

NOTE**Estimation of Rutin and Quercetin in
Amaranthus spinosus by HPLC**

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Flavonoids present in the *Amaranthus spinosus* are rutin and quercetin. Rutin and quercetin possess many biochemical properties like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, anticancer, antihepatotoxic, protection of cardio vascular system. An HPLC method was developed for estimation of rutin and quercetin from herbal extract of *Amaranthus spinosus*.

Key Words: Flavonoids, *Amaranthus spinosus*, Quercetin, Rutin.

Amaranthus spinosus Linn, also called as spiny amaranth and belongs to family amaranthaceae. *A. spinosus* has been used in traditional medicines for the treatment of constipation, inflammation, eczema, bronchitis, anaemia, leprosy¹⁻⁵. It contains many phytochemicals such as flavonoids, anthraquinones, cardiac glycosides, saponins and tannins. Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. To date about 300 varieties of flavonoids are known⁶. Many have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity⁷. Rutin, *i.e.*, 5,7,3',4'-tetrahydroxy flavonol-3- rhamnoglucoside and quercetin *i.e.*, 5,7,3',4'-tetrahydroxy flavonol exhibit antiinflammatory, antihepatotoxic⁸, anti-ulcer⁹, antiallergic, antiviral actions and some of them provides protection against cardiovascular mortality^{10,11}. Both possess antioxidant activity and reduce low density lipoproteins oxidation¹², quercetin in combination with other flavonoids, are inhibiting a number of enzymes like bradykinin¹³, tyrosine kinase¹⁴ and 5'-nucleotidase activity¹⁵. Rutin and quercetin have shown regulatory activity of hormones like affect the transport, metabolism and action of thyroid hormones.

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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 a Chromquest Version 3.0 software on Pentium computer (Hewlett Packard).

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

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.

Amaranthus spinosus leaf 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, this solution was used for analysis.

Chromatography¹⁶: Flow rate: 0.9 mL/min; Detection: 340 nm; Injection quantity: 50 µL; Column used: Hichrom C₁₈ (150 mm × 4.6 mm i.d. 5 µ); Column temperature: 35 °C; Mobile phase ration: 70:30 % v/v; Mobile phase: 0.5 % Formic acid:Acetonitrile.

The retention time of standards rutin and quercetin were found to be 4.072 and 19.104. The retention time of rutin and quercetin in *Amaranthus spinosus* was found to be 4.629 and 19.168, which are matching with standard R_t values, respectively. Then the amount of rutin and quercetin in *Amaranthus spinosus* was found to be 57.47 and 9.22 % w/v, respectively.

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