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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY ANALYSIS OF *EUPHORBIA HIRTA* LINN

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Abstract:
Euphorbia hirta Linn is a medicinal plant belonging to the family Euphorbiaceae. It is widely used in traditional medicine to treat various ailments such as fever, headache, and stomach pain. The plant is rich in phytochemicals such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds. High-performance thin-layer chromatography (HPTLC) is a powerful technique that can be used to analyze the phytochemical constituents of plant extracts. HPTLC is a simple, rapid, and cost-effective method for the qualitative and quantitative analysis of phytochemicals. In this study, HPTLC was used to analyze the flavonoids (Quercetin) in *Euphorbia hirta* Linn These results are in agreement with previous studies which have reported the presence of these phytochemicals in Euphorbia hirta Linn. HPTLC is a useful tool for the rapid and accurate analysis of the phytochemical constituents of medicinal plants. The results of the TLC and HPTLC analysis of Euphorbia hirta suggest the presence of bioactive components such as flavonoids (Quercetin). However, the exact composition of these components is still unknown. Further studies are needed to identify the exact composition and quantify the amounts of these bioactive components present in the plant.

Key words: Quercetin, TLC, HPTLC, *Euphorbia hirta*, Validation

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INTRODUCTION:

Quercetin is a naturally occurring flavonoid present in a variety of fruits, vegetables and herbs, including *Euphorbia hirta* Linn (*E. hirta*). It is widely used in traditional medicine due to its anti-inflammatory, anti-oxidant, anti-allergic and anti-cancer properties. Many studies have demonstrated that quercetin has multiple beneficial effects on human health and has been used to treat various health conditions such as allergies, cancer, cardiovascular diseases and diabetes. High performance thin layer chromatography (HPTLC) is an efficient and widely used technique for the analysis of quercetin in plant extracts. This method is based on the separation of compounds in the sample, which is then monitored and quantified using a special detector. HPTLC has been successfully used for the qualitative and quantitative analysis of quercetin in *E. hirta* extracts. For example, a study by Chaturvedi et al. (2017) used HPTLC to determine the quercetin content of E. hirta extracts[1]. This method has also been used to study the stability of quercetin in *E. hirta* under different environmental conditions. A study by Singh et al. (2016) demonstrated that quercetin was stable under light and dark storage conditions, and in the presence of various solvents[2].

However, no TLC or HPTLC method for the quantitative determination of *Euphorbia hirta* was available and so the present work involves the use of HPTLC method for the quantification of this drug as TLC/HPTLC, a well recognized routine analytical technique proves to be more economical for analysis of pharmaceuticals than other chromatographic methods because of its advantages like a Disposable stationary phase, static detection free of time constraints, storage device for chromatographic information wider range of detection possibilities, utilization of smaller volumes of solvents, minimum sample clean up and simultaneous estimation of several components in a short time is also possible. Therefore, the main aim of the present work is to develop a validated HPTLC method for the determination of phytoconstituent present in selected plant. The proposed HPTLC method was validated as per ICH guidelines.

MATERIAL AND METHODS:

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. The leaves extract of *Euphorbia hirta* were collected from local area of Bhopal in the month of May, 2022. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves extract of *Euphorbia hirta* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

Extraction from plant materials is an important step in phytochemical processing for discovering bioactive secondary metabolite. Selection of a suitable extraction technique is also important for the standardization of herbal products. Extraction is used to extract suitable soluble constituents, with the aid of the chosen solvents except those not necessary. The products obtained from the plant were thoroughly washed in tap water and rinsed in purified water. The cool, stable samples obtained from the plants were cut into small pieces and dried under shade for 3 to 4 weeks.

Defatting of plant material

65 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by Soxhlet's apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted powdered of *Euphorbia hirta* has been extracted with hydroalcoholic solvent (Ethanol: water; 70:30) using Soxhlet's apparatus for 48 hrs, filtered and dried using vacuum evaporator at 40°C [3].

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\textbf{Percentage yield} = \frac{\textbf{Weight of Extract}}{\textbf{Weight of powdered drug}} x100$$

Phytochemical Screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for

extract. Phytochemical examinations were carried out for all the extracts as per the standard methods [4].

Separation and Identification of phytoconstituents by TLC

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used[5]. After pre-saturation with mobile phase for 20 min for development were used. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples. The developed thin layer chromatographic plates were visualized in normal light, short UV light (254nm), and long UV light (365nm) using TLC cabinet (Electronic India).

Detection and Calculation of R_f Value

Once the chromatogram was developed the R_f Value of the spot was calculated using the formula:

$$\mathbf{Rf} = \frac{\mathbf{Distancetraveledbysolute}}{\mathbf{Distancetraveledbysolvent}}$$

Estimation of Quercetin using HPTLC method

A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl), CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study. The MFP-PARC, HPTLC facility used for present study[6].

Preparation of the Standard

1mg/ml of the standard, quercertin was prepared with methanol. From this 100µl was diluted with 950µlof methanol and hence the concentration of the standard was 100 µg/ml.

Preparation of the extract Sample

1mg/ml of the all extract was prepared with methanol seperately. From this 100µl was diluted with 950µlof methanol and hence the concentration of the extract was 100 µg/ml.

Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCKKGaA). 100 µg/ml of the Standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 2, 4, 6, 8, and 10µl.The concentration of the sample was 10 mg/ml, and the different amounts were 2µl. The mobile step used was toluene: formic acid (5:4:1) ethyl acetate. Built the chromatograph For 15 minutes, dried at room temperature and scanned at 254 nm. The normal maximum peak area was measured. Average peak area of the standard was calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software.

Estimation of quercetin in herbal extracts

Estimation of quercetin in hydroalcoholic extract of *Euphorbia hirta*. The mean peak height/area of the sample was calculated and the content of quercetin was quantified using the regression equation obtained from the standard curve.

Validation of Developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

D. Precision

The precision are established in three differences:

1. Repeatability

2. Intermediate precision
a) Day to Day
b) Analyst to Analyst
3. Reproducibility
1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for drug indicates the precision under the same operating condition over short interval time.

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentration.

b) Analyst- To- Analyst Precision

Analyst to analyst variation was performed by different analyst in five replicate at five concentrations.

Reproducibility

The reproducibility was performed by chemical to chemical (use of rankem chemicals in place of merck chemicals) variation in five replicate at five concentrations.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

RESULTS AND DISCUSSION:

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using pet. Ether and hydroalcoholic solvents.

Small portion of the dried extracts was subjected to the phytochemical tests using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml.

Qualitative phytochemical analysis clearly demonstrated the presence of number important active constituents and revealed that *Euphorbia hirta* have similar phytochemical constitution. The results revealed that the plant is a rich source of different secondary metabolites like flavonoids, saponins, carbohydrates, proteins and tannins. The assorted phytochemicals are common compounds to give pharmacological benefit. However, there were certain compounds present in this herb are likely to be different from the other plants. Therefore, they can be recommended to be used as therapeutic agent to certain illnesses. HPTLC studies revealed well resolved peaks of extracts containing quercetin. The spots of the entire chromatogram were visualized under UV 254 nm and the percentage of quercetin (Rf 0.48±0.2) in *Euphorbia hirta*hydroalcoholic extract, was found to be 0.0061% (w/w) respectively.

Table 1: % Yield of hydroalcoholic extract of*Euphorbia hirta*

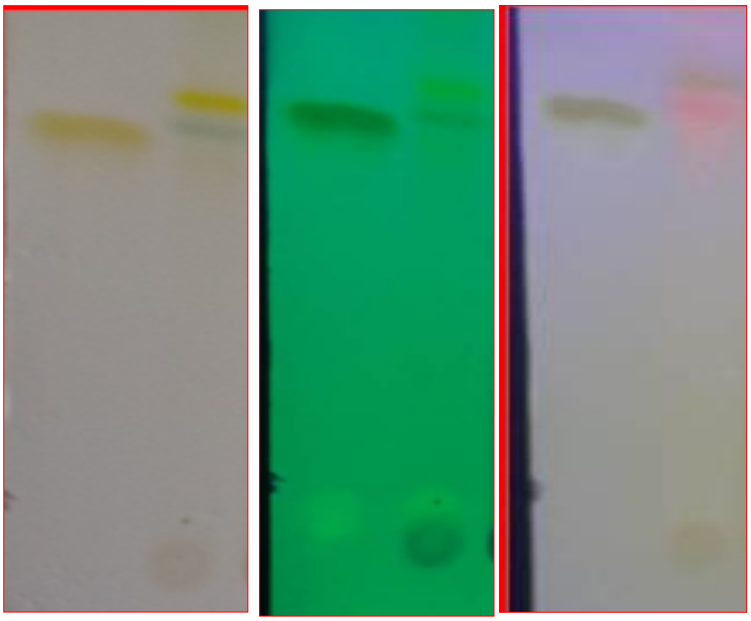
S. No.	Extracts	% Yield (w/w)
1.	Pet. Ether	2.43%
2.	Hydroalcoholic	7.85%

Table 2: Phytochemical screening ofextractof*Euphorbia hirta*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Mayer's Test Wagner's Test Dragendroff's Test Hager's Test	 -ve -ve -ve -ve
2.	**Glycosides** Legal's Test	 +ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenol** Ferric chloride test	 -ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Molisch's Test Benedict's Test Fehling's Test	 -ve +ve +ve
7.	**Saponins** Froth Test	 +ve
8.	**Diterpenes** Copper acetate test	 +ve
9.	**Tannins** Gelatin Test	 +ve

Table 3: Identification of phytoconstituents by TLC of *Euphorbia hirta*

TLC of *Euphorbia hirta*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)** Dis. Travel by mobile phase= 5.5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.63 Short- 0.63 Normal- 0.63
2.	**(Hydroalcoholic extract)** Dis. Travel by mobile phase= 5.5cm No. of spot at long UV = 2 No. of spot at short UV = 1 No. of spot at normal light= 3	 Long- 0.63,0.74 Short- 0.63 Normal- 0.58,0.63,0.74
	Spot Sequence	
	Quercetin	1st
	Euphorbia hirta extract	2nd



Normal light **Short UV** **Long UV**

Figure 1: TLC of *Euphorbia hirta* extract

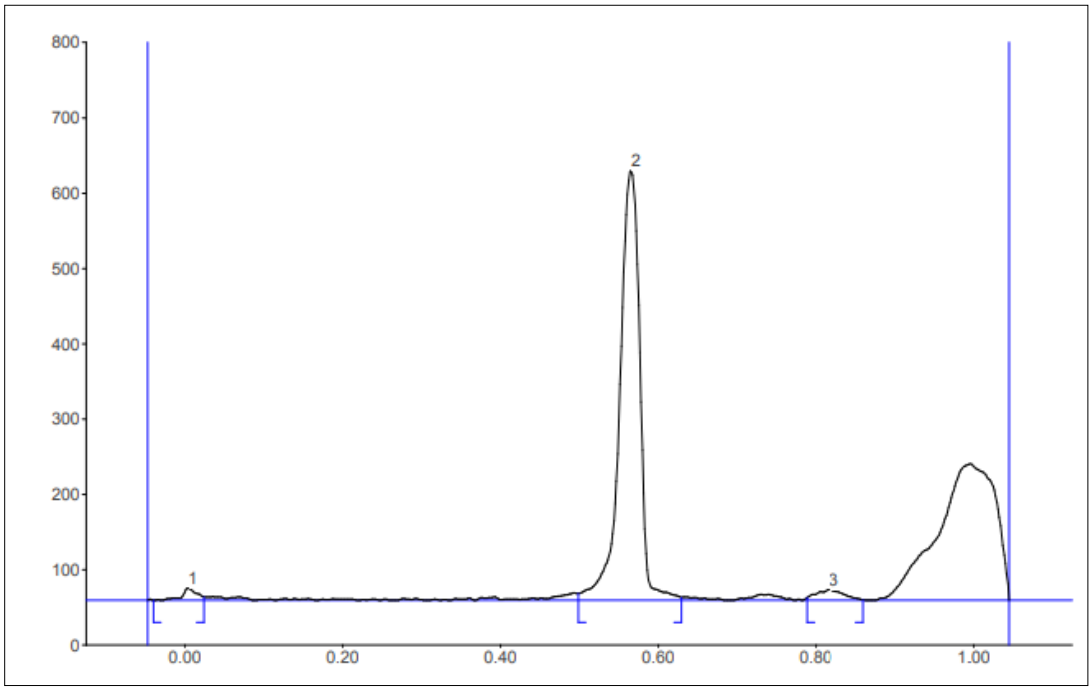


Figure 2: Chromatogram of standard quercetin 0.2µg

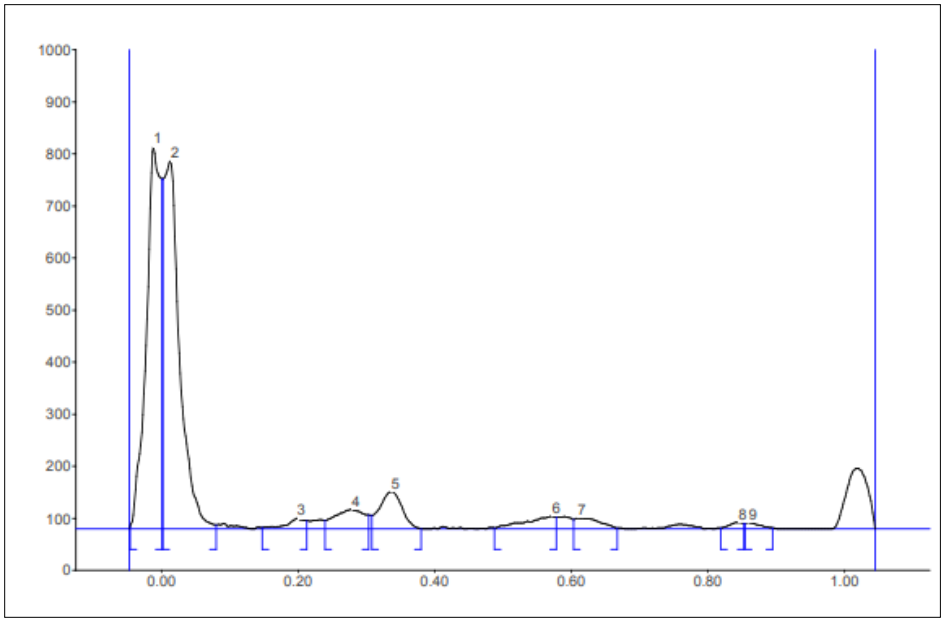


Figure 3: HPTLC chromatogram of Hydroalcoholic extract of *Euphorbia hirta*

Table 4: Results of HPTLC estimation

Estimation	Hydroalcoholic extract
Area	244.6
Percentage Found	0.0061

Table 4: Results of validation parameters

1.	**Recovery study**	
	80%	98.056±0.241
	100%	97.435±0.494
	120%	98.603±0.717
2.	**Repeatability**	96.210±0.010
3.	**Day-to-day variation**	96.490±0.010
4.	**Analyst- to-analyst variation**	96.310±0.010
5.	**Reproducibility**	95.710±0.020
6.	**Robustness**	95.370±0.020
7.	**LOD (µg/ml)**	0.010
8.	**LOQ (µg/ml)**	0.030

CONCLUSION:

Euphorbia hirta is a plant with many medicinal uses. HPTLC (High Performance Thin Layer Chromatography) has been used to identify and quantify the various phytoconstituents present in the plant. HPTLC analysis has also revealed that Euphorbia hirta contains some potential bioactive compounds such as Quercetin, which could be useful in the treatment of certain diseases. HPTLC analysis is a useful tool for identifying and quantifying the various phytoconstituents present in Euphorbia hirta and can help in the development of potential therapeutic agents derived from this plant. The results of the TLC and HPTLC analysis of *Euphorbia hirta* suggest the presence of bioactive components such as flavonoids (Quercetin). However, the exact composition of these components is still unknown. Further studies are needed to identify the exact composition and quantify the amounts of these bioactive components present in the plant.

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