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Simultaneous Determination of Rutin and Quercetin in Different parts of *Capparis spinosa*

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ABSTRACT
Capparis spinosa is a multipurpose plant which contains a number of chemically active and diverse secondary metabolites, in particular, flavonoids. Rutin and quercetin are two major flavonoids in caper plant. In this study the HPLC method was developed for simultaneous determination of rutin and quercetin content in different parts of C.spinosa at the floral budding stage .Collection of plant materials were made from Tafresh, Iran. Plant were separated into root, stem, leaves, floral bud which were dried separately, and subsequently assayed for total rutin and quercetin content. The highest amount of rutin (25.82 mg/g) and quercetin (10.4 mg/g) was measured in the leaf of caper plant. The significant amounts of these antioxidants confirm the nutritional and medicinal value of caper.
Key words: *Caper, Rutin, Quercetin, HPLC*

INTRODUCTION

C. spinosa L. (*Capparidaceae*) is a common aromatic plants growing wild in the dry regions around the Mediterranean basin. From ancient times, the floral buttons of Caper has considerable nutritional value and the floral buds are extensively used in diet as vegetable [21]. The ripened fruit are rich from protein, lipid, carbohydrates,and vitamins and minerals. Capers are one of the plant sources high in flavonoid compounds rutin (or rutoside) and quercetin. Also it contains phytosterols, tocopherols, carotenoids, flavonoids and glucosinolates in different parts of this plant [6][16] [25][26]. The two flavonoid in the extracts of leaves and buds of caper, rutin and quercetin, exhibit notable pharmacological activities. Rutin and quercetin structural was shown in figure 1&2.

Flavonoids are polyphenol compounds, widely found in the plant kingdom. Flavonoids, as a major active constituent, display a remarkable role in various pharmacological activities including antiallergic, anti-inflammatory and antioxidant effects [2][6][27][22]. As intrinsic components of fruits, vegetables and beverages such as wine and tea many of the 4000 different flavonoids known to-date are part of a regular diet[5]. In recent years, scientists have accomplished extensive research on the flavonoid biological activities such as antibacterial, antifungal, antiviral, anticancer and anti-inflammatory effects [12][15].

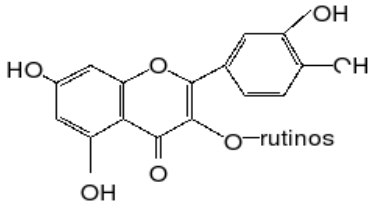


Figure1; Rutin structure

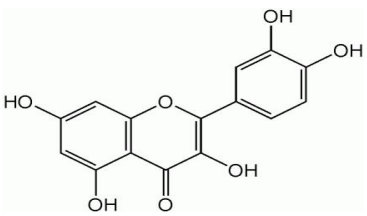


Figure2; Quercetin structure

Until to date, flavonoids have been analyzed by gas chromatography (GC) [4], high-performance liquid chromatography (HPLC) [13][28], and capillary electrophoresis (CE) [28][19], capillary electro chromatography [19],high-speed counter-current chromatography [29],capillary isotachophoresis and

capillary zone electrophoresis [14].HPLC greatly improved the determination value of the active ingredients in plants.

MATERIALS AND METHODS

Reagents and materials

Reagents and chemicals

Methanol and acetic acid were of HPLC grade and were purchased from Merck Company. Deionized water was prepared by a Milli-Q Water Purification system. Rutin and quercetin standards were obtained from Sigma Company.

Plant materials

Caper plants were collected from Tafresh, Iran, in June at the floral budding stage of plant development. Collections were done in these populations by a randomized collection of 10 individual within floral budding stage. After collection, plants were separated into root, stem, leaf and floral bud. The plant materials were dried in shade separately.

Preparation of sample solution

An amount of 0.1-0.5 g of ground plant material was extracted with 10 ml of solution (methanol-acetic acid- water (100:2:100) for 1 hour on a shaker at laboratory temperature. 2 ml of the extract were centrifuged for 10 min at 2000 rot/min.Then solution was filtered through a micro filter with a regenerated cellulose membranes of the pore size 0.22 .The filtrate was applied for HPLC.

Preparation of standard solutions

Standard stock solutions of rutin and quercetin were prepared in ethanol, at concentration of 1,5,10 and 15 ppm. All sample solutions were filtered through 0.22 µm membrane filter and injected directly. Rutin (RU) and quercetin (QU) were quantified by HPLC separation at 355.5 and 368nm and the retention time for RU and QU was 6.7 and 9.8 min respectively.

The standard response curve for each standard was a linear regression, fitted to triplicate values obtained at each of four concentrations. The linearity relationship between peak areas and concentrations was good and the correlation coefficient (r2) was0.9996 for RU and 0.9994 for QU.

HPLC condition

Chromatographic analysis was carried out by using C18 column (4.6mm × 250mm) as the stationary phase and methanol: acetonitrile: water (10:10:75) containing 5% acetic acid as the mobile phase. Flow rate and injection volume were 1.0 ml/min and 10 µm respectively.The chromatographic peaks of the analytics were confirmed by comparing their retention time and UV spectra with those of the reference standards. All chromatographic operations were carried out at ambient temperature.

RESULTS AND DISCUSSION

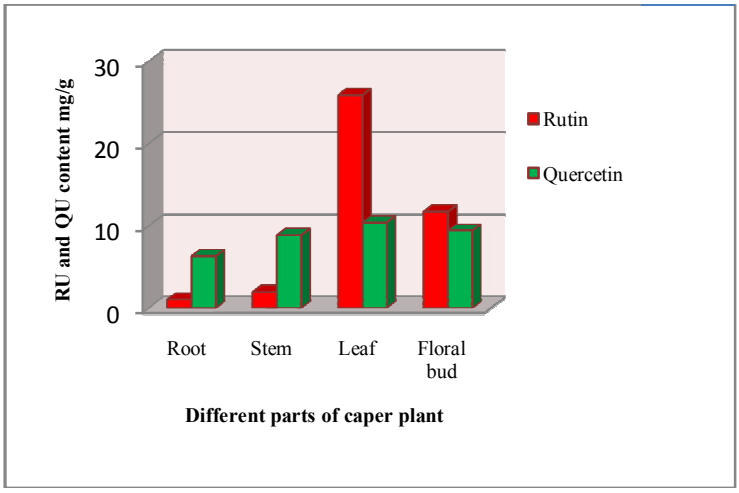
The results of this study showed significant differences among different parts of plant in regard to their RU and QU content. The mean for RU and QU content in different plant parts is given in (table 1).In our study, the highest amount of RU and QU was observed in the leaves of caper. RU content values ranged from 1.02 mg/g for root to 25.82 mg/g in the leaves. Also this variation for QU ranged from 6.3 mg/g for root to10.4 mg/g in leaves (Fig3).

Table 1.RU and QU content in different parts of caper

Plant organ	Rutin content mg/g	Quercetin content mg/g
Root	1.02	6.3
Stem	1.95	8.82
Leaf	25.82	10.4
Floral bud	11.7	9.4

Similar to primary metabolites tissue-dependence of secondary metabolites is very common among medicinal plants [1]. Leaves and flowers generally contain greater levels of phenolic acids and

terpenoids than stems and roots [8].In the present study, leaves had the highest level of flavonoid (rutin and quercetin). Also similar findings about rutin were reported in previous study [15][20][23].



Figur3: Rutin and Quercetin in different parts of plant

The plant kingdom includes about 35 species with the described rutin content. However, there are only a few nutritionally important species [9]. Fagopyrum esculentum and *Rutagraveolens*(53.5 mg/g) belong to the richest sources of rutin[24]. We observed the presence of rutin in all tested plant sample: root, stem, leaf, floral bud. Rutin was distributed in caper plant parts similarly as in common buckwheat [24] and amaranth which highest amount of rutin was found in the leaves[11].In our study caper leaves contained up to 2.5% rutin per dry weight. It is less than in common buckwheat leaves where it was established from 4% to 9% of rutin per dry weight in dependence on the stage of development[10].The amount of rutin in *C.spinosa* is similar to leaves of thyme (24.9 mg/g), herbsof *thymussibthorii* (22.6 mg/g) and more than other plant like flower of dog rose(14.0 mg/g), herbs of dandelion (14.0mg/g)[24]. So caper use, especially as a culinary agent,could be a great source of rutin in human nutrition.

According our study leaves and floral buds of caper plant are a rich source of quercetin in comparison other plants. In amaranth,highest amount of quercetin was reported in the leaves [9]. Also quercetin content was reported 1.46-4.96 mg/g in *Hypericum maculatum*[3]. Quercetin was found in all the berries such as bog whortleberry (158 mg/kg fresh weight), lingon berry (74 and 146 mg/kg), cranberry (83 and 121 mg/kg), chokeberry (89 mg/kg), sweet rowan (85 mg/kg), rowanberry (63 mg/kg), sea buckthorn berry (62 mg/kg) and crowberry (53 and 56 mg/kg)[7]. The highest total flavonoids content was found in onion leaves, which contained 1497.5 mg/kg quercetin [17].

CONCLUSION

The results of the present study suggest caper plant as a natural source of rutin and quercetin. Rutin and quercetin content brings attention to caper plant as a new potential for commercial production. High content of rutin and quercetin content in the leaves of caper plant in compared to other parts of plant encourage introduction and application this part Plant for industrial and culinary purpose.

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