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Investigation of Rutin Content in Different Plant Parts of Wild Caper (*Capparis spinosa* L.) Populations from Jordan

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ABSTRACT

Capparis spinosa L. is a common perennial shrub in the Mediterranean region. The plant is known for its medicinal and aromatic properties. It contains a number of chemically active and diverse secondary metabolites, in particular, rutin. In this study, the content of rutin was quantitatively determined in different plants parts of *C. spinosa* using High Performance Liquid Chromatography (HPLC) analysis. Collection of plant material was made from twenty four populations of *C. spinosa*. The studied *C. spinosa* populations evidenced a great variability of rutin content among plant parts and among locations. Rutin content averaged on dry basis was 2.76% for leaves, 1.8% for flowering buds and 0.28% for fruits. Among the populations investigated in this study rutin content was found to vary significantly among and within regions. The data show both the value of plant genetic resources for breeding programs and the importance of exploring chemical variation before introducing wild plant in cultivation and breeding programs. The study also underlines the nutritional value of the caper plant which is widely used as a source of condiment.

Key words: *Capparis spinosa*, flavonoids, rutin, Jordan

INTRODUCTION

Caper bush is grown commercially to obtain unopened flower buds (capers) that are picked and used as a condiment in many dishes. The plant is also used in the manufacture of cosmetics and medicines. The genus *Capparis* belongs to the Capparaceae family which comprises of 250 species (Fici and Gianguzzi, 1997). Most of capers are presumably of the species *C. spinosa* and they considered as such in international food legislation (Inocencio *et al*., 2002). Around the world numerous *Capparis* species are collected from their natural habitats to be sold in the herb's seller shops. Some of these species like *C. zeylanica* (Sini *et al*., 2011; Mishra *et al*., 2011), *C. decidua* (Ali *et al*., 2011; Keymanesh *et al*., 2009), *C. pepiaria* (Malabadi and Vijay Kumar, 2007) and *C. erythrocarpus* (Woode *et al*., 2009; Danquah *et al*., 2011) were investigated and scientifically validated for their medicinal uses. The first use of the caper bush for medicinal purposes was recorded in 2000 B.C. by the Sumerians (Alaoui, 2003). The ancient Greeks and Romans also used the plant for this purposes. As early as the first century A.D., the Greek physician Dioscorides explained the medicinal properties and use of capers in his treatise "The Medicinal Use of Capers"

(Hadid *et al*., 2004). Recently, several reports have shown the pharmacological properties of caper and its great potential as a medicinal plant (Germano *et al*., 2002; Kim *et al*., 2005; Musallam *et al*., 2011; Paulickova, 2010; Shahidi and Naczk, 2004). The crude extract of the flower buds contains 162 volatile constituents; the major components of them were identified to be isothiocyanates, thiocyanates, sulphides and their oxidative products (Batanouny and Shams, 2006). The caper plant metabolizes a large number of secondary metabolites mainly flavonoids, alkaloids, glycosides, organic acids and glucosinolates (Batanouny and Shams, 2006). The seeds are rich in protein, lipids and fiber with a high content of palmitic, oleic, linolenic, stearic, lauric, myristic and linoleic acids (Batanouny and Shams, 2006; Matthaus and Ozcan, 2005; Tlili *et al*., 2009b). The oil content of the seeds ranged from 27.3 to 37.6 g/100 g and the dominating fatty acid was linoleic acid which accounted for 26.9-55.3% (Matthaus and Ozcan, 2005). The flowering buds of the plant also contain polyunsaturated fatty acids and among them linoleic acid and γ-linolenic acid were identified as the major components (Giuffrida *et al*., 2002). Khanfar *et al*. (2003) investigated the chemical constituents of *C. spinosa* plant in Jordan and found that it has afforded 18 compounds two of which are isolated for the first time from a natural source (β-sitosterylglucoside-6-octadecanoate and 3-methyl-2-butenylglucoside).

C. spinosa has been subjected to many phytomedicinal studies. Its extract can be used as anti-oxidative (Germano *et al*., 2002) antifungal, anti-diabetic, antileishmania, anti-inflammatory (Rajesh *et al*., 2009), antimicrobial (Mahasneh, 2002), antihepatotoxic (Gadgoli and Mishra, 1999) antipyretic (Ageel *et al*., 1986; Rimbau *et al*., 1999), antiallergic (Trombetta *et al*., 2005) and anticancer (Esiyok *et al*., 2004). It is also used for the treatments of cardiovascular diseases and diabetes mellitus (Eddouks *et al*., 2004; Eddouks *et al*., 2005). Capers have been used or still being used in reducing flatulence, in the treatment of rheumatism, anemia, arthritis and gout (Alkire, 1998; Peter, 2004).

C. spinosa contains phytosterols, tocopherols, carotenoids, flavonoids and glucosinolates in different parts of this plant (Germano *et al*., 2002; Matthaus and Ozcan, 2005; Tlili *et al*., 2009a, b). Flavonoids, as a major active constituent, display a remarkable role in various pharmacological activities including antiallergic, anti-inflammatory and antioxidant effects (Ageel *et al*., 1986; Germano *et al*., 2002; Trombetta *et al*., 2005; Panico *et al*., 2005). Furthermore, flavonoids have been suggested to affect the function of immune system (Middleton, 1998; Middleton and Kandaswami, 1992). The most abundant flavonoid in *C. spinosa* is rutin. The presence of the flavonoid rutin makes caper a valuable medicinal herb, as it is believed to improve capillary functions and permeability "Vitamin P" and as a general free-radical antioxidant (Inocencio *et al*., 2000).

Rutin has been found to occur abundantly in plants but only a small number of plant materials contain quantities sufficient for industrial extraction such as *Sophora japonica*, *Eucalyptus macrorhyncha* and buckwheat (Paulickova, 2010). Many beneficial health effects of rutin have been demonstrated. Such effects have been attributed to anti-inflammatory, analgesic (Pietta and Gardana, 2003), anti-mutagenic (Brindzova *et al*., 2009), anti-tumor (Molnar *et al*., 1981), anticarcinogenic (Webster *et al*., 1996), antifungal (Han, 2009) and partial protective effect against the development of diabetes (Srinivasan *et al*., 2005). Rutin can also be used as a natural coloring agent, an oxidation inhibitor, sunburn preventative in cosmetics (rutin absorbs ultra violet rays) and as an ingredient in functional food applications (WIPO, 2004). Some published data refer to specific aspects of the qualitative composition of flavonoids and content in capers flowering buds (Hamed *et al*., 2007; Inocencio *et al*., 2000; Giuffrida *et al*., 2002) but up to now there is little information available regarding the differences in rutin content in the

different plant parts or populations. The objectives of this study was to investigating the variation in rutin content among plant parts and among populations of *C. spinosa*.

MATERIALS AND METHODS

Plant materials: Two experiments were conducted. In the first experiment samples of flowering bud, leaves and fruits of wild plant were collected from different ecotypes in Jordan to study the variation in rutin content of *C. spinosa* plant parts. Sampling was undertaken across seven locations (Ajloun, Al-Balqa, Al-Karak, Al-Mafraq, Amman, Irbid and Jarash) in Jordan during the period from May to July 2007. The second experiment was done to investigate the chemical diversity of rutin content among the wild populations and a samples of 100 buds were collected in May 2007 from twenty four populations that cover different geographical regions (North, Center and South) of Jordan (Table 1). The study area included the different four bio-geographical regions (Mediterranean region, Irano-Turanian, Saharo-Arabian and Sudanian) present in Jordan. Elevation of locations varied from 299 to 1358 and the rainfall varied from 200 to 650 mm (Table 1).

Rutin extraction, analysis and quantification: Dry material (0.5 g) was extracted with 50 mL of HPLC grade methanol/water (70:30 v:v) for 48 h, filtered through Albet® no. 150 filter paper and

Table 1: Variation of rutin content among and within Jordanian Caper (*Capparis spinosa*) populations

		Coordinates				Rutin content (% w/w)	
Region	Population	Latitude N	Longitude E	Elevation (m)	Rainfall (mm)*	Population	Region
North	Um Qays	32°39'18.60"	35°40'34.74"	310	500	1.55fghi**	1.50c
	Kufr Yuba	32°33'10.44"	35°47'06.90"	555	550	0.94i	
	Ash-Shajara	32°038'0.30"	35°56'15.30"	299	350	1.38ghi	
	Rasun	32°24'53.76"	35°45'47.28"	895	600	1.58efghi	
	Um Al-Yanabi'	32°22'04.50"	35°46'12.06"	994	650	1.93defg	
	Anjara	32°17'14.58"	35°43'56.94"	935	550	1.55fghi	
	Al-Khanasry	32°24'27.12"	36°03'28.74"	674	250	2.10cdef	
	Juba	32°20'06.06"	35°54'47.46"	967	400	1.46ghi	
	Ar-Rumman	32°12'08.76"	35°52'00.78"	374	350	1.05hi	
Center	Zay	32°05'52.92"	35°42'58.14"	875	550	1.78defg	1.90b
	Al-Salt	32°03'10.44"	35°45'45.18"	903	550	2.20cde	
	Mahis	31°59'47.40"	35°45'49.32"	860	500	1.87defg	
	Marj Al-Hamam	31°54'57.18"	35°48'59.76"	780	350	2.02defg	
	Tariq	32°00'50.10"	35°57'05.70"	826	450	1.62efgh	
	Al-Rosayfah	32°00'54.66"	36°00'08.40"	655	250	2.17cdef	
	Bilal	31°56'40.32"	35°46'07.80"	750	400	1.90defg	
	Wadi Shu'ayb	31°58'15.18"	35°43'42.78"	348	400	1.98defg	
	Mukawir	31°35'10.86"	35°37'53.46"	495	250	1.53fghi	
South	Al-Lajjun	31°12'34.38"	35°49'10.10"	663	300	2.70bc	2.76a
	Al-Karak	31°10'55.44"	35°42'22.92"	955	350	2.91b	
	El Huseiniya	30°59'55.26"	35°42'58.86"	989	300	4.08a	
	At-Tafila	30°55'00.48"	35°41'56.10"	760	300	2.42bcd	
	Dana	30°40'27.78"	35°36'32.40"	1225	350	2.66bc	
	Wadi Musa	30°19'57.30"	35°29'53.16"	1358	200	1.79defg	
Mean						1.97	
LSD 0.05%						0.64	0.26

*From Jordan rainfall contour map (GIS database, National Center for Agricultural Research and Extension). **Means within each column followed by different letter are significantly different at p≤0.05 according to LSD

stored at refrigerator until analysis. The HPLC analyses were performed on a Lachrom® MERCK-HITACHI (Tokyo, Japan) using a reversed phase Supelcosil™ (USA) LC-18 (15 cm×4 mm, 5 μm) column. The mobile phase consisted of two eluents: (W) water and (A) acetonitrile, adjusted to pH 3.1 with acetic acid. To achieve separation, flow rate was set at 1.0 mL min^{-1} and adopting a gradient solvent system of water (A) and methanol (B), from 95 to 5% of A using a linear gradient over 80 min (Giuffrida *et al*., 2002) and detection at $\lambda = 340$ nm. Rutin was identified by its retention time and its UV spectrum, as compared to those of the authentic standard (Sigma-Aldrich, Germany). Its content was quantified on the basis of a calibration curve obtained from standard solutions of reference compounds.

Statistical analysis: Analysis of variance over locations was performed. STATISTICA 7.0 program (StatSoft, Inc.) was used to carry out statistical analysis. Treatment means were compared using Fisher's test (LSD). A significant level of 5% was used for all statistical analysis. Probability of significance was used to indicate significance among treatments and interactions according to Steel and Torrie (1980). The correlation coefficients of average rutin content versus rainfall and altitude were also calculated.

RESULTS AND DISCUSSION

Quantitation of rutin was performed at 340 nm as the absorbance obtained at this wavelength was the maximum. Assessment of peak purity, monitored between 250 to 380 nm, showed peak homogeneity thereby, excluding the possibility of the presence of interfering components and rendering the method specific.

Among the several solvent system tested in this study, the highest percent yield of rutin (1.98%) in the tested samples was obtained when a mixture of methanol/water (70:30) was used (Table 2). Hamed *et al*. (2007) also found that percent yield of rutin from *C. deserti* and *C. cartilaginea* extract vary widely depending on the solvent used. In the literature, several solvent systems were used for extraction of flavonoids, include absolute methanol, ethanol, acidified methanol, acetone, water, ethyl acetate, propanol, dimethyl sulfoxide (Inocencio *et al*., 2000) and their combinations (Shahidi and Naczk, 2004). No satisfactory solvent extraction systems are suitable for isolation of all classes of plant phenolics or even a specific class of phenolics. This is attribute to the variable chemical nature of these compounds. But, as found in this study, in cases of flavonoids like rutin, methanol can be chosen as the extraction solvent due to its high polarity index, facilitating the extraction of both the polar glycosides as well as the aglycone components (Sticher *et al*., 2000).

Rutin content in the different plant parts: Rutin was found in all plant parts. An example of an HPLC chromatogram of the separation of flavonoids of flowering buds of *C. spinosa* is illustrated

Table 2: Effect of different solvent systems on rutin yield from *Capparis spinosa* buds

Solvent system	Rutin content (% w/w)
Methanol	1.54
Ethanol	1.50
Acetone	1.51
Water	0.62
Ethyl acetate	0.83
Propanol	1.42
Methanol/water (70:30)	1.98

in Fig. 1. There were significant differences among different plant parts in regard to their rutin content. The mean for rutin content in different plant parts is given in Table 3. Determined on dry weight basis, it is apparent that leaves contain the highest rutin content. Rutin content values for leaves ranged from 1.57 to 3.57% with an average of 2.76%, for buds ranged from 0.79 to 4.41% with an average of 1.80% and for fruits from 0.17 to 0.43% with an average of 0.28%. This is in agreement with rutin content recorded in other plants. For example, the content of rutin differs in different parts of the buckwheat plant (*Fagopyrum esculentum* Moench) in ascending order from hulled grains, unhulled grains, germinated hulled grains, root, germs, stalk, flower, young plants and tops up to leaves (Kreft *et al*., 2006). Abreu *et al*. (2004) also found that the *Hypericum brasiliense* accumulated more rutin in the shoots than other plant parts.

While the highest rutin content in this study was found in the leaf of *C. spinosa*, different plant parts show variable flavonoid profile (Fig. 2). By comparing chromatogram profiles of leaves, buds

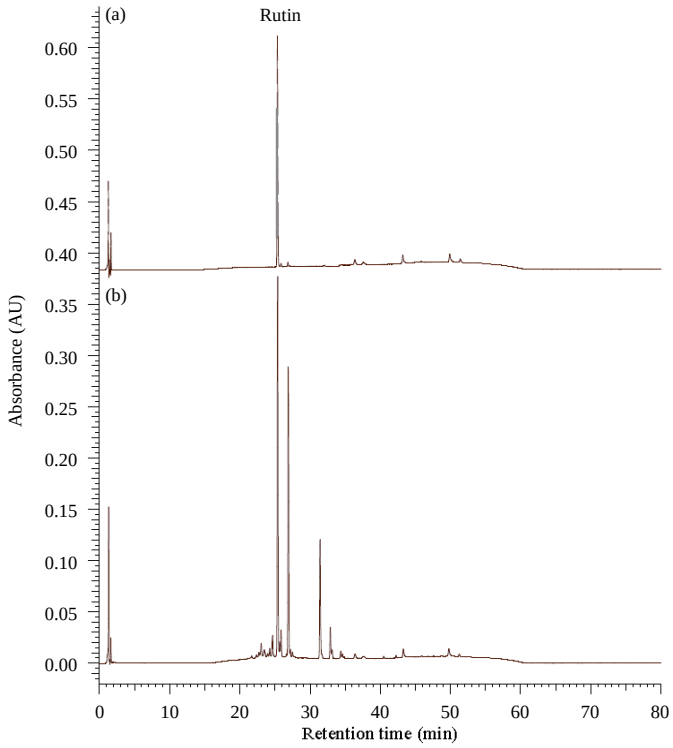

Fig. 1: HPLC chromatograms of (a) rutin standard and (b) extract of *Capparis spinosa* flowering buds (λmax = 340 nm)

Table 3: Rutin content in different plant parts (bud, leaf and fruit) of *Capparis spinosa*

Plant Part	Mean rutin content (% w/w)
Bud	1.80±1.05b
Leaf	2.76±0.66a
Fruit	0.28±0.07c
Mean	1.61

Values are Mean±Standard deviation. Means within each column followed by the same letter are not significantly different at p≤0.05 according to LSD

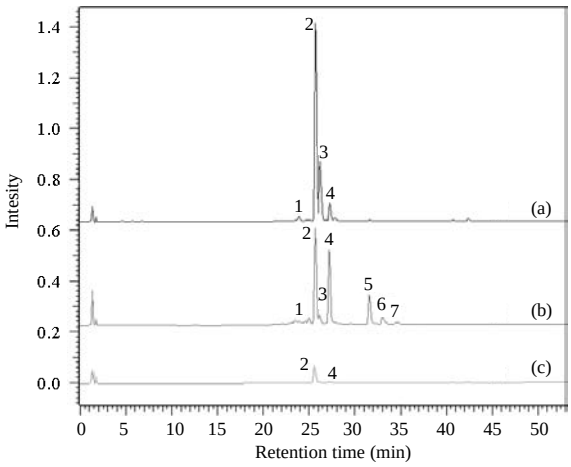

Fig. 2: HPLC chromatograms of *Capparis spinosa* extracts from (a) Leaves, (b) Buds and (c) Fruits

and fruits we can stipulate that the highest number of major flavonoids can be found in the buds of *C. spinosa*. However, the fruit seems to contain only rutin and in low amounts. A decrease in flavonoid glucoside content of *C. spinosa* buds with maturation has been reported in previous study by Giuffrida *et al.* (2002). Abreu *et al.* (2004) studied *Hypericum brasiliense* in different developmental stages (vegetative, flowering and fructification), they observed significantly higher content of rutin in flowering stage as compared with other growth stages of the plant. The higher flavonoid glucoside content in buds of *C. spinosa* as compared to fruits may be due to active phenolic biosynthesis in young growing flower buds, in which these compounds act as defense and protective agents. Some chemical or enzymatic degradation of the glycosides occur during fruit formations, therefore, flavonoid content was found to be low in fruits (Giuffrida *et al.*, 2002).

Chemical diversity of rutin content among wild populations: In the second experiment, the results clearly indicate that the methanolic extract of *C. spinosa* buds exhibited a good quantity of rutin Table 1. This is in agreement with previous studies in which *C. spinosa* flowering buds were found to contain a good quantity of flavonoids in particular rutin (Germano *et al.*, 2002; Inocencio *et al.*, 2000). Rutin content of *C. spinosa* found in this study is comparable to other plants considered as major commercial sources of rutin, including buckwheat, *Sophora japonica* and *Eucalyptus* spp. (Paulickova, 2010). Buckwheat leaf flour contains about 2.7% rutin (Kreft *et al.*, 2006). Rutin contain in dried flower buds of *Sophora japonica* and leaves of *Eucalyptus jumanii* reach about 6.4 and 5.5%, respectively (Yin *et al.*, 2008; Yarosh *et al.*, 2001).

Among the populations investigated in this study rutin content was found to vary significantly among and within regions (Table 1). Among regions in Jordan, the southern region populations gave the highest mean for rutin content (2.76%); it exceeded the grand mean of rutin with about 40%. However, northern and center regions did not differ widely in their rutin content mean (1.50 and 1.90%, respectively).

Among the populations, rutin content was ranged from 0.94 to 4.08%. This indicates that there is a wide variation in the relative amounts of rutin content in caper flower buds and that

environmental factors or genetic influences can have important effects on rutin content. The same significant variations in rutin content and other flavonoids were found when several accessions of *Hypericum perforatum* cultivated in three different localities were investigated (Buter *et al*., 1998). The authors suggested that climatic conditions or different physiological stages might account for the variation of the flavonoids. In addition, they also detected a strong genetic influence in the studied accessions. Hamoudova *et al*. (2006) attributed the variation of rutin content in their collected material of *Hypericum perforatum* to the place of collection.

All the analyzed samples showed a common flavonoid profile; nearly in all samples rutin was always the major flavonoid. However some differences in the relative amounts of the individual flavonoids were noticed. Figure 3 shows an example which demonstrates how the rutin content in the plant can be affected inversely with the content of other flavonoids in the plant. Abreu *et al*. (2004) studied the rutin and quercetin content in *Hypericum brasiliense* and observed a negative relationship ($r = -0.8$ and $r^2 = 0.64$) between rutin and quercetin concentrations. The same negative relationship between these flavonoids was also found in *H. perforatum* (Buter *et al*., 1998). Inocencio *et al*. (2000) reported similar finding for flavonoid (kaempferol 3-rhamnosyl-rutinoside, quercetin 3-rutinoside and kaempferol 3-rutinoside) profile of *C. spinosa*, *C. sicula* and *C. orientalis*. These variations might be explained by several factors, from endogenous regulation of physiological processes to environmental characteristics (Abreu *et al*., 2004).

In this study, result did not show a significant correlation between rutin content and altitude ($r = 0.39$ and $r^2 = 0.15$) or rutin content and rainfall ($r = -0.34$ and $r^2 = 0.12$). Although, the correlation between rutin content and rainfall was not significant but it was negative which means that rainfall may negatively affect rutin content in the plant.

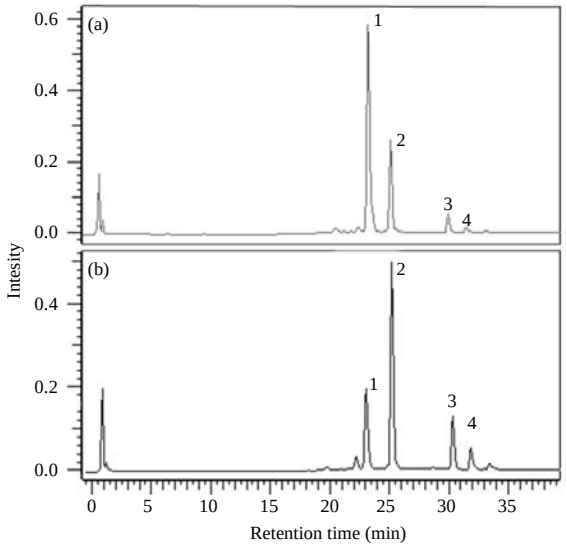

Fig. 3: HPLC chromatograms of *Capparis spinosa* from two locations. (a) Sample from Amman and (b) Sample from Jarash. Peak number (1) is for Rutin

CONCLUSIONS

The study showed that capers are rich source of the flavonols rutin and was 4.08% in the flowering buds of one population. The information obtained in this study might be useful to obtain higher amount of these substances. Moreover, high rutin content found in capers increases the potential of this species for commercial production. It also brings attention to the nutritional value of caper flowering buds which are widely used as a source of flavor. This study showed high content of rutin in the leaves compared to the flowering buds which encourage the consumption and introduction of the leaves as food in the countries where only the buds are consumed.

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