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Estimation of Rutin and Quercetin in *Amaranthus viridis Linn* by HPLC

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Abstract : *Amaranthus viridis*, is traditionally used for treatment of constipation, inflammation, eczema, bronchitis, anemia, leprosy. Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. Flavonoids like Rutin and quercetin possess many biochemical effects like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, and anticancer, antihepatotoxic, protection of cardio vascular system. An HPLC method was developed for the estimation of rutin and quercetin from methanol herbal extract of *Amaranthus viridis*.

Key words : *Amaranthus viridis*, Rutin, Quercetin, Antioxidant activity, HPLC.

Introduction

Amaranthus viridis Linn, belongs to family amaranthus. *Amaranthus viridis*, is traditionally used for treatment of constipation, inflammation, eczema, bronchitis, anaemia, leprosy (Sivarajan and Balachandran. 1994; Kirtikar and Basu, 1987; Anonymous, 1996). Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. To date about 300 varieties of flavonoids are known (Anonymous, 1996). Many have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity (Kuhnau, 1976). Rutin, 5,7,3', 4',tetrahydroxy flavonol -3-rhamnoglucoside and quercetin 5,7,3',4',- tetrahydroxy flavonol are exhibits anti-inflammatory, antihepatotoxic (Cesarone,1992), antiulcer (Clack *et al*., 1950), antiallergic, antiviral actions and some of them provides protection against cardiovascular mortality (Colergie Smith *et al*., 1980; Hertog, *et al*., 1993). Both rutin and quercetin possess antioxidant activity and reduce low density lipoproteins (LDL) oxidation (De-Whalley *et al*., 1990), quercetin in combination with other flavonoids, are inhibiting a number of enzymes like bradykinin (Bamard *et al*., 1993), tyrosine kinase (Hur *et al*., 1994), and 5'-nucleotidase activity (Beladi *et al*., 1989). Rutin and quercetin have shown regulatory activity of hormones like affect the transport, metabolism and action of thyroid hormones. High performance layer chromatography (HPLC) method is the suitable method for estimation of chemical constituents present in plant materials. Hence *Amaranthus viridis* contains rutin and quercetin are important active constituents and is estimated by HPLC method.

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Materials and Method

Instrumentation

The Shimadzu class LC-10AT HPLC, Hichrom C18 and a Rheodyne 7725i injector fitted with a 20 µl loop, column oven, and a photodiode array detector. The output signal was monitored and processed using chromquest version3.0 software on Pentium computer (Hewlett Packard).

Solvent and Chemicals

Rutin and quercetin obtained from natural remedies (Bangalore) chromatographic grade methanol, formic acid and acetonitrile (AR), were obtained from Merck (Mumbai, India).

Extraction of Plant material

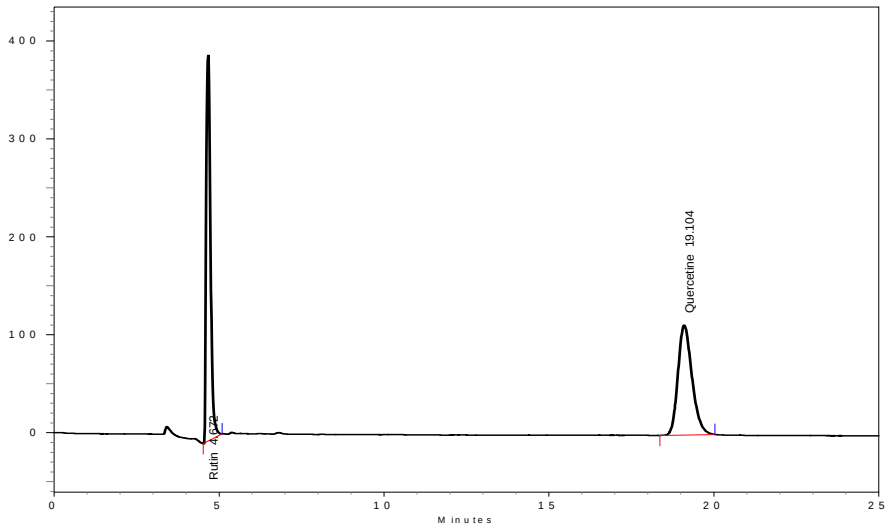
Plant material was collected from Chickballapur surroundings and was authenticated by Dr. B.K.Vekatesh, Department of Botany, Government First Grade College, Chickballapur. Specimen was stored in college herbarium (SKVCP-20). *Amaranthus viridis* leaves were extracted with distilled Methanol by Soxhlet apparatus. The pooled Methanolic extract was evaporated under vacuum to dryness, yielding was noted. Methanolic extract was subjected for estimation of rutin and quercetin.

Preparation of Standard and Sample Solutions

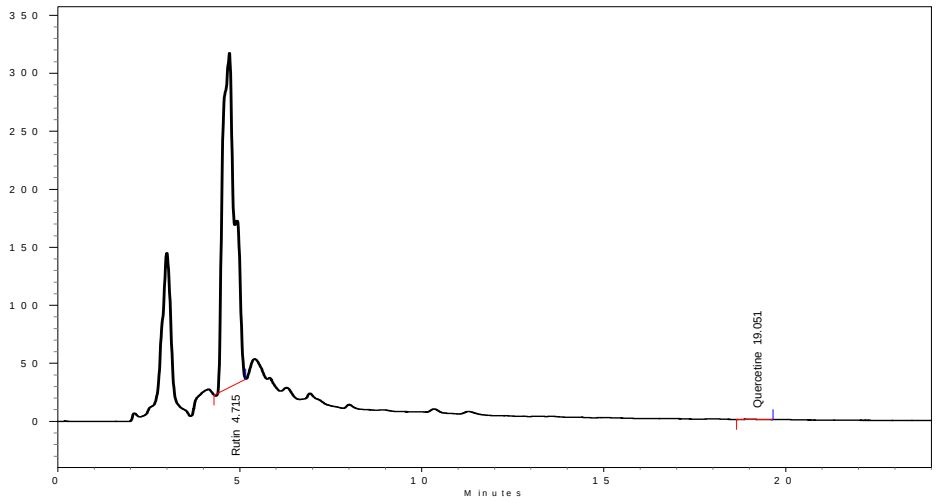
Rutin and quercetin 10 mg were accurately weighed into a 10 ml volumetric flask, dissolved in 5 mL methanol and the solution was made up to 10 ml with the same solvent (1 mg/ml). *A. viridis* fruit extract was accurately weighed (10 mg) into a 10 ml of volumetric flask and shaken on a mechanical shaker for 10 min filtered through Whatman filter paper No. 42 and the filtrate was used for analysis.

Chromatography

Flow rate	:	0.9 ml/min
Detection	:	340 nm
Injection quantity	:	50 µl
Column used	:	Hichrom C18 (150 mmx4.6 mm i.d., 5µ)
Column temperature	:	35°C



Graph 1:Standards (Rutin and Qurcetin)



Graph 2: Methanolic extract of *Amarnthus Viridis* Linn.

Mobile phase ration	:	70:30 % v/v
Mobile phase	:	0.5% Formic acid: Acetonitrile

Results and Discussion

The retention time of standards rutin and quercetin were found to be 4.072 and 19.104 (Graphs1). The retention time of rutin and quercetin in *Amaranthus viridis* were found to be 4.715 and 19.051 (Graphs2), which are matching with standard R_t values respectively. Then the amount of rutin and quercetin in *Amaranthus viridis* was found to be 58.52 and 9.12 % w/v respectively graphs

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