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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (5): 768-772, 2008
ISSN 1028-8880

Rutin from Different Parts of *Capparis spinosa* Growing Wild in Khuzestan/Iran

[1]Zahra Ramezani, [2]Nasrin Aghel and [1]Hossein Keyghobadi
[1]Department of Medicinal Chemistry,
[2]Department of Pharmacognosy, School of Pharmacy,
Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract: A qualitative and quantitative analysis of rutin from leaves, fruits and flowers of *Capparis spinosa* growing wild in Khuzestan was achieved. After soxhelet extraction of fats in diethyl ether, rutin was extracted by maceration using 50% EtOH. The ethanol extracts of these parts were separated by preparative TLC on silica gel precoated plate with a mixture of butanol: acetic acid (4:1, by volume) as the developing solvent. The spots were visualized under ultraviolet light (254 nm). Rutin was qualified by comparison of its R_f value with that of standard. UV/Vis spectrum of separated rutin was also compared with those of standards and showed characteristic wavelengths at 260 and 360 nm. Purified rutin was quantified by UV/Vis spectrophotometric determination at 360 nm. The calibration curve was linear in the range of 0.156-2.5 μg mL^{-1} with detection limit of 0.0731 μg mL^{-1}. The purity of extracted rutin from leave, flower and fruit determined by high performance liquid chromatography were 90.41, 87.25 and 64.56%, respectively. The amounts of rutin in leaves, fruits and flowers were 61.09, 6.03 and 43.72 mg per 100 g of dried powder, respectively. By analyzing the spiked samples of leave, flower and fruit the recovery of the UV/Vis method was in the range of 102-107.6%.

Key words: *Capparis spinosa*, rutin, UV/Vis spectrophotometry, HPLC

INTRODUCTION

In recent years, the increase in the residential and agricultural areas and the decrease in medical plants have triggered the interest in ethno-botanical studies throughout the world (Harsha *et al*., 2002). Capparidaceae are a medium-sized family of approximately 40-45 genera and 700-900 species, whose members present considerable diversity in habit, fruit and floral features (Cronquist, 1981; Heywood, 1993; Mabberley, 1997). *Capparis spinosa* L. (Capparidaceae), a winter-deciduous species, is one of the few perennial shrubs that grow and flower entirely during summer. In Dioscorides's herbal, *C. spinosa* (caper) is referred to as a species distinct enough not to be confused with anything else (Raven, 1990; Rhizopoulou, 1990). It is likely that *C. spinosa* is a stenohydric plant, largely free of competition for water with other species in the Mediterranean region (Rhizopoulou *et al*., 1997; Sozzi, 2001). Fermented capers fruits are often served as an appetizer with meat, olives, cheese and nuts, or as a complement to salads, pasta and other foods. Fermentation of caper fruits is often done by traditional artisanal ways (Luna and Perez, 1985).

Capparis spinosa is used in phytomedicine around the world as anti-oxidative (Germano *et al*., 2002), antifungal (Ali-Shtayeh and Abu Ghdeib, 1999), antihepatotoxic (Gadgoli and Mishra, 1999), anti-inflammatory (Al-Said *et al*., 1988; Ageel *et al*., 1986), anti-diabetic (Yaniv *et al*., 1987; Ziyyat *et al*., 1997) and antileishmania (Jacobson and Schlein, 1999) drug.

The flavonoid rutin is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. Rutin is found in many plants.

Rutin is a solid substance, pale yellow in appearance and only slightly soluble in water. It is, however, much more soluble in water than its aglycone quercetin. Rutin's molecular formula is $C_{27}H_{30}O_{16}$, its molecular weight is 610.53 daltons and its structural formula is presented in Fig. 1.

Rutin may have antioxidant, anti-inflammatory, anticarcinogenic, antithrombotic, cytoprotective and vasoprotective activities (Cruz *et al*., 1998; Deschner *et al*., 1993; Kostyuk and Potapovich, 1998; Ihme *et al*., 1996). Rutin may be useful in the management of venous edema (Kostyuk *et al*., 1996). It may help strengthen capillaries, protect against some toxins and have anti-inflammatory effects, as well as some anticancer

Corresponding Author: Zahra Ramezani, Department of Medicinal Chemistry, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
Tel: +98-611-3342197 Fax: +98-611-3340988

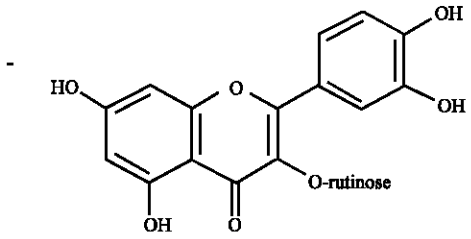

Fig. 1: Structure of rutin

effects. It may also help prevent the oxidation of vitamin C and have some positive lipid effects (Schmitt *et al*., 1995; Webster *et al*., 1996).

Different extraction and determination methods have been used for the quantitation of rutin in a variety of plants and pharmaceutical preparations (Kim *et al*., 2005; Eremina *et al*., 2004; Dubber and Kanfer, 2004; Jensen *et al*., 2002). In most of them HPLC is used. In this study it was decided to use the UV/Vis spectrophotometric which is less expensive and more users friendly. So a simple method for the extraction, isolation and quantitation of rutin from different parts of *Capparis spinosa* living wild in Khuzestan, South West of Iran, which its rutin contents is not yet determined, are introduced. The error introduced by this method is also evaluated by HPLC.

MATERIALS AND METHODS

Chemicals and reagents: Ethanol, butanol, acetic acid, diethyl ether, acetonitril, dichloromethane and rutin were purchased from Merck, Germany. Silica gel plates GF257 and 60F254 were from Merck. All solvents used in HPLC analysis were HPLC grade. Doubly distilled water was used through out this study.

Stock solutions of 1000 μg mL^{-1}; 10 mg rutin was transferred into a 10 milliliters volumetric flask completely dissolved in 50% EtOH, then diluted to the mark with 50% EtOH.

Working solution of 5, 3.75, 2.5, 1.9, 1.25, 0.625, 0.312 and 0.156 μg mL^{-1} were prepared from stock solution with step by step dilution using 50% EtOH as diluents.

Plant material: *Capparis spinosa* L. was collected during June to August 2006 from Hamiedieh (Khuzestan province) South West of Iran. The plant was authenticated by Khuzestan Agriculture and Natural Resources Research Center, Ahvaz, Iran and the voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences. Ahvaz, Iran.

Apparatus: The UV/Vis measurements were made on Shimadzue UV/Vis 1650, Japan. Solvents were evaporated in Heidolph Laborota 4011, Germany. CE1000, Cecil (UK) HPLC equipped with C18 eurospher-100, Herbert Knaver (125×4 mm, 5 μm) using gradient elution were used for HPLC analysis of the extracts and standard.

Extraction, identification and isolation of rutin
Extraction: The plant's different parts were dried at ambient temperature in the shade. Fifty gram of leaves, fruits and flowers were defatted separately using diethyl ether and soxhlet apparatus for 4 h. Then the residue was extracted with 50% (v/v) EtOH in 80°C for 3 h with stirring. The marc was re-extracted with 50% EtOH/H_2O three more times. The filtrates were combined and were filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure (yield 18.22, 18.42 and 25.40% for leaves, fruits and flowers, respectively).

TLC of extracts: Samples of extracted rutin from different parts and standard were introduced on a precoated silica gel aluminum sheets plate, $60F_{254}$ (5×5 cm, 250 μm). The plate was transferred to the developing chamber and chromatogram was developed by the flow of butanol: acetic acid (4:1, by volume) over the surface. After developments are judged to be complete, the flow of mobile phase is disconnected and the solvent front is marked. The positions of separated species were located under UV light at 254 nm. The rutin was identified by comparing the R_f values with that of standard (R_f = 0.58).

Preparative TLC: One hundred milligram of each extract were dissolved in minimum amount of 50% EtOH and then introduced on silica gel GF_{257} (20×20 cm, 15 μm) plates. After drying, the plate was transferred into a chamber containing butanol: acetic acid (4:1, by volume). Linear ascending development was carried out in twin through glass chamber. The length of chromatogram run was 8 cm. Spots were scraped and dissolved in EtOH. Silica gels were separated from solution by centrifugation. In order to completely separate rutin from silica gel, the separated silica gels were transferred into a glass column and elute three times with 10 mL 50% EtOH. The solvents were combined and diluted to the volume with 50% EtOH in a 100 mL volumetric flask. This was ready for analysis by UV/Vis spectrophotometry and HPLC.

UV/Vis spectrophotometric determination: UV/Vis spectrum of rutin in the range of 200-500 nm was recorded. Wavelength of 360 nm was selected from the spectrum. The calibration curve at this wavelength was linear in the range of 0.156-2.5 μg mL^{-1} of rutin. The calibration was

Table 1: Amount of rutin per 100 g dry powder of leave, flower and fruit of *Capparis spinosa* growing wild in Khozestan (results are the mean of three measurements)

	HPLC		UV/Vis
Parts	(mg/100 g)	(Purity %)	(mg/100 g)
Leave	61.09±0.99	90.41	67.57±1.36
Flower	43.72±0.80	87.25	50.11±1.18
Fruit	6.03±0.54	64.56	9.34±0.35

Table 2: Recovery of the spectrophotometric determination of extracted rutin. (Results are the mean of three measurements)

Parts	Standard added ($\mu g\ mL^{-1}$)	Standard recovered ($\mu g\ mL^{-1}$)	Recovery (%)
Flower	0.625	0.71±0.05	107.6
Fruit	1.25	1.33±0.25	102.4

Table 3: Gradient elution program for HPLC analysis of rutin

Time (min)	Solvent A (%)	Solvent B (%)
0	80	20
40	55	45
46	0	100
49	0	100
50	80	20

In all steps flow rate is 1 mL min^{-1}

linear in the range of 0.156-5 $\mu g\ mL^{-1}$ with the equation of $y = 0.3349x-0.0195$ ($R^2 = 0.9972$) but because of absorbances more than one, concentration of more than 2.5 $\mu g\ mL^{-1}$ was removed from calibration curve. The amount of rutin extracted from leaves, flowers and fruits are determined using this calibration curve (Table 1).

Accuracy: In order to evaluate the accuracy of the extraction and determination method, each sample part were spiked with a defined amount of standard. All the procedure was repeated again and the total amounts of rutin were measured. The amount of standard recovered was calculated by subtracting the amount determined for the sample from total. The results are obvious in Table 2.

Limit of detection: LOD was experimentally determined by reading absorbances of dilute solutions. The LOD signal was considered as mean black signal plus three times its standard deviation. So the limit of detection was $7.31\times10^{-2}\ \mu g\ mL^{-1}$.

Precision: The precision of the method was determined by analyzing the samples and standards 5 times in one day and four successive days. The mean coefficients of variation for within and between days were 2.17 and 2.67, respectively.

HPLC determination: HPLC method which was previously set up for the determination of flavonoids and isoflavonoid in our laboratory were used. The gradient elution using solvent A (5% acetic acid) and Solvent B

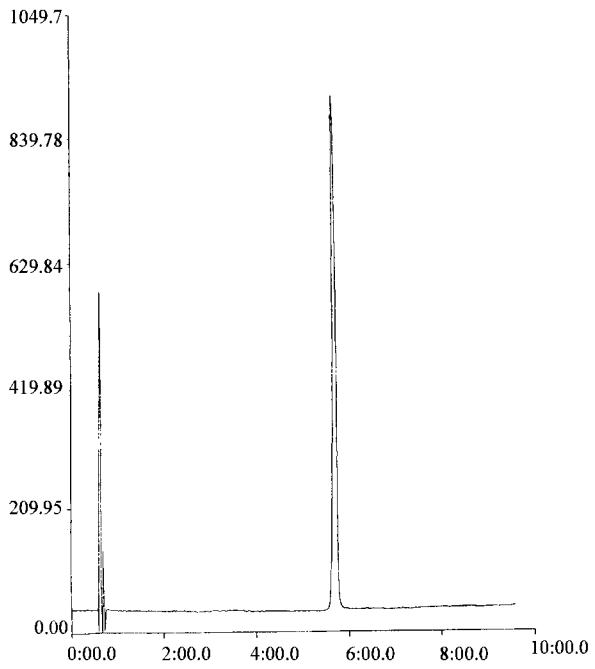

Fig. 2: HPLC chromatogram of standard (pure) rutin

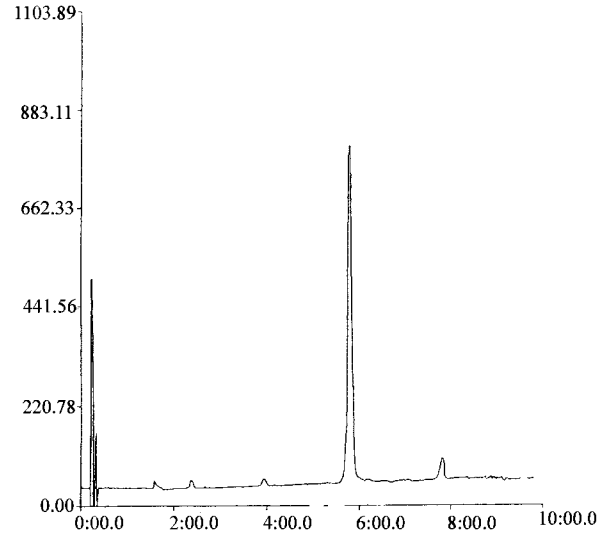

Fig. 3: HPLC chromatogram of rutin extracted from *Capparis spinosa* leaves

($MeOH:CH_3CN:CH_2Cl_2$, 1:5:10) with the program shown in Table 3, flow rate of 1 mL min^{-1}, injected volume: 20 µL, UV detector at 360 nm and sensitivity of 0.2 a.u.f.s. were used. Typical chromatograms of standard and extracted rutin are shown in Fig. 2-5.

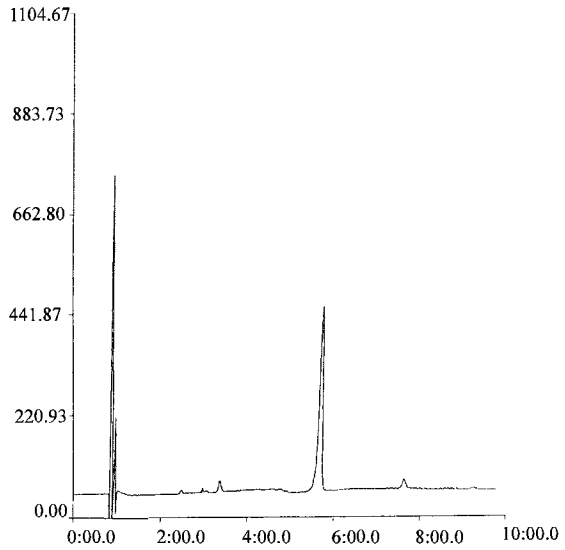

Fig. 4: HPLC chromatogram of rutin extracted from *Capparis spinosa* flowers

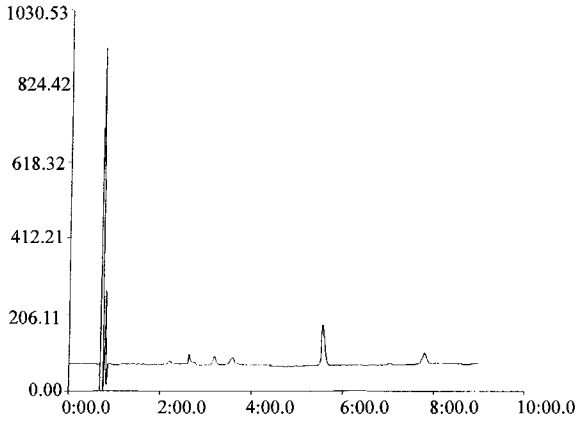

Fig. 5: HPLC chromatogram of rutin extracted from *Capparis spinosa* fruits

RESULTS AND DISCUSSION

The purpose of this study was to use a simple, low cost and fast method of separation and determination of rutin from different parts of *Capparis spinosa* grown wild in Khozestan/Iran.

At first, liquid-liquid extraction of rutin was used but the comparison of UV/Vis spectrum of the purified rutins with that of standard showed so much deviation while the characteristic wavelengths of the rutin was seen in all the extracts. Analytical TLC of liquid-liquid extraction technique also showed that besides rutin so many compounds have been extracted. So it was decided to use preparative TLC for purification step instead of liquid-liquid extraction.

To achieve best separation, composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The best results were obtained using butanol: acetic acid (4:1, by volume). The selected mobile phase showed good resolution. The calculated R_f value for standard was 0.58. Then, in preparative TLC the $R_f = 0.58$ was scrapped and dissolved in extracted solvent. The UV/Vis spectrum of rutin extracted from leaf and flower were as that of standard but a small deviation in spectrum was seen for the fruit. The lower purity was also obtained for rutin extracted from fruit by HPLC as it is obvious in Table 1.

The UV/Vis method for quantitative analysis of rutin was validated with regard to its accuracy, precision and linearity. As it is illustrated from the results, good accuracy and linearity is obtained using this method (Table 2). The calibration curve was linear from 0.156-2.5 μg mL^{-1} with the equation of $y = 0.3097x+0.0041$ ($R^2 = 0.9933$). As it is explained in the experimental parts, the precision of the method was also good.

In order to define the percent of error introduced in the results using UV/Vis method for the analysis of extract, the purity of the rutin obtained from different parts was evaluated with HPLC method. As it is obvious from the Fig. 2-5 and Table 1, rutin extracted from leaf is more pure than other parts. However the error introduced in the results when UV/Vis method is used is low. The purity of rutin in fruit is less than all other parts (Table 1). So this part must be more purified used repeated chromatographic method or providing a suitable SPE method before analysis by the UV/Vis spectrophotometry. Otherwise HPLC must be used for its quantitation. A SPE clean up procedure of extracted rutin is under investigation in our research group.

ACKNOWLEDGMENT

The authors acknowledged the research council of Jundishapur University of medical sciences who support this work.

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