10 min. The supernatant was discarded, and the remaining residue was extracted from ethanol. A yellowish - green amorphous powder was obtained (0.109% w/w). [19]

HPTLC analysis

Standard Preparation

10 mg of standard reference quercetin was dissolved in methanol and filtered through Whatmann filter paper no.1. Final volume was made up to 10 ml with methanol in volumetric flask.

Sample Preparation

500 mg of crude extract of *Euphorbia hirta* was accurately weighed and dissolved in 10ml of methanol in a 50 ml beaker with stirring for few minutes. The solution was filtered through Whatman filter paper to get a clear solution and used for HPTLC analysis.

Procedure

The analysis was carried out using a Camag HPTLC system equipped with Linomat-V applicator and 100 µl syringe. 2 µl of the test solution and 2µl of standard solution were applied to the layers as 7 mm-wide bands positioned 15 mm from the bottom and 20 mm form the side of the plate. The sample was spotted against the standard using microliter syringe over the precoated silica gel 60 F254 HPTLC plates, and development of the applied plate was carried out in Camag twin-trough chamber which was pre-saturated with solvent vapours. Hexane: Acetone: Ethyl acetate (16:1:1) was used as solvent system. Ascending mode was used for the development of HP thin layer chromatography. [20, 21] HPTLC plates were developed upto 90 mm. Then the plates are dried and

visualized in UV light at 366 nm wavelength and the results were interrelated in Table 1 and Figure 1.

Table 1: HPTLC results of alcoholic extract from the roots of euphorbia hirta

Band no.	R_f (Std.)	R_f (alcoholic root extract)
1.	-	0.35
2.	-	0.50
3.	0.76	0.75
4.	-	0.84

Figure 1: HPTLC chromatography profile of quercetin and alcoholic extract from the roots of *Euphorbia hirta* viewed at 366nm.

Calibration Curve of Standard (Querectin)

1 mg of reference standard of querectin was dissolved in 100 ml of methanol to yield stock solution of 100 µg/ml concentration. Calibration curve in the concentration range of 10-50µg/ml was prepared and checked for reproducibility, linearity and for validation of the proposed method. [22, 23]

RESULT AND DISCUSSION

Using the proposed method, the R_f value of querectin was found to be 0.76. Chromatogram of standard was shown in Figure 2. The extract of *Euphorbia hirta* had produced 4 peaks with R_f values 0.35, 0.50, 0.75 and 0.84 and was shown in Figure 3. The total querectin content present in extract was found to be 0.109% w/w. Quercetin, a yellow amorphous powder (Melting point – 305^{0}C), was isolated from the root extract using solvent - solvent extraction method. Shinoda and Ferric chloride tests were found to be positive, thus confirming the isolated compound as a flavonoid. Furthermore, the alcoholic extract of *Euphorbia hirta* shows peak in the chromatogram at same R_f value as that of standard. The precision, linearity and accuracy of the method was checked with standard querectin with the calibration curve in the concentration range of 10-50µg/ml. Calibration curve of standard querectin was shown in Figure 4.

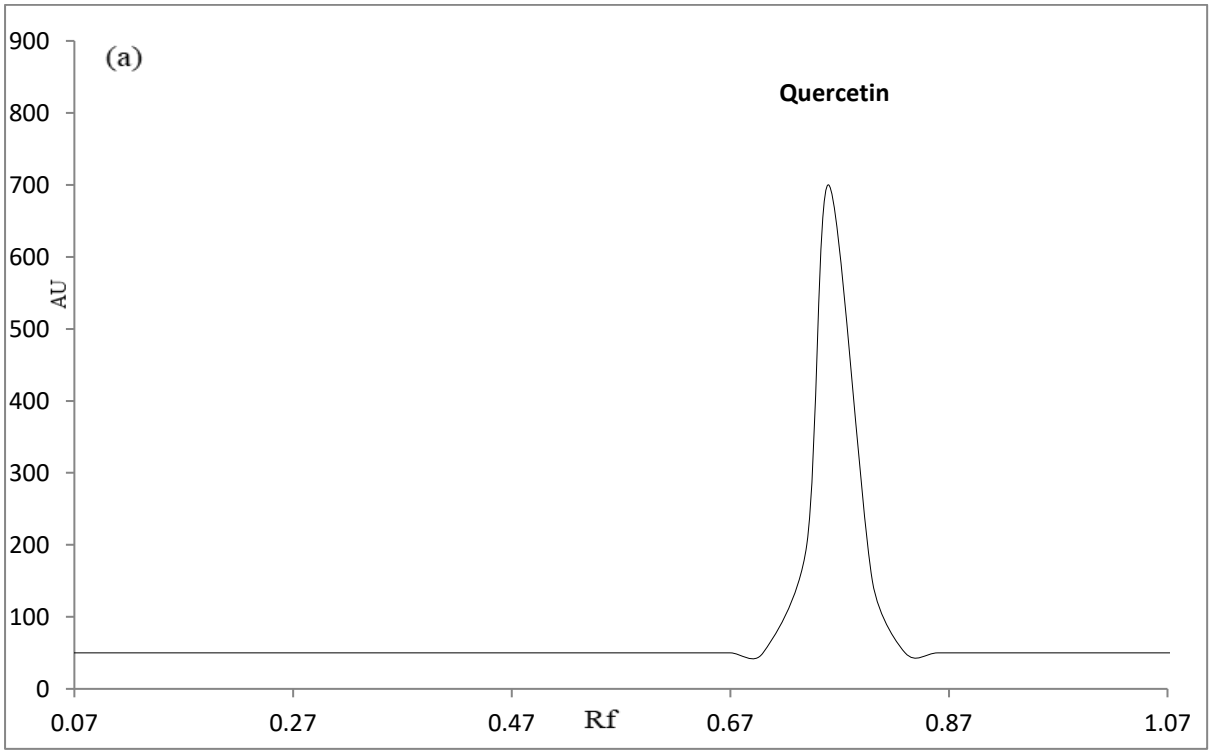

Figure 2: HPTLC Chromatogram of Standard Quercetin

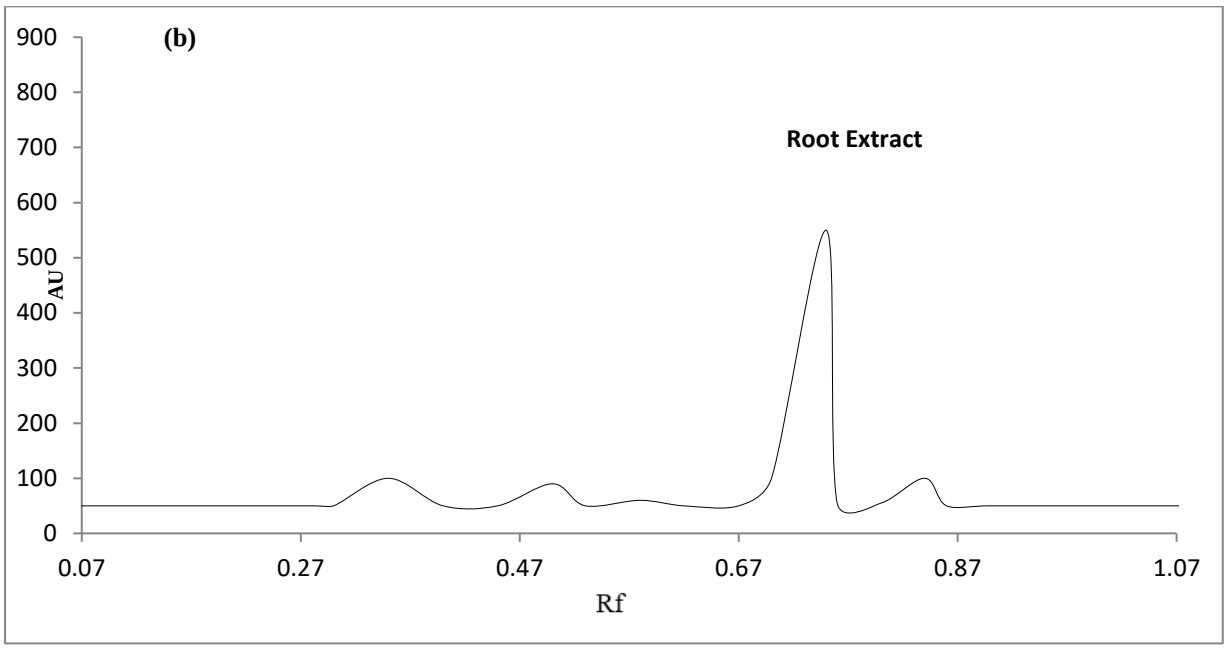

Figure 3: HPTLC Chromatogram of Alcoholic Root Extract

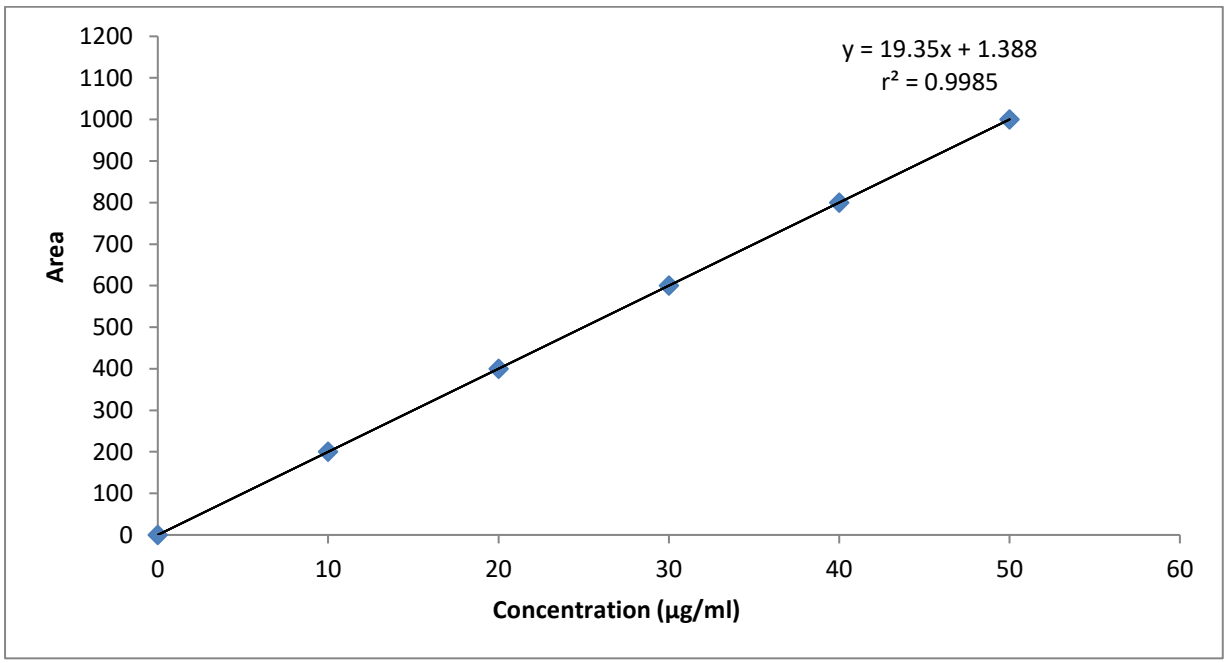

Figure 4. Calibration Curve of Quercetin

CONCLUSION

The interest in herbal drug medicines is increased now a days due to their low cost and lesser side effects. Hence, for standardization of plant extracts, chromatographic techniques have been developed like HPLC and HPTLC etc. The present study evidences the occurrence of flavonoid compound in *Euphorbia hirta*. As *Euphorbia hirta* have been reported to possess various pharmacological activities, so, the data reported here could be useful in standardizing the extract of *Euphorbia hirta*.

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