High Performance Liquid Chromatographic Analysis of Rutin in Some Turkish Plants II

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Summary: The determination of rutin in *Cydonia oblonga, Ecballium elaterium, Fraxinus angustifolia, Jasminum fruticans, Marsdenia erecta, Mimosa pudica, Nicotiana tabacum, Paliurus spina-christi and Ruta montana* was carried out by using a sensitive reversed-phase high performance liquid chromatographic method. Chromatographic analysis was achieved on a LiChrospher 100 RP18 5 μm column by using an isocratic mixture of water, methanol and glacial acetic acid (65:30:5) at a flow rate of 0.8 ml/min as a mobile phase. The UV detection was performed at 259 nm.

Introduction

Recently, naturally occurring flavonoids as well as those synthesized are used in modern medicine for various therapeutic effects. Especially in U.S.A., Western and Central Europe, rutin and its derivatives have found a wide application area in some venous diseases. It is reported as an active compound for the treatment of capillary fragility, rheumatic fever of hemorrhagic conditions, the cases of coronary thrombosis, apoplexy, retinal hemorrhage and radiation injuries [1-3].

A few plants containing rutin, for example *Fagopyrum esculentum, Sophora japonica, Eucalyptus macrorrhynca* are used as the sources for obtaining rutin in some countries.

Rutin is found in varying amounts in some plant species cultivated and wild in Turkey. Most of these plants have been used in tradition of folk medicine [4].

Because of the pharmacological activities of rutin, its qualitative and quantitative analysis have been extensively studied. Generally the quantitative determination of rutin in plants and preparations has been carried out by gravimetry, polarography, fluorometry, UV spectrophotometry and high performance liquid chromatography [5-10]. According to our previous studies, we examined the rutin content of some plants by using several of these methods [5,6,8,9,11].

The purpose of this investigation was to apply a simple, rapid and reproducible HPLC procedure for quantitative analysis of rutin and to find out new rutin sources.

Results and Discussion

HPLC analyses of rutin were performed on a LiChrospher 100 RP 18 5 μm column by using water:methanol:glacial acetic acid (65:30:5) as a mobile phase and isocratic elution. This method was applied to 12 plant materials containing rutin. HPLC chromatograms of the plant samples showed many resolved peaks. The peaks were identified by comparison of retention time of standard rutin which was found to be 19 min. HPLC chromatogram of methanol extract of the aerial parts of *Marsdenia erecta* is given in Fig. 1.

The detector response was linearity correlated with concentration in the ranges 1.25-40 μg/ml. The regression equation and correlation coefficient were determined as Y=163676.9 X - 94031.6 (r^2=0.998). Y is peak area of rutin and X is concentration of rutin in μg/ml. The precision of the system was tested three successive injections of standard solutions and plant samples. The detection limits were 1 μg/ml and 40 μg/ml for rutin. The Table-1 showing the names of plants, used parts and rutin contents.

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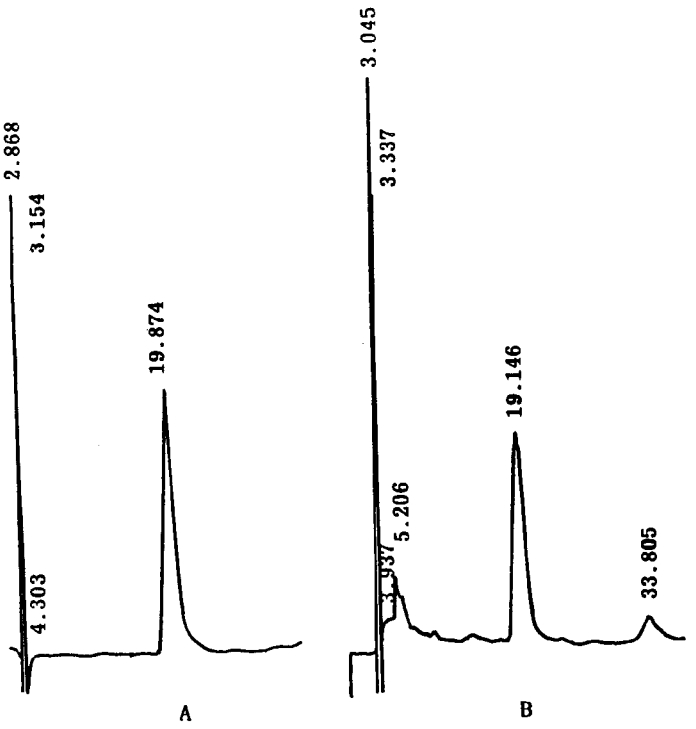

Fig. 1: HPLC Chromatogram of methanol extract of aerial parts of *M. erecta*. A: Rutin B: *M.*

Table-1: Rutin contents of some plants

Plants	Used Parts	Rutin contents (%)
Cydonia oblonga	leaf	0.148
	peel of fruit	0.270
Ecballium elaterium	leaf	0.465
Fraxinus angustifolia	leaf	0.387
Jasminum fruticans	flowers	1.243
Marsdenia erecta	aerial part	0.960
Mimosa pudica	leaf	1.505
	flowers	1.578
Nicotiana tabacum	leaf	0.218
Paliurus spina-christi	leaf	0.473
	fruit	0.255
Ruta montana	aerial part	0.283

Statistical evaluation revealed relative standard deviations at different values for three injections which are shown in Table-2.

Previously, the determination of rutin in plant samples and pharmaceutical preparations was achieved by some classical methods, such as gravimetry, polarography and UV spectroscopy. Recently this has been performed by using high performance liquid chromatographic methods. Thus we can put off some extraction and purification procedures, sometimes followed by a derivatisation step or by a chemical reaction prior to the actual determination.

Table-2: The determination of rutin contents (μg/ml) in different samples with relative standard deviations (RSD)

Plants	Mean ± SEM*	SD**	RSD(%)
Cydonia oblonga (l)	2.96 ± 0.02	0.02	0.67
(p.fr)	5.41 ± 0.52	0.73	13.49
Ecballium elaterium (l)	5.58 ± 0.03	0.04	0.71
Fraxinus angustifolia (l)	9.29 ± 0.08	0.11	1.18
Jasminum fruticans (fl)	9.95 ± 0.39	0.67	6.73
Marsdenia erecta (a.p)	11.52 ± 0.31	0.54	4.68
Mimosa pudica (l)	24.09 ± 0.26	0.37	1.53
(fl)	12.63 ± 0.10	0.14	1.10
Nicotiana tabacum (l)	3.49 ± 0.14	0.19	5.44
Paliurus spina-christi (l)	5.68 ± 0.22	0.31	5.45
(fr)	5.10 ± 0.16	0.23	4.50
Ruta montana (a.p)	6.79 ± 0.29	0.41	6.03

*SEM: Standard Error of Mean, **SD: Standard Deviation, l(leaf), p.fr. (peel of fruit, fl(flower); a.p (aerial part), fr (fruit).

Most of the plants investigated in present study are native and have been used as traditional

medicine in Turkey. In Table-3 the plants, their families, used part and traditional uses are given [4,12-15]. Flavonoids may be responsible for some of these uses e.g. diuretic, diaphoretic etc.

Marsdenia erecta growing naturally [16], has not been used remedy because of its toxicity. But it has high content of rutin as the major component. *Mimosa pudica* is not native but cultivated in garden and parks as ornamental plant in Turkey. Leaves and flowers of this plant have rutin in high amount. There is no information about their usage in folk medicine.

Table-3: The plants used in folk medicine.

Plants	Families	Used parts	Traditional uses
Cydonia oblonga	Rosaceae	fruit leaf	diuretic, antidiarrhoeic for common colds, antitussive, sedative, for dysuria, abdominal pain, hypoglycemic, bronchitis [4,12,14]
Ecballium elaterium	Cucurbitaceae	leaf fruit root	diuretic, to treat sinusitis, to treat jaundice and sinusitis diuretic, laxative [4,12,14,15]
Jasminum fruticans	Oleaceae	flower branch	diuretic, antihelmintic antiparasiter [4,12]
Nicotiana tabacum	Solanaceae	leaf	to treat wounds insecticide [4]
Paliurus spina-christi	Rhamnaceae	fruit	antidiarrhoeic, diuretic, for sore throat, to pass kidney stone [4,13]
Ruta montana	Rutaceae	aerial part	diaphoretic, sedative, against rheumatic pain, emmenagogue [4]

In conclusion, according to our findings *Mimosa pudica, Jasminum fruticans* and *Marsdenia erecta* have high amounts of rutin and so these plants can be used as sources of rutin.

Experimental

Chemicals

Rutin (Merck) was employed as a standard and no further purification was carried out. HPLC grade solvents (Merck) and bidistilled water were used for chromatographic studies. The mobile phase was degassed in an ultrasonic bath.

Plant materials

Plants were collected from different regions in Turkey and identified by Prof. Dr. O. Ketenoglu at the Faculty of Science, Ankara University. Voucher specimens have been deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

The plants used in this study were collected in different periods (flowering and fruiting) from the following places:

Cydonia oblonga Miller	Kergah Vineyard, Kayseri
Ecballium elaterium (L.) Rich.	The garden of Ankara University, Faculty of Science
Fraxinus angustifolia Vahl	Hipodrom, Ankara
Jasminum fruticans L.	The garden of Ankara University, Faculty of Science
Marsdenia erecta (L.) R.Br.	Kemer to Antalya, The entrance of Antalya
Mimosa pudica Banks and Sol.	Adana to Mersin, The entrance of Mersin
Nicotiana tabacum L.	Mugla to Kale. The exit of Mugla
Paliurus spina-christi Miller	Kalecik, Ankara
Ruta montana L.	Amasya

HPLC analysis

Hewlett-Packard higher performance liquid chromatograph was used. The analysis of rutin was performed on a isocratic system consisted of a model 1050 pump. The model 1050 UV detector set at 259 nm. A Rheodyne 7125 injection valve was fitted with a 20 µl loop. The integrator was a Hewlett-Packard 3396A. The analytical column was a LiChrospher 100 RP18, 5 µm, 250x4.0 mm i.d., stainless steel from Hewlett-Packard Chemical Industries, Ltd. The mobile phase was consisted to water:methanol:glacial acetic acid (65:30:5) delivered isocratically at a flow rate 0.8 ml/min resulting in a column head pressure of about 2600 psi. The chromatographic analyses were performed at room temperature.

Preparation of samples

Powdered and weighted plant materials were extracted with diethyl ether in a Soxhlet apparatus for 12 hours. After removed the solvent, materials were air dried and extracted three times with methanol for 48 hours at room temperature. Combined methanol extracts were evaporated to dryness in *vacuo*. The crude extracts were dissolved and diluted with HPLC grade methanol, passed through membrane filters (0.45 µm, Alltech) and suitable dilutions were prepared for each sample for HPLC analyses.

Calibration curve of rutin standard solution

1 mg of Rutin (Merck) was dissolved with 25 ml HPLC grade methanol to give a standard solution of concentration 0.04 μg/ml. Standards ranging in concentrations from 1.25 to 40 μg/ml were prepared by serial dilutions using methanol. These standards were stored at 0°C. Triplicate injection of standard solutions were applied. The area counts of individual peaks and the corresponding concentrations were used to construct the standard curve for rutin. Six standard points were used for each graph and standard linear regression was used to determine the slope and intercept.

Precision of the system

Three replicates of standard rutin solutions were injected successively and the retention times of each was recorded. Precision of method was determined by measuring the retention times of the sample solutions corresponding to the rutin standard.

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