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DETERMINATION OF QUERCETIN IN *EUPHORBIA THYMIFOLIA* AND *MANILKARA HEXANDRA* EXTRACTS USING RP-HPLC

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Keywords:

HPLC, Quercetin estimation, *E. thymifolia*, *M. hexandra*

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ABSTRACT: High-Performance Liquid Chromatography is an important and powerful analytical technique for the identification, quantification and isolation of natural products. In the present investigation, a Reversed-Phase-HPLC method was developed for the identification and quantification of the marker compound quercetin in *Euphorbia thymifolia* Linn. (whole plant) and *Manilkara hexandra* Roxb. (leaves). Both the plants have been used to cure various ailments since a long time in India. The results confirmed the presence of quercetin in both the plants, and its concentration was found higher in *M. hexandra* than *E. thymifolia* extract. Thus, the study justified the traditional use of these plants as medicines, and both the plants could be used in the formulation of some quality drugs in the future; however, further studies are required in this direction.

INTRODUCTION: Medicinal plants are regarded as an important source of pharmaceuticals for the formulation of cost-effective drugs due to the presence of biologically active compounds. The medicinal value of the plant lies in some of its chemical substances that produce a definite physiological action on human body [1]. Quercetin, a flavanol from the flavonoid group of polyphenols, is found in plants. It is one of the most researched flavonoids, well-known for its antioxidant and anti-inflammatory properties. It is found to have a broad range of health benefits and is used to treat various diseases. *Euphorbia thymifolia* Linn. belongs to the family Euphorbiaceae. It is usually referred to as 'laghududhika' or 'chhoti-dudhi'. In India, it is found to grow along roadsides, wastelands, and semi-arid regions and is considered a weed.

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It is a small branched, hispidly pubescent, prostrate annual herb. *Euphorbia thymifolia* is traditionally used as a blood purifier, sedative, hemostatic; aromatic, stimulant, astringent in diarrhea and dysentery, anthelmintic, demulcent, laxative and also in cases of flatulence, constipation; in chronic cough; as an antiviral in bronchial asthma and paronychia [2, 4]. *Manilkara hexandra* (Roxb.) Dubard belongs to the family Sapotaceae.

It is commonly called as Rayan/ Khirni. The plant is an evergreen tree, native of Central India and Deccan peninsular. It is also found growing at Sri Lanka, Thailand, Indochina, and Hainan [1, 5]. M. Hexandra is an underutilized fruit tree that falls under the category of endangered plants. The fruit is edible; the seed is used as cooking oil by the natives. The Koli tribe uses the decoction of the bark in diarrhea for children. The stem bark is also recommended for fever, jaundice, helminthiasis, flatulence, stomach disorder, *etc.* [1, 6]. Keeping in view, the importance of both the plants in the Indian traditional medicinal system to cure various ailments, the present study was conducted. Previous studies conducted on the preliminary

phytochemical analysis of *E. thymifolia* and *M. hexandra* aqueous extracts revealed the presence of important bioactive compounds, including (flavonoids) [1, 7]. TLC profiling for identifying and separating phytochemicals and estimation of total flavonoid content (TFC) in both the plants has also been done earlier [1, 7]. On the basis of these previous findings, the present study was carried out on *E. thymifolia* Linn. (whole-plant extract) and *M. hexandra* Roxb. (leaf extract), for the identification and quantitative estimation of quercetin with the help of the HPLC technique.

FIG. 1: (A) *EUPHORBIA THYMIFOLIA* LINN. (B) *MANILKARA HEXANDRA* ROXB

MATERIAL AND METHODOLOGY:

Collection, Identification and Authentication of the Plants: *Euphorbia thymifolia* was collected from a village named 'Nitardi' and *Manilkara hexandra* was purchased from 'Santosh nursery Shujalpur', located in Shajapur district, Madhya Pradesh. Further, the plant materials were identified, authenticated and voucher specimens submitted in 'Herbarium', Department of Botany, Dr. Hari Singh Gour University, Sagar, M.P. The registration number allotted to *Euphorbia thymifolia* specimen is Herbarium number P1 (bot/BG/201198) and to *Manilkara hexandra* P2 (bot/BG/201199).

Preparation of Extract: The plant materials were shade dried at room temperature for about 15 days, then powdered by a mechanical grinder and sieved to give particle size 40-100 mm. The dried and powdered plant materials (50 g each) were extracted with hot continuous percolation method (Soxhlet extraction). The temperature during extraction was maintained at 70 °C. The extraction process was carried out by using water as solvent. The extract was filtered through a paper filter (Whatman no.1) and then evaporated to dryness under the reduced pressure developed by the rotary evaporator. The obtained crude extracts were stored in dark glass bottles for further processing.

Identification and Quantification of Marker Compound (Quercetin) by RP-HPLC: H.P.L.C. or High-Performance Liquid Chromatography, formerly referred as High-Pressure Liquid Chromatography, is a technique in analytical chemistry used to separate, identify and quantify the components in a mixture. HPLC is useful for compounds that cannot be vapourised or that decompose under high temperatures. HPLC provides both quantitative and qualitative analysis in a single operation [8]. High pressures of up to 400 bars are required to elute the analyte through the column before they pass through the detector. HPLC fingerprinting includes a recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases [9]. In Normal-Phase Chromatography, the compounds separate on the basis of their interaction with solid particles of a tightly packed column (polar stationary phase) and the solvent of the mobile phase (non-polar). In contrast, Reversed-Phase Chromatography is a newly evolved type of HPLC in which a non-polar (hydrophobic) stationary phase and a polar (hydrophilic) mobile phase is used. Nowadays, the RP-HPLC technique is much widely used by pharmaceutical industries to qualify drugs prior to their release.

Reagents and Chemicals: Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol, acetonitrile, and water used were of HPLC grade and purchased from Merck Ltd, New Delhi, India.

Instrumentation: A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for the determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C18 (250 × 4.6 mm, 5µm) column, a Data Ace software.

Chromatographic Condition: The chromatographic analysis was performed at ambient temperature on an RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min^{-1}.

A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

Determination of λ_{max}:

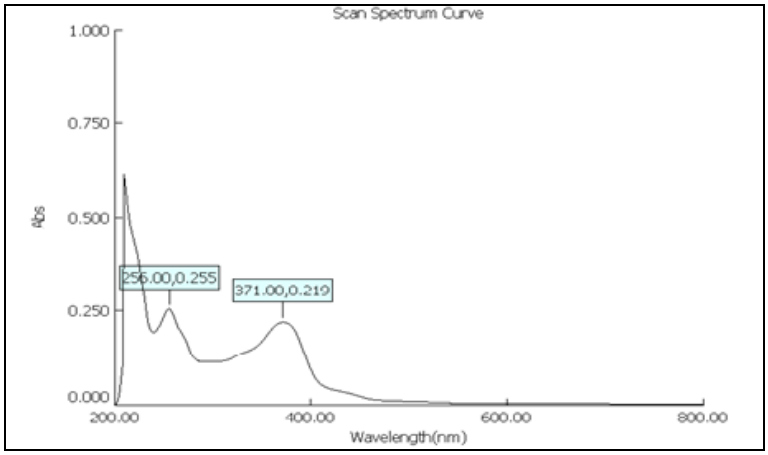

FIG. 2: DETERMINATION OF λ_{MAX}

Selection of Separation Variable:

TABLE 1: SELECTION OF SEPARATION VARIABLE

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Acetonitrile	50
Methanol	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	256 nm
Retention time	2.50± 0.5 min

Preparation of Standard Stock Solution: 10mg of quercetin was weighed accurately and transferred to a 10 ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000 ppm.

Preparation of Working Standard Solution: From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10 ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Sample Preparation: 10 mg extract was taken in 10 ml volumetric flask and dilute up to the mark with Methanol; the resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with the same solvent to obtain a concentration of 1000 µg/ml. The resulting solution was again filtered using Whatman filter paper no. 41 and then sonicated for 10 min.

RESULTS AND DISCUSSION:

Identification of Marker Compound (Quercetin) By RP-HPLC: A reverse phase C-18 column equilibrated with mobile phase methanol: acetonitrile (50:50, v/v) was used. The mobile

phase was filtered through Whatman filter paper and degassed. The mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 256 nm. The sample was injected using a 20 µl fixed loop and the total run time was 10 min. The sample solution was chromatographed and a concentration of quercetin in the extracted sample was found out using a regression equation.

Preparation of the Calibration Curve of Quercetin: Each of the standard drug solutions was injected 3 times, and the mean peak area of the drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

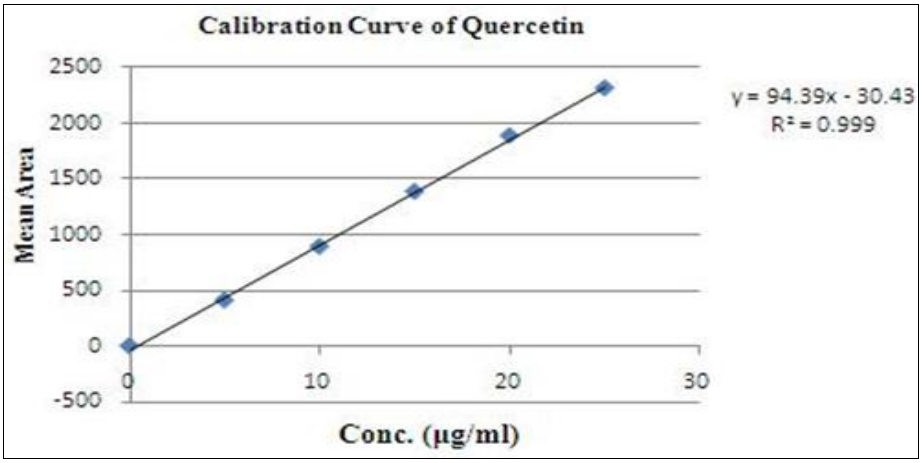

FIG. 3: CALIBRATION CURVE OF QUERCETIN

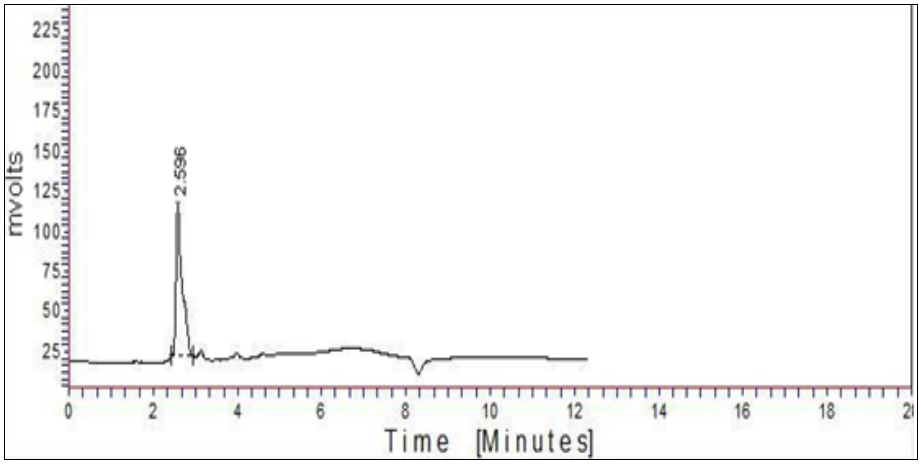

FIG. 4: CHROMATOGRAM OF STANDARD QUERCETIN

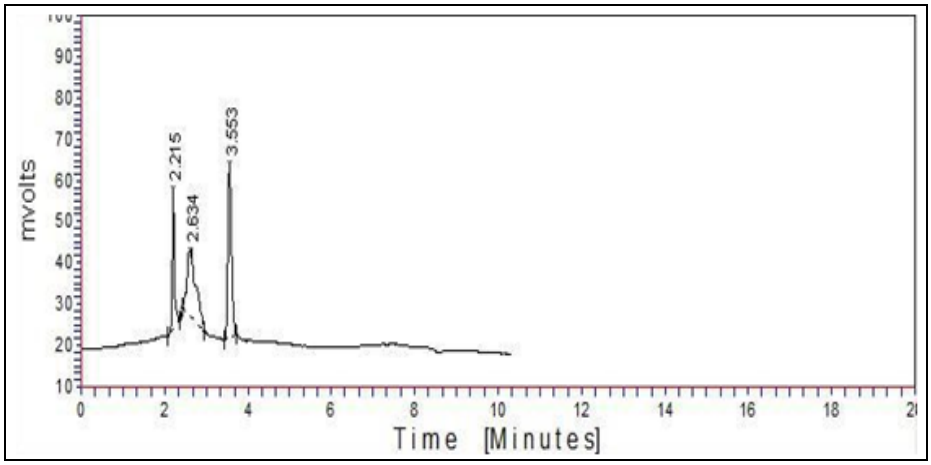

FIG. 5: CHROMATOGRAM OF *E. THYMIFOLIA* WHOLE EXTRACT

Quantitative Estimation of Quercetin: The results of quantitative estimation of quercetin in both the plant extracts are presented in the table below:

TABLE 2: PREPARATION OF CALIBRATION CURVE

S. no	Conc.	Mean AUC
1	0	0
2	5	410.235
3	10	892.541
4	15	1387.324
5	20	1889.548
6	25	2317.181

TABLE 3: CHARACTERISTICS OF THE ANALYTICAL METHOD DERIVED FROM THE STANDARD CALIBRATION CURVE

Compound	Linearity Range	Correlation	Slope	Intercept
	µg/ml	co-efficient		
Quercetin	5-25	0.999	94.39	-30.43

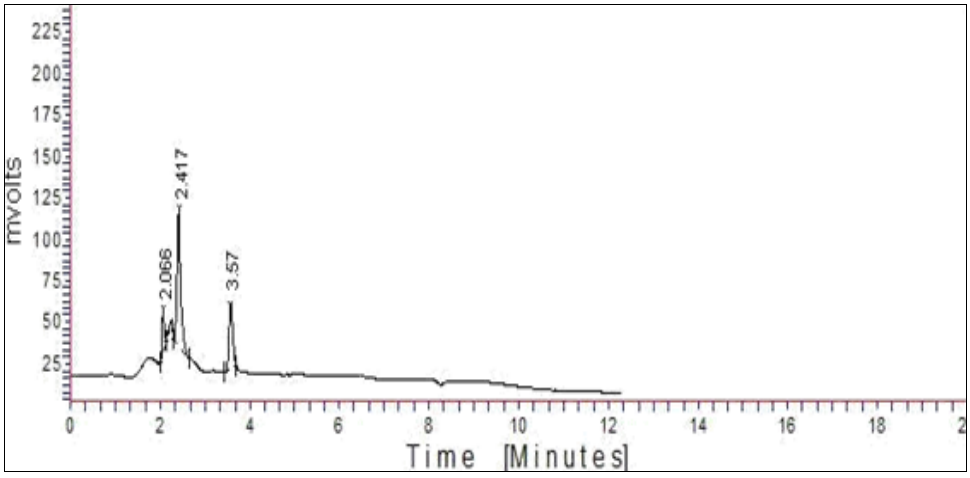

FIG. 6: CHROMATOGRAM OF *M. HEXANDRA* LEAF EXTRACT

TABLE 4: QUANTITATIVE ESTIMATION OF QUERCETIN IN DIFFERENT EXTRACTS

S. no	Extract	RT	Area	% Assay
1	*Euphorbia thymifolia L.*	2.634	222.864	0.268
2	*Manilkara hexandra R*	2.417	411.407	0.468

Chromatograms of the standard quercetin and the plant samples were recorded and the retention time of the peak in the samples was compared with the standard used. The measure of the area under the peak is proportional to the amount of the compound present in the sample and % assay indicates the purity of the compound. The results revealed that the concentration of quercetin is higher in *M. hexandra* than *E. thymifolia* extract.

CONCLUSION: This report confirmed the presence of an important bioactive flavonoid, quercetin, and its quantitative estimation in *E. thymifolia* and *M. hexandra* extracts was successfully and rapidly achieved using the RP-HPLC technique. The present study could lead to the development of some novel drugs in the future by the pharmaceutical industries to accomplish the unsupplied therapeutic needs. The data presented here could be helpful in standardizing extracts of these plants. However, further researches are required for the standardization, identification, and isolation of some useful bioactive phyto-compounds present in the selected plants with the help of available primary information.

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CONFLICTS OF INTEREST: The author declares no conflict of interest.

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