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DIRECT HPLC ANALYSIS OF QUERCETIN IN EXUDATES OF *ABUTILON INDICUM* (LINN). MALVACEAE

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Abstract

Quercetin, one of the most abundant natural flavonoids, presents in daily food. Quercetin is of interest because of its pharmacological function. The isolation and purification of Quercetin in the dried leaves and flowers of *Abutilon indicum* (Malvaceae) is developed. A simplified method for the detection of flavonoids was also developed using Reversed -Phase HPLC. Concentration of quercetin in *Abutilon indicum* leaves and Flowers was calculated based on calibration curve.

Keywords : *Abutilon indicum*, HPLC, Flavonoids, Quercetin

Introduction

Flavonoids (flavus- yellow) or bioflavonoids, are a ubiquitous group of poly phenolic substances which are present in most plants, concentrated in the seeds, fruit skin, peel, bark, and flowers[1]. *Abutilon indicum* commonly known as Mudrica, have analgesic. [2, 3], hypoglycemic activity [4]. and literature site the presence of the sesquiterpine lactone, Gallic acid, and eugenol[5]. Extract of the whole plant is said to possess decreasing peroxidative damage in liver through free radical scavenging activity due to its flavonoids[6]. Petroleum ether extract of this plant is also a potent source of natural mosquito larvicidal agent[7]. Seven flavonoid compounds including quercetin and its glycosides have been isolated from flowers of *A.indicum* [8]. No data has been reported for the separation of the flavonoid quercetin from the ethanol exudates of *Abutilon indicum* using the HPLC method.

The objective of this work was to analyze the flavonoid content of leaves and flowers of *A.indicum* using HPLC.

Materials and Methods

Plant material

The EtOH extracts of leaves, stem and flowers of *A. indicum* were obtained by maceration method. The above parts of the plant were collected from vivekanandha college campus, Tiruchengode and identified from the Department of Botany, Vivekanandha college of Arts and Sciences for women, Tiruchengode and placed in the Herbarium for future reference (Voucher No. RUBL- 19910).Quercetin (RM 6191) and Ketaconazole (RM 4322) were purchased from Himedia Laboratories (Mumbai, India).

Standards and chemicals

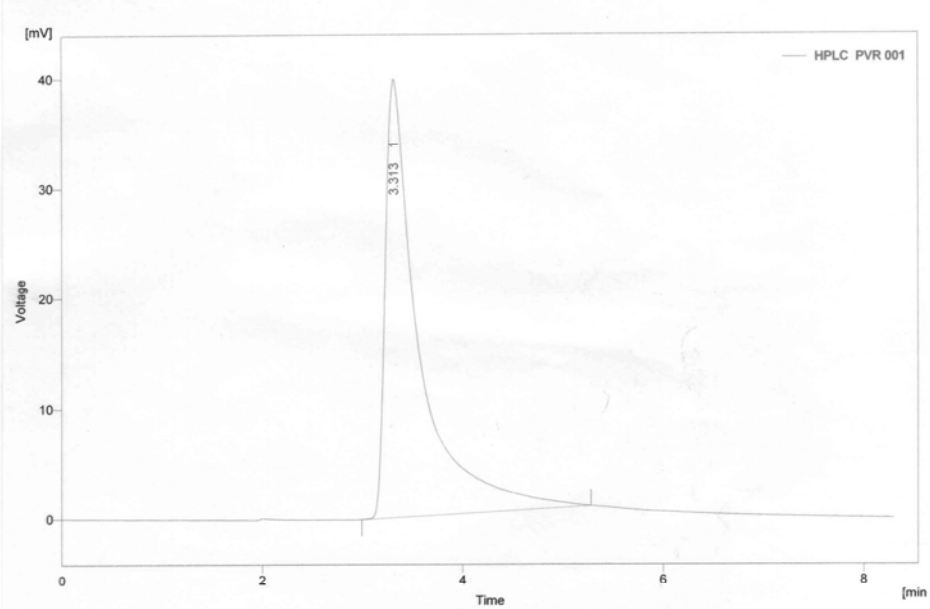
HPLC - gradient grade methanol, other chemicals (acetone, ethanol, benzene, petroleum ether) and Authentic standards Quercetin (RM 6191) were purchased from Himedia Laboratories (Mumbai, India).

Sample preparation and HPLC Analysis

The EtOH extract of plant exudates prepared and were evaporated to dryness. The samples were then heated under reflux for 1 hour with 6 ml of 25% hydrochloric acid and 20 ml MeOH, the hydrolysate was diluted with methanol to 100ml and filtered. (PTFE Syringe filter, Whatman, UK). 1 ml of this solution was injected for HPLC analysis. Analysis was performed after three separate extraction of each sample and each extract was diluted and injected in triplicate. Quercetin in the samples was identified by comparison of their retention times (t_R) with the standard Quercetin.

Chromatographic equipment and condition

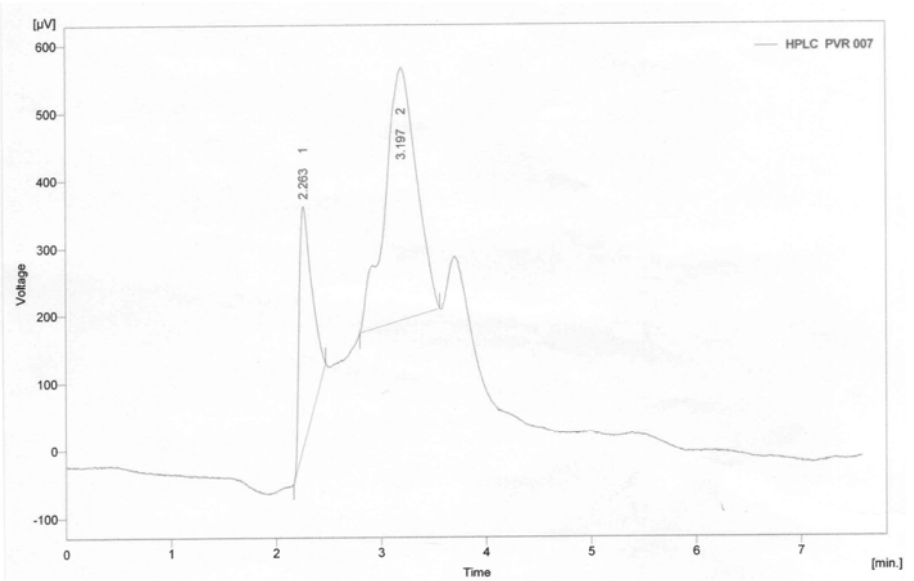
The chromatographic analyses were performed on a 250 mm × 4.6 mm i.d., C18 (ODS), Shimadzu, Japan with 0.5% aqeuous



Result Table (Uncal - HPLC PVR 001)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.313	937.910	39.802	100.0	100.0	0.28
	Total	937.910	39.802	100.0	100.0	

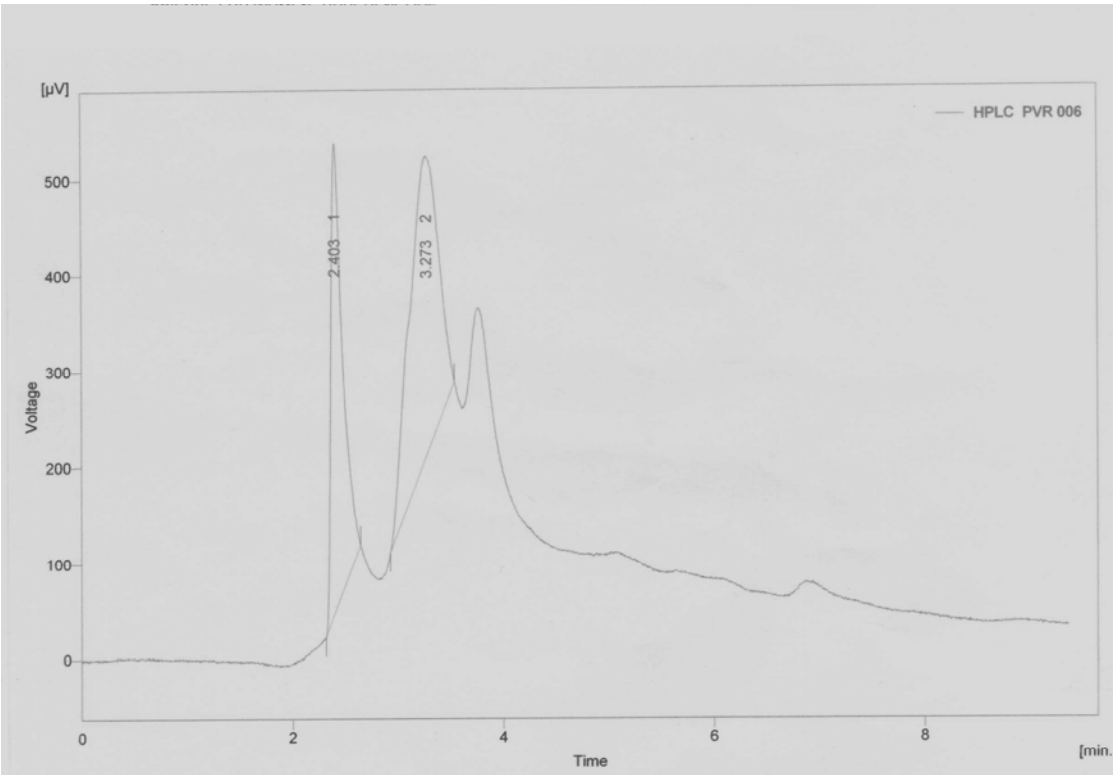
Fig.1: HPLC Analysis of Quercetin



Result Table (Uncal - HPLC PVR 007)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.263	2.969	0.355	27.4	48.7	0.18
2	3.197	7.854	0.374	72.6	51.3	0.32
	Total	10.823	0.729	100.0	100.0	

Fig.2: HPLC Analysis of *A.indicum* Leaf Extracts



Result Table (Uncal - HPLC PVR 006)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.403	3.796	0.489	38.2	61.1	0.13
2	3.273	6.138	0.311	61.8	38.9	0.32
	Total	9.934	0.800	100.0	100.0	

Fig.3: HPLC Analysis of *A.indicum* flower Extracts

solution of Orthophosphoric acid and Methanol (HPLC Grade) as mobile phase at a flow rate of 1 mL min^{-1}. The HPLC equipment comprised Hewlett-Packard (HP) 1050 ChemStation Software, an HP model 35900 interface unit, an HP 9000 Series 300 computer, and an HP DeskJet 500 Printer. A Waters 486 tuna-ble absorbance detector was operated at 254 nm; detector sensitivity was 0.05 AUFS and the column oven temperature was 30°C. Determinations were performed after three separate extractions of each sample, and each extract was injected in triplicate (n = 3).

Result and Discussion:

Reversed-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[9]. It has been applied especially for the identification of flavonoid derivatives[10]. In the present investigation, flavonoids were quantified at 254nm using peak area by comparison to a calibration curve derived from the quercetin. Comparing the HPLC chromatograms from leaves and flowers of *A. indicum*, the main difference was in peak eluted at 3.27min. External flavonoid aglycones were already analysed using HPLC method in various plant extracts[11]. The peaks in this study shown marked increase in peak area in case of *A. indicum* leaves and compared with standard quercetin (Fig-1).

From the calibration curve results, the amount of Quercetin, in the sample injected

was calculated. *A. indicum* leaves contain quercetin 72% (Fig-2) than A. indicum flowers(Fig-3). Other peaks (#1) in both the HPLC chromatogram A. indicum leaves and flowers extracts indicated the presence of other chemical constituents (euginol, etc.,). The present method was applicable for determining quercetin in any aerial part of plant material using HPLC technique.

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