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PHYTOCHEMICAL STUDIES OF *MELIA AZADIRACHTA* & *MURRAYA KOEINGI*

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ABSTRACT

Quercetin is the most abundant natural flavonoid, present in medicinal plants. Quercetin is of interest because of its pharmacological function. Quantification of quercetin from the dried leaves of *Melia azadirachta and Murraya koiengi* was carried out based on chromatographic separation. Sensitive, simple, and accurate high-performance liquid chromatographic method has been established for detection of quercetin in *Melia azadirachta* & *Murraya koeingi* dry leaf powder.

INTRODUCTION: Neem *(Melia azadirachta)*, curry leaves *(Murraya koeingi)* are perhaps the most useful traditional medicinal plants in India. *Melia azadirachta* belongs to the family Meliaceae and *Murraya koiengi* belongs to the family Rutaceae. *Melia azadirachta* is known as mahanimbin in Ayurvedic system of medicine and is used for management of diabetes. The plant is reported to contain nimbidine a bioactive alkaloid. Hypoglycemic property of fruit and bark of this tree has been already reported [1, 2, 3, 4]. Biologically active ingredients of these plants have diverse applications.

Murraya koenigii (Rutaceae) commonly known as "Curry Patta" (Hindi) is widely used as a spice and condiment in India and other tropical countries. Various parts of *Murraya koenigii* have been used in traditional or folk medicine for the treatment of rheumatism, traumatic injury and snake bite and it has been reported to have antioxidant, anti-diabetic and anti-dysenteric activities [5]. Mahanimbine is a carbazole alkaloid and present in leaves, stem bark and root of *Murraya koenigii* [6, 7]. These compounds belong to triterpenoids. Flavonoids (flavus- yellow) orbioflavonoids, are a ubiquitous group of poly phenolic substances which are present in most plants, reported in the seeds, fruit skin, peel, bark, and flowers [8, 9, 10, 11, 12].

The thorough study of literature resulted in insufficient data regarding the separation of the flavonoid from the chloroform extracts of *Melia azadirachta* and *Murraya koeingi* using the HPLC method. The objective of this work was to develop a simple, effective method to analyze the flavonoid content of leaves of *Melia azadirachta & Murraya koeingi* using HPLC.

PLANT MATERIALS AND SAMPLE PREPARATION: The leaves of *Melia azadirachta & Murraya koeingi* were collected from Kothanur (Bangalore, India) at an altitude of 949 meters (3113 ft.). Plant samples were authenticated by the Department of Life sciences, Kristu jayanti college, Bangalore. 5 kg of the fresh plant material was collected; shade dried and powdered in mixer.500gms of the dry powder was taken for further studies (**Plate 1 & 2**).

LEAVES OF *MURRAYA KOEINGI* (PLATE 1)

LEAVES OF *MELIA AZADIRACHTA* (PLATE 2)

Crude Extraction: Leaf powder of *Melia azadirachta & murraya koeingi* was extracted with Chloroform following the method of Bakus [13] with certain modifications. The sample was dried in air for 2 days and after complete drying, 10 g of sample was put into 200 ml of chloroform, covered and kept standing for 5 hours. The solvent was then removed after squeezing the sample and filtered through Whatman filter paper No 1. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 4^{o}C and stored in refrigerator for further use as crude chloroform extracts. The filtrate was diluted and subjected to screening for phytochemical constituents using standard procedures.

Standards and chemicals: HPLC-gradient 0.5% phosphoric acid in 40% aqueous chloroform, other

solvents such as chloroform, acetone, petroleum ether, ethanol and Authentic standard Quercetin (RM 6191) were purchased from Himedia Laboratories (Mumbai, India).

Quantification using HPLC: Based on the literature the compounds possessing hypoglycemic activity reported from the leaf extracts of *Melia azadirachta* and *Murraya koeingi* were quantified using HPLC.

Chromatographic equipment and condition: The chromatographic analyses were performed on 8 x 100 mm Bond pack C18 (a normal phase column) Shimadzu, Japan with 0.5% aqueous solution of Orthophosphoric acid and 40% chloroform (HPLC Grade) as mobile phase at a flow rate of 1.2 mL min-1. The column effluent was monitored at 280 nm with aL-2400 series multi-wavelength UV Detector.

RESULT AND DISCUSSION: The chloroform extract of leaves of *Melia azadirachta* and *Murraya koiengi* was carried out and yield percentage was calculated (**Table 1**). The major phytochemical constituents of Melia & Murraya were analyzed using standard procedures (**Table 2**).

TABLE 1: YIELD OF CRUDE EXTRACT

Name of the solvent	Yield (in grams for 500 g of sample)	
	Sample I (*M. Azadirechta*)	Sample II (*Murray koeingi*)
Chloroform	4.8g (0.96%)	5.98g (1.19%)

TABLE 2: PHYTOCHEMICAL CONSTITUENTS

Tests	Aqueous		Chloroform		Acetone		Petroleum ether		Ethanol	
	S-I	S-II	S-I	S-II	S-I	S-II	S-II	S-II	S-I	S-II
Reducing sugar	+	+	+	-	-	+	-	+	-	+
Anthraquinone	+	+	+	+	+	+	+	+	-	+
Flavonoids	+	+	-	+	-	+	+	+	+	-
Saponins	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	-	+	+	+	-	+
Alkaloids	+	-	+	+	+	-	+	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+	+

Sample: S-I-*Melia Azadirachta,S-II-Murraya Koiengi*

Quercetin is the most abundant natural flavonoid, present in medicinal plants. Quercetin is of interest because of its pharmacological function. The quantification of quercetin from the dried leaves of *Melia azadirachta and Murraya koiengi* was carried out based on chromatographic separation. Normal-phase HPLC has been used in a number of occasions for the analysis of flavonoids in plants; it was used to distinguish species based on the quantitative variation of flavonoids among them. It has been applied especially for the identification of flavonoid derivatives. In the present investigation, flavonoids were quantified at 280nm using peak area by comparison to a calibration curve derived from the quercetin. The retention time recorded for quercetin in Melia and Murraya leaf extracts was between 81-82mints (**Fig. 2 & 3**). From the calibration curve results, the amount of Quercetin, in the sample injected was calculated. Melia leaves contained 36% and Murraya leaves showed the presence of 21%quercetin when compared to standard (**Fig. 1, 2 & 3**). In the present investigation, quercetin was identified based on the absorbance at 280nm for both samples and standard. Quantification was done based on peak height and peak area obtained. The present method is applicable for quantifying quercetin in any plant material using Normal phase HPLC technique.

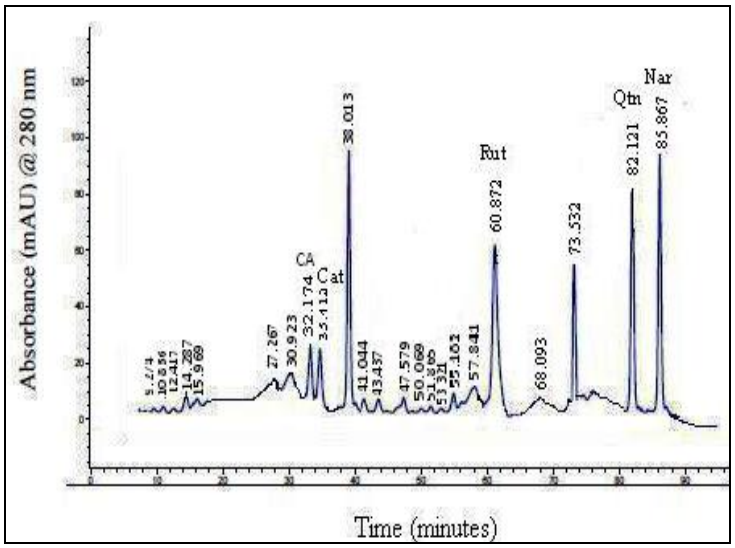

FIG.1: HPLC ANALYSIS OF STANDARD QUERCETIN

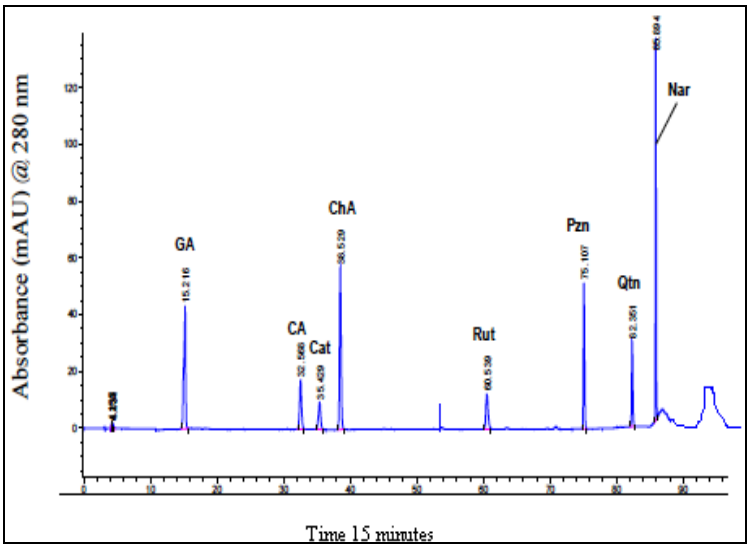

FIG.2: HPLC ANALYSIS OF MELIA AZADIRACHTA LEAVES

Standard	Retention time (min)	Height (mAV) @280 absorbance	% of yield
Quercetin	82	80	97.5

Flavonoid	Retention time (min)	Height (mAV) @280 absorbance	% of yield
Quercetin	82.56	30	36.37

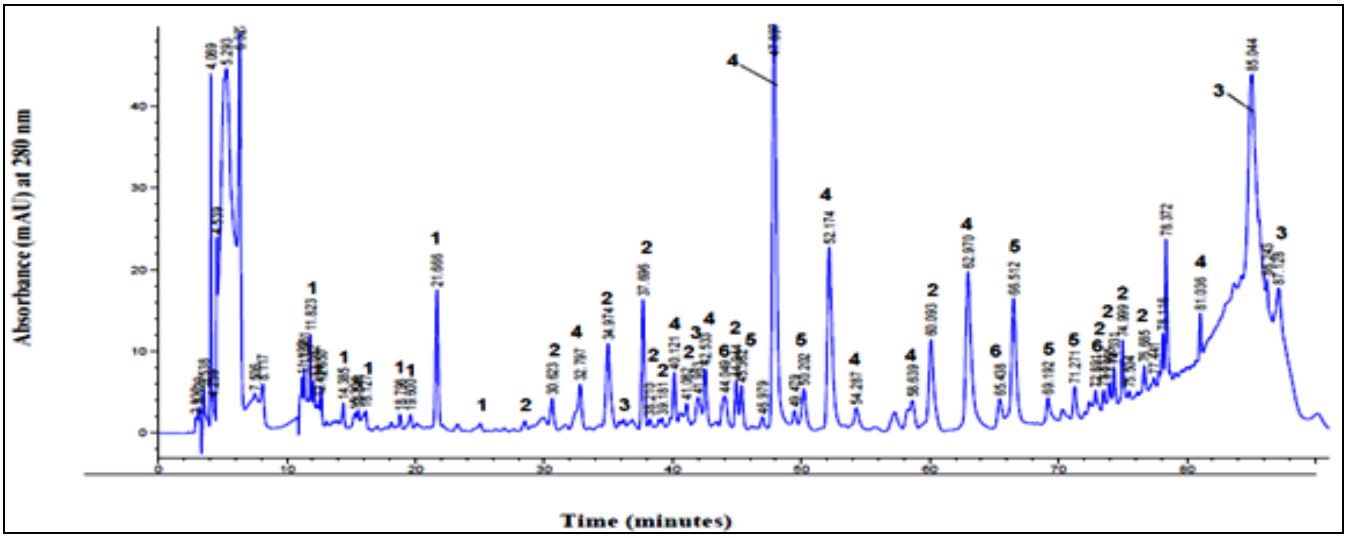

FIG. 3: HPLC-DAD ANALYSIS OF *MURRAYA KOEINGI* LEAVES

Flavonoid	Retention time (min)	Height (mAV) @280 absorbance	% of yield
Quercetin	81.03	18	21.95

Fig. 2 Peak identities:

GA, gallic acid; Cat, catechin; CA, caffeic acid; ChA, chlorogenic acid; Rut, rutin; Pzn, phloridzin; Qtn, quercetin; Nar, naringenin.

Fig. 3 Peak assignment:

1) hydroxybenzoic acids; 2) hydroxycinnamic acids; 3) flavanols; 4) flavonols; 5) flavones.

Spectral profiles of phenolic standards;

A. Gallic acid, a hydroxybenzoic acid;

B. Caffeic acid, a hydroxycinnamic acid; C. catechin, a flavanol, D. quercetin, a flavonol;

E. Phloridzin, a dihydrochalcone; F. apigenin, a flavone; G. naringenin, a flavanone;

H. Cyanidin 3-glucoside, an anthocyanin.

CONCLUSION: The application of a simple, rapid and accurate HPLC method for the quantification of quercetin in Melia & Murraya leaf powder was standardised. The method was validated to track the active principles in the complex mixture of herbal ingredients. The method could be extended for the marker-based standardization of other herbal products. The method was found to be simple, precise, accurate, specific, sensitive and can be used for routine quality control of herbal raw materials and also for the quantification of these compounds in plant materials.

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