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HPLC Analysis of Ascorbic Acid in the Fruits of *Rosa* Species growing at Işık Mountain

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Summary : *Reversed phase high pressure liquid chromatographic method was used to quantify ascorbic acid in the fruits of some Turkish Rosa species growing around Ankara. Ascorbic acid content was estimated as 0.37 % for R. villosa L., 0.37 % for R. canina L. form c, 1.30 % for R. canina L. form a, 1.51 % for R. pulverulenta Bieb. , 1.53 % for R. dumalis Bechst. ssp. boissieri (Crepin) Ö. Nilsson X R. canina L.*

Key words: *RP-HPLC, Ascorbic Acid, Rosa*

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Işık Dağında Yetişen Rosa Türlerinin Meyvalarında YBSK Yöntemiyle Askorbik Asit Miktar Tayini

Özet : *Ankara civarında yetişen 5 Rosa örneğinin (R. villosa L. , R. canina L. form c, R. canina L. form a, R. pulverulenta L. ve R. dumalis Bechst.ssp. boissieri (Crepin) Ö. Nilsson X R. canina L.) meyvalarında Ters Faz-Yüksek Basınç Sıvı Kromatografisi (TF-YBSK) metoduyla askorbik asit (Vitamin C) miktarları tayin edilmiştir. Askorbik asit miktarı; R. villosa' da % 0.37, R. canina form c' de % 0.37, R. canina form a' da % 1.30, R. pulverulenta' da % 1.51 ve R. dumalis ssp. boissieri X R. canina' da % 1.53 olarak tesbit edilmiştir.*

Anahtar kelimeler : *TF-YBSK, Askorbik Asit, Rosa.*

INTRODUCTION

Fruit and vegetables are the major sources for ascorbic acid (AA)and it is well known that rose hips are rich in vitamin C. *Rosa* species are widely distributed in the world and used for various purposes including food and folk medicine.

Twentyfour *Rosa (Rosaceae)* species are recorded in Turkey[1], most of them being abundant in Turkey. Rose hips are still a valuable natural source of vitamin C and commonly used in rural parts of Turkey, as juice, marmalade, tea bags and powdered rose hips. Fruits of *Rosa* species (Fructus Cynosbati) are known by the following vernacular names in Anatolia; Kuşburnu, gül burnu, gül elması, it burnu, köpek gülü meyvası and yabangülü meyvası[2]. Titrimetric, colorimetric, spectrophotometric and chromatographic methods are generally used for determination of AA in Rose hips[3]. The older classical chemical methods of analysis are gradually being replaced by separation techniques such as High Performance Liquid Chromatography (HPLC), which may eliminate the problem of interfering compounds in determination. Since High Pressure Liquid Chromatographic (HPLC) method is more sensitive and has high selectivity than the other methods, this modern method was preferred in this study[4].

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Fresh rose hips, the fruit of *Rosa* species usually contain high amounts of a AA ranging from 0.250-4.00 %. But the content of AA in commercially available products are highly variable, depending on the origin of *Rosa* species, its habitat, time of collection, methods of drying and storage. As a matter of fact some commercial samples no longer contain detectable quantities of vitamin C[5]. This report deals with the determination of AA in the fruits of five *Rosa* specimens (*R. villosa, R. canina* form c, *R. canina* form a, *R. pulverulenta, R. dumalis ssp. boissieri* X *R. canina*).

EXPERIMENTAL

Material and methods

The research materials were collected from Işık Mountain located in the North of Ankara. The locations and AEF No. of collection are given in Table 1. Voucher specimens are deposited in Ankara University Faculty of Pharmacy Herbarium - AEF.

Table 1. The Location of *Rosa* Species

Rosa species	AEF No	Location
R. villosa	15571	Ankara,Kızılcahamam,Işık Dağı, 1630 m. 23.10.1991
R. canina form c	15574	Ankara,Kızılcahamam to Çerkeş, 1080m. 23.10.1991
R. canina form a	15576	Ankara,Kızılcahamam to Çerkeş, 1510m. 23.10.1991
R. pulverulenta	15570	Ankara,Kızılcahamam,Işık Dağı, 1630 m. 23.10.1991
R. dumalis ssp. boissieri X *R. canina* hybrids	15572	Ankara,Kızılcahamam,Işık Dağı, 1630 m. 23.10.1991

To avoid decomposition of AA, all fresh fruits of *Rosa* species were immediately frozen at -20 C and frozen material was directly subjected to extraction. The amount of AA is expressed in g per 100 g of dried fruit of *Rosa* species. Loss on drying was calculated in an oven at 105°C according to European Pharmacopoeia[6].

An external standard method was used for quantitative determination. The calibration curve was obtained by analysing five dilutions(n=5) of authentic AA (Merck Cat. No. 500074). The linearity of AA was confirmed by regression analysis. The correlation coefficient for AA was r^2=0.9997. Results are expressed as the mean of three determinations.

Sample Preparation

After removing seeds and hairs, 1 g rose hips were crushed and powdered in a porcelain mortar with 50 ml of 2 % metaphosphoric acid. After filtration, 5 ml of the filtrate was passed through a Sep-pak C_{18} Cartridge(Waters). Ascorbic acid was completely eluted with 4 ml of 2 % metaphosphoric acid. The solution was then brought to a final volume to 10.00 ml in a volumetric flask[4]. 5 µl were used for the HPLC analysis on the same day.

Chromatographic Conditions

A model 600 Liquid Chromatograph equipped with 6000A pump, M-440 UV detector, U6K injector(Waters) and an electronic integrator (M-745B-Waters) were used. Column pressure and detector sensitivity were 1800 psi and 0.05 AUFS respectively. Novapak C_{18} column (3.9 x150 mm, Waters) was used. The mobile phase was 0.5% metaphosphoric acid, filtered through a 0.22 µm filter (Milipore, Bedford, MA, USA) and degassed in an ultrasonic bath. The flow rate and chart speed were 0.8 ml/min. and 0.5 cm/min. respectively, detection of AA was performed at UV 254 nm. The identification of the AA peak was based on retention time and cochromatography with an authentic sample.

RESULTS AND DISCUSSION

HPLC is mostly preferred to other methods for AA determination because it is more selective and fast and there is no need for derivatization before analysis [7,8].

The HPLC analysis (Fig. 1) of the different samples enabled a precise and quick quantification of ascorbic acid (AA). The results are given in Table 2.

Table 2. Results of AA Assays of Rose Hips (% g dry weight).

Rosa species	% AA ± S.D.*
R. villosa	0.37 ± 0.001
R. canina form c	0.37 ± 0.007
R. canina form a	1.30 ± 0.013
R. pulverulenta	1.51 ± 0.035
R. dumalis ssp. *boissieri* X *R. canina*	1.53 ± 0.021

* Each value is the average of three runs ± Standart Deviation (S.D.)

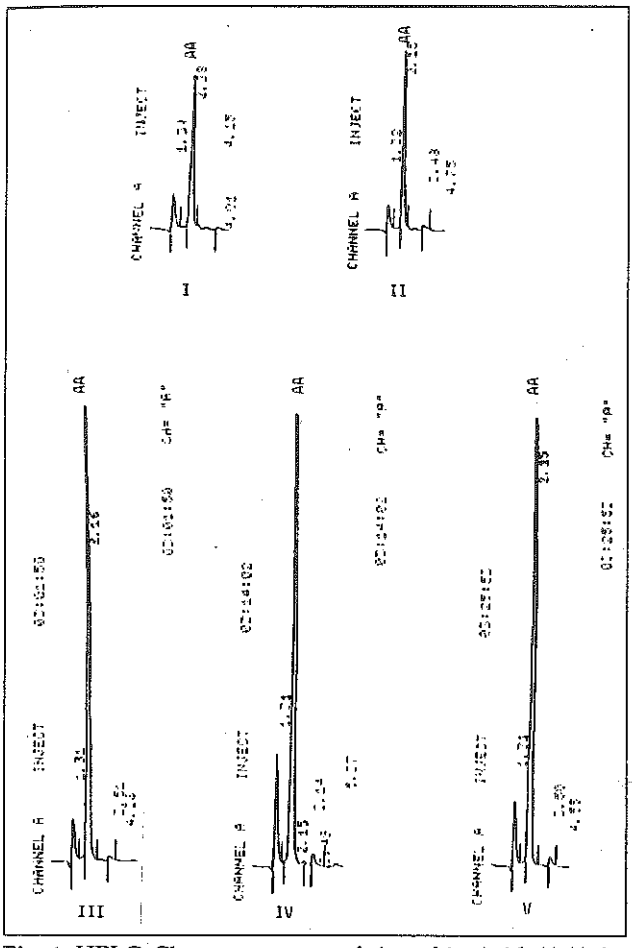

Fig. 1. HPLC Chromatograms of Ascorbic Acid (AA) in *Rosa* Species

I. *R. villosa*, II. *R. canina* form c,
III. *R. canina* form a, IV. *R. pulverulenta*,
V. *R. dumalis* ssp. *boissieri* X *R. canina*
(HPLC condition see experimental section)

The Ascorbic acid content was reported to be 1.335-2.145% in *R. villosa*, 0.250-2.411% in different forms of *R. canina* and 1.233-3.258% in *R. pulverulenta* by using classical titration methods. AA content in the Turkish *Rosa* species up to date have been determined by titration methods also and have been found to be 0.57-2.4 % in different forms of *R. canina*, 2.0-3.1 % in varieties of *R. dumalis*, 2.12 % in *R. montana* and 3.06 % in *R. pulverulenta* [9,10].

It is obvious that titration methods are limited by interfering substances found in the extract. Therefore, end points are ill-defined and problems with color development or fading are common[11]. As a result of these factors AA content is usually found to be higher than the original.

Thus, the results found in the study (Table 2) are somewhat lower than the previous results as expected. On the other hand, *Rosa canina* L. is a very polymorphic species without a formal infraspecific classification. At least 4 main groups may be distinguished in Turkish samples and transitions are frequent[1].

As a result, AA content in the hips of this species indicate a wide range. Among the studied species, hips of *R. canina* form a, *R. pulverulenta* and *R. dumalis ssp. boissieri* X *R. canina* hybrids are found be rich in AA (over 1 %). This is the first report on the determination of AA in *R. dumalis ssp. boissieri* X *R. canina hybrid*.

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