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Diurnal change in rutin content in *Capparis spinosa* growing wild in Tafresh/Iran

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ABSTRACT

Capparis spinosa has received considerable scientific interest in recent years, as it is as a source of a variety of biologically active compounds including flavonoids in special rutin. The present study was conducted to determine diurnal change in rutin content in different parts of C.spinosa at fresh fruiting stage. The plants were collected from Tafresh/Iran in August, after dissected into root, stem, leaf, floral bud, flower and fresh fruit they were dried at room temperature and subsequently assayed for rutin content by HPLC. Among different plant parts, floral buds and leaves were found to be the principle organ for rutin. Diurnal changes of rutin were also observed and slightly higher concentration of rutin in whole plant was observed in the morning.

Keywords: *Capparis spinosa*, rutin, diurnal change, HPLC

INTRODUCTION

Increased attention has been paid to genus *Capparis spinosa* family Capparidaceae due to its significant medicinal uses via anti-inflammatory[Al-said et al,1988], anti-fungal[Ali-shtayeh et al,1999]anti-diabetic[Ziyyat et al,1997] ,anti-spasmodic, analegesic, expectorant [Rajesh et al,2009]. *C. spinisa* is an important species for our natural surrounding and economy[Baytop,1984].the antioxidative effect of plant extracts is mainly due to phenolic components such as flavonoid[Rice-Evans et al 1997].The most abundant flavonoid in *C.spinosa* is rutin[Inocencio et al,2000]. Rutin is an important therapeutic substance that favorably influences the increase of blood vessel elasticity and the treatment of circulatory disorders and atherosclerosis. It reduces blood pressure, stimulates vitamin C utilization and has antioxidant activity. [Abeywardena and Head, 2001] (Schilcher et al, 1990][Wojcicki et al,1995][Holasová et al, 2001]. Rutin, a flavonoid glycoside (3-O-beta-rhamnoglucoside---a form of quercetin) has been found to occur abundantly in plants, but only a small number of plant materials contain quantities sufficient for industrial extraction (*Sophora japonica, Eucalyptus macrorrhyncha,* buckwheat*)* [Bruneton ,1999].

Nowadays, the development of drugs from natural sources is recommended to overcome the side effect of many of the synthetic drugs. Recent research on medicinal plants has generated a great deal of information about biologically active chemical components that are responsible for the claimed medicinal effects [Ataa said et al.2011].

Previous chemical studies on *C.spinosa* have reported the richness with flavonoid in special rutin [Musallam et al, 2012][Ramezani et al, 1998]. Hence, focusing our attention on natural sources of rutin, this study was carried out to evaluation of rutin content in different parts of caper .Also diurnal change in rutin content was determined.

MATERIALS AND METHODS

2.1 Plant materials

The caper plants were collected from Tafresh/Iran in August at fresh fruiting stage. Collection was done four times a day 06.00, 11.00, 16.00, 21.00). Samples were separated into; stem, leaf, floral bud, flower and fruit then were dried at ambient temperature in the shade. Dried organs were grounded into powder for 30 seconds.

2.2 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.

2.3 preparation of sample

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 was centrifuged for 10 min at 2000 rot/min.Then solution was filtered through a micro filter with a regenerated cellulose membrane of the pore size 0.22. The filtrate was applied for HPLC.

2.4 Preparation of standard solutions

Standard stock solutions of rutin 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) was quantified by HPLC separation at 355.5 and the retention time for RU was 6.7 min.

The standard response curve for rutin was a linear regression and the linearity relationship between peak areas and concentrations was good and the correlation coefficient (r2) was 0.9996 for rutin.

2.4 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

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RESULTS AND DISCUSSION

Rutin content was affected by the different parts of plant. Floral buds contain the highest amount of rutin (1.97%) when compared to other organs and followed by leaves (1.91%) and flowers (1.84%) (Figure 1).

Diurnal fluctuation in rutin content did not follow the same trend in different organs and it varied with plant tissues (Figure2). Flower and leaf samples collected in the morning and at night gave the highest values. For floral bud the highest amount of rutin was measured in the morning(2.66%) while rutin content of stem and fresh fruit was higher at 11.00 (0.87%) and 16.00(0.4%) respectively. The mean for diurnal change in rutin content in different plant parts is given in table1.

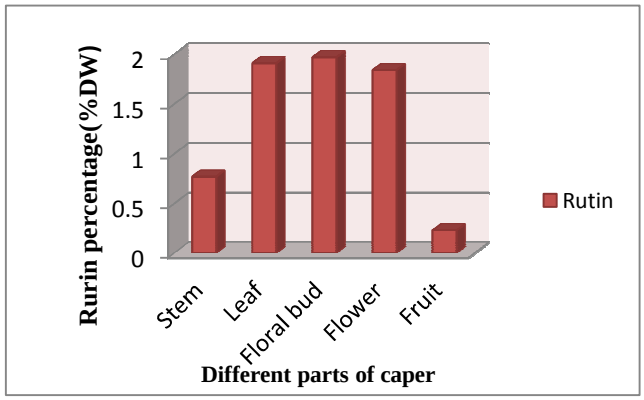

Fig1: Rutin in different parts of caper

Table1: Diurnal change in rutin content in different parts of plant(%DW)

	Diurnal collection times				
Plan organs	06.00	11.00	16.00	21.00	Mean
Stem	0.72	0.87	0.81	0.66	0.77
Leaf	2.00	1.81	1.69	2.12	1.91
Floral bud	2.66	1.20	1.32	2.70	1.97
Flower	2.20	1.83	1.66	1.67	1.84
Fresh fruit	0.33	0.19	0.40	0.03	0.23
Mean	1.6	1.18	1.18	1.43	

As to variations during the day, the rutin content in whole plant varied but, the changes are relatively small (Figure2, 3).

Our results in this study about rutin content in different parts of caper are in agreement with previous study. Some researcher reported that rutin content varied in different parts of caper [Musallam et al2011][Ramezani et al, 1998]. Also it was proved that rutin differs in different parts of buckwheat plant [Kreft et al, 2006].

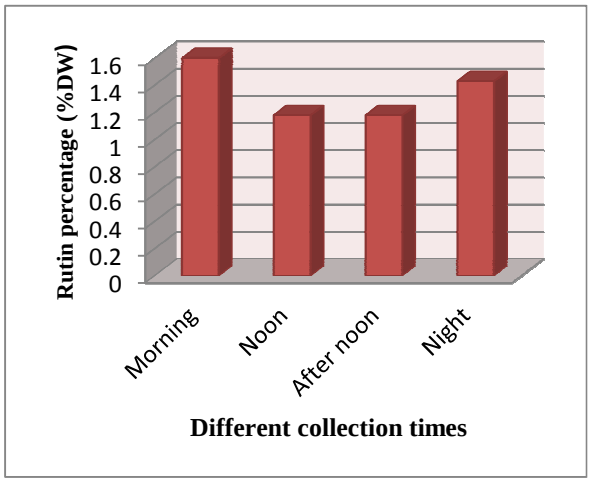

Fig 2: Fluctuation of rutin content in whole plant

Of course tissue-dependence of secondary metabolites is very common among medicinal plants. Our finding in the present study about highest amount of rutin in flower bud is in contrast with the result of [Musallam et al,2011][Ramezani et al,1998].They determined highest amount of rutin in the leaves of caper while in our results there was only small difference between rutin content in leaves and flower budding.

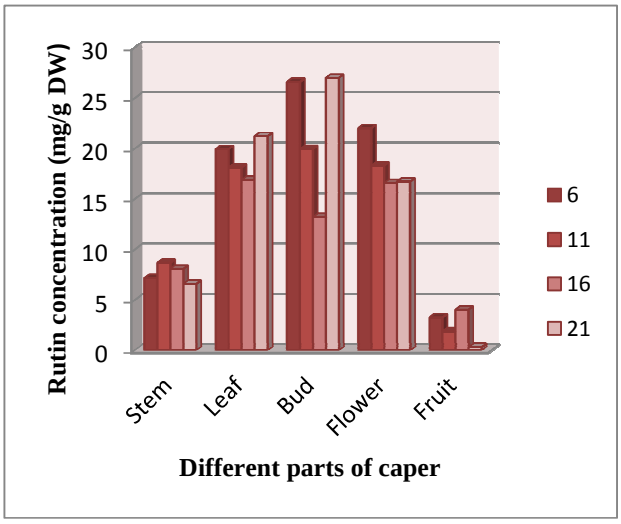

Fig3: Diurnal change of rutin in different parts of caper

The diurnal change of determined rutin in *Capparis spinosa* seems to be brought about by fluctuations in environmental factors, such as day and night temperatures and intensity of sunshine. In *Bellis perennis* ultraviolet light influences dry weight of flowers, leaves, and roots, leaf area, photosynthetic parameters, and transpiration rate depending on wavelength and intensity of radiation[Cooley et al,2000a][Cooley et al,2000b], and temperature affects plant total dry mass, leaf area, and root respiration [Gunn et al,1999] so secondary metabolism in *Bellis perennis* may be influenced as well.

The influence of environmental conditions on quantity of active constituents in various plants has been reported. Phenolic acid, flavonol, and anthocyanin contents and antioxidant activity in fruit juice of *Fragaria* x *ananassa* were considerably influenced by different day/night growing temperature combinations[Wang and Zheng ,2001].A positive correlation between the duration of exposure to sunshine and flavonoid content in leaves of *Angelica keiskei* has been demonstrated (Lee et al,2003).

CONCLUSION

Thus, diurnal change in rutin content reported in the present study can be explained by qualitative and quantitative changes of environmental circumstances during the day. It is difficult to determine which environmental factor is mainly responsible for observed variations because the changes are small and irregular. Moreover, the plants were collected from plants growing in natural conditions, so it is not easy to separate effects of individual factors from multifactorial influence of the environment. This would be possible in greenhouse or laboratory experiments where the factors can be set and regulated exactly.

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