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Quantitative Analysis of Rutin and Quercetin in *Capparis spinosa and Brassica oleracea* by HPLC

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Abstract

This paper describes a validated High performance liquid chromatographic (HPLC) detection method for quantitative analysis of flavonoid, rutin and quercetin in aqueous and ethanolic extracts of *Capparis spinosa* Linn. and *Brassica oleracea* Var. Italica. Separation was achieved by means of water's HPLC system equipped with universal injector, injection volume 20 µl, and PDA (UV-Vis) detector. The stationary phase is Octadecylsilane (c18) column with small particle (3 – 50 mm) packing and small bore (2 – 5 mm), which is attached to a source of pressurized mobile phase, Methanol: Acetonitrile: Water (60:20:20). The reference standers and test samples were injected at a flow rate 0.5 ml/min and scanned at wavelength 257 nm at ambient temperature. The run time was set at 35 minutes. The data obtained from our study conclude that the ethanolic extracts of *Capparis spinosa* consist of more amounts of rutin and quercetin compared to aqueous extract. Ethanolic extracts of *Brassica oleracea* also shows more amount of quercetin compared to aqueous extract, however rutin is not seen in *Brassica oleracea* extracts. The significant amounts of these antioxidants confirm the nutritional and medicinal value of *Capparis spinosa* Linn. and *Brassica oleracea* Var. Italica.

Key-Words: *Capparis spinosa*, *Brassica oleracea*, Quercetin, Rutin

Introduction

Herbs are rich source of flavonoids. Flavonoids are polyphenolic compounds that are abundance in nature and are categorized according to their chemical structure, into flavones, flavonols, flavanones, isoflavones, catechins and anthocyanidins. Rutin and quercetin are the plant derived flavonoid, specifically flavones which are found in high amount in various nutritional vegetables and fruits including caper and broccoli. They are the most active antioxidants because of their high ability to scavenge free radicals [(1)].

Capparis spinosa Linn, the caper bush belonging to family Capparaceae is a perennial winter-deciduous plant that bears rounded, fleshy, alternative leaves and thick, shiny, large white to pinkish-white complete flowers. *Capparis spinosa* is present in almost all the circum-mediterranean countries including India and China. The buds of plant, cooked and pickled, are used as a flavoring in cooking and the stem is used to treat arthritis because of its analgesic, anti-inflammatory & coagulant effect. Caper root, bark and leaves may have some antirheumatic and anticarcinogenic activity [(2,3)].

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Brassica oleracea belonging to family brassiceae is rich in essential nutrients including flavonoid quercetin and thus recognize one of the health-promoting vegetable [(4)]. The previous phytochemical investigation shows presence of alkaloids, steroids, carbohydrates, flavonoids, saponins, tannins, triterpenoids and glycosides in *Capparis spinosa* linn. and *Brassica oleracea* var. italica [(5,6)]. In our previous finding we also reported the hyperglycemic activity of *Capparis spinosa* linn. and *Brassica oleracea* var. italic in diabetic rats, which is considered to be because of their flavonoid content [(7)]. Thus in the present study the aqueous and ethanolic extracts of *Capparis spinosa* linn. and *Brassica oleracea* var. italica were investigated for their rutin and quercetin content which are supposed to be accountable for their hyperglycemic action.

Material and Methods

Solvents and chemicals: Rutin and quercetin dihydrate were purchased from Sigma-Aldrich, USA. HPLC grade methanol, Acetonitrile and water were obtained from Merck (Mumbai, India).

Collection of plant and preparation of extract: The fresh plant specimen of *Capparis spinosa* Linn. and *Brassica oleracea* Var. Italica were obtained and authenticated by Dr. P. Jayaraman, Plant Anatomy

Research Center, Chennai, India in the month of Jan. 2014. The shade-dried specimens were powdered and 100 gm. of the coarsely powdered drugs were treated separately with 250 ml. distilled water and 250 ml. 90% ethanol by the method of continuous hot extraction at around 60ºC. The extracts were cooled at room temperature, filtered and concentrated under reduced pressure in a rotary evaporator, then dried. The residual extracts were used for estimation of rutin and quercetin [8,9].

Preparation of Standard and Sample Solutions: 10 mg rutin and quercetin 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 (1000 µg/ml). *Capparis spinosa* and *Brassica oleracea* extract were accurately weighed (10 mg) into a 10 ml of volumetric flask and Sonicate in ultra sonicator for 10 min, filter through 0.45 µm memb. filter and the filtrate were used for analysis [10,11].

Chromatographic Conditions: Chromatographic analysis was carried out by water's HPLC system equipped with universal injector and PDA (UV-Vis) detector. The stationary phase is Octadecylsilane (c18) column with small particle (3 – 50 mm) packing and small bore (2 – 5 mm). The mobile phase was methanol:acetonitrile:water (60:20:20 v/v/v). The mobile phase was degassed ultrasonically and filtered through a 0.45µm membrane filter. Flow rate and injection volume were 0.5 ml/min and 20µl respectively. The chromatogram of rutin and quercetin were recorded at 257 nm for 35 minutes [12,13]. All chromatographic operations were carried out at ambient temperature. The Chromatographic peaks of samples were confirmed by comparing their retention time with those of the reference standards. Percentage of rutin and quercetin content were calculated from the peak area of chromatogram using the following formula [14,15].

Concentration of Sample = Area of sample / Area of standardx Concentration of Standard x Dilution Factor

Results and Discussion

In our present study, HPLC profile detected at 257 nm showed that rutin and quercetin representing the major flavonoids in *Capparis spinosa* while quercetin is the only flavonoid present in Brassica oleracea extract, however there were significant differences among different extracts regarding their rutin and quercetin content (Fig. 1-5).

Quantity of rutin and quercetin was found to be more in ethanolic extracts compared to aqueous extracts of the plants. Moreover Capparis spinosa extracts contains higher concentration of quercetin compared to rutin. Brassica oleracea extracts lacks rutin whereas quercetin is the major flavonoid present in it, however amount of quercetin is higher in capparis spinosa compared to Brassica oleracea (Table 1).

Flavonoids are the major bioactive compounds useful against free radical derived oxidative stress and presence of ample amount of quercetin and rutin in *Capparis spinosa* linn. and *Brassica oleracea* var. italica confirmed their effectiveness against various oxidative stress mediated disorders including diabetes mellitus.

Table 1. Quantative estimation of rutin and quercetin in different extracts

S/No	Chromatogram	Peak No	Retention time in min.	Area of peak	Conc. in µg/mg of dry Wt.	Conc. in %
1	Reference standards	1	9.37	91358	1000	100
		2	20.33	453269	1000	100
2	Aq. Extract of *Capparis spinosa* linn.	1	9.21	15639	171.1	17.11
		2	20.40	190359	419.96	41.99
3	Etho. Extract of *Capparis spinosa* linn.	1	9.13	30489	333.73	33.37
		2	20.50	213641	471.33	47.13
4	Aq. Extract of *Brassica oleracea* ver. Italica	1	19.81	153260	338.12	33.81
5	Etho. Extract of *Brassica oleracea* ver. Italica	1	20.19	170219	375.53	37.55

Conclusion

Hence from the HPLC analysis it was conclude that *Capparis spinosa* extracts contains significant amount of rutin and quercetin, while *Brassica oleracea* extracts shows the presence of quercetin only. Thus these plants can be used as the major source of flavonoids against various ailment caused by oxidative stress. This is an accurate, validated and reproducible HPLC method which can be used in quality control of herbal preparation containing rutin and quercetin.

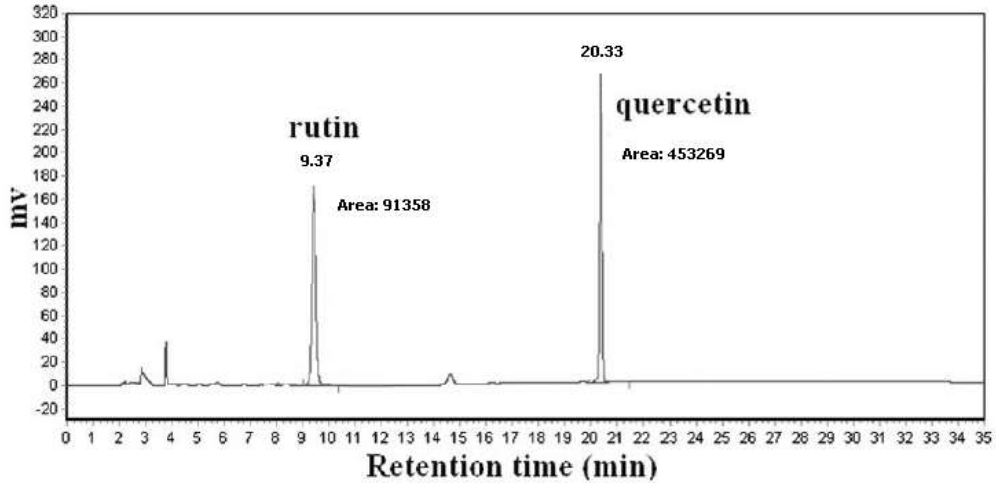

Fig. 1: HPLC chromatogram of reference standards (1) Quercetin (2) Rutin

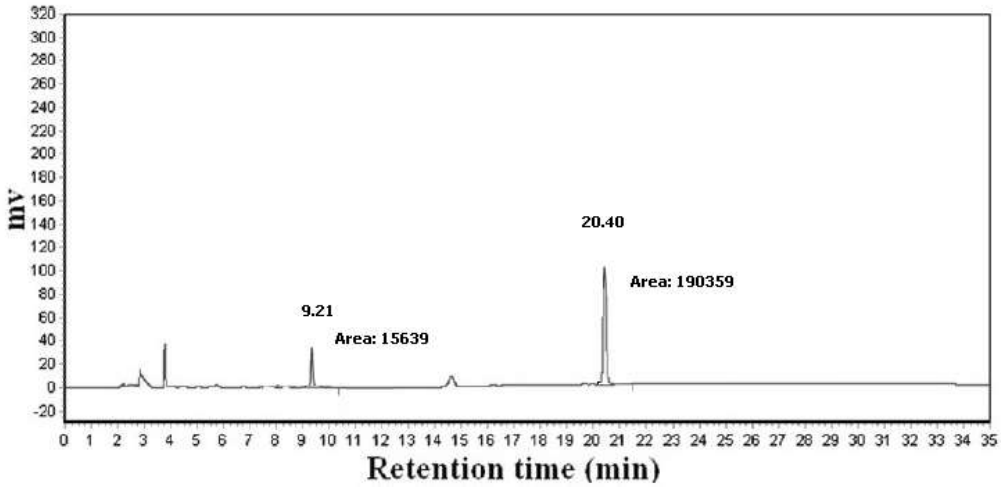

Fig. 2: HPLC chromatogram of aq. Extract of *Capparis spinosa* Linn.

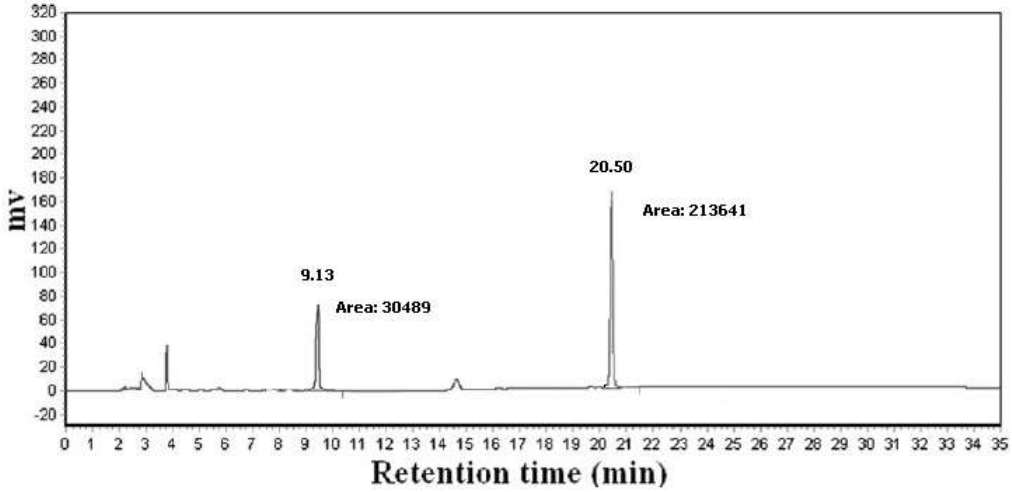

Fig. 3: HPLC chromatogram of etho. Extract of *Capparis spinosa* Linn.

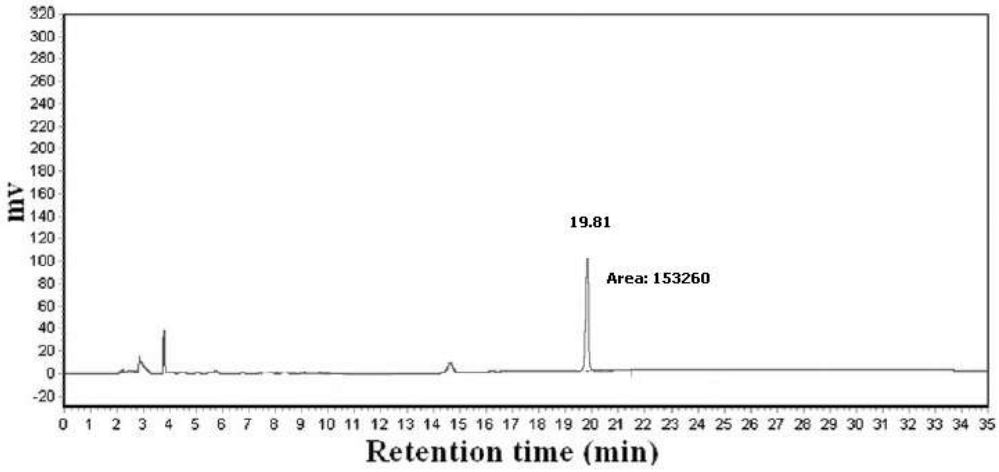

Fig. 4: HPLC chromatogram of aq. Extract of *Brassica oleracea* ver. Italica

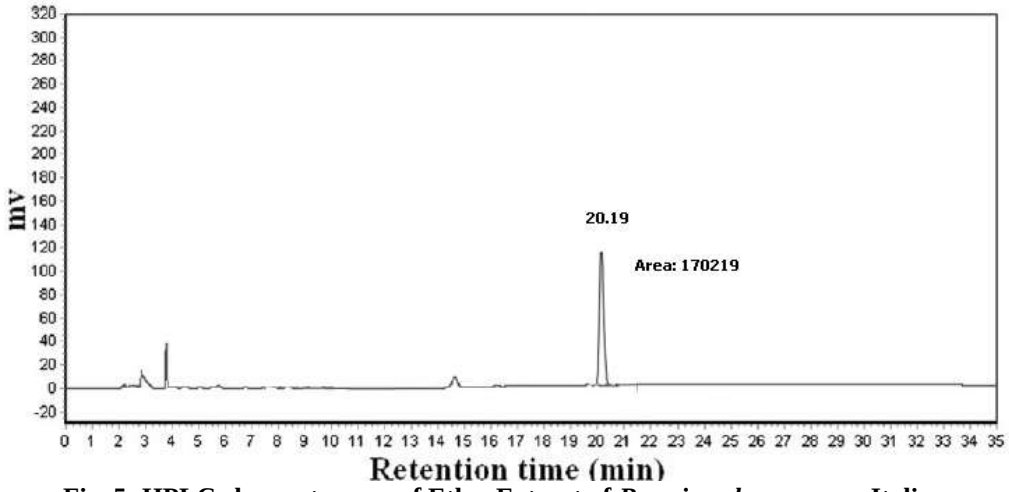

Fig. 5: HPLC chromatogram of Etho. Extract of *Brassica oleracea* ver. Italica

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