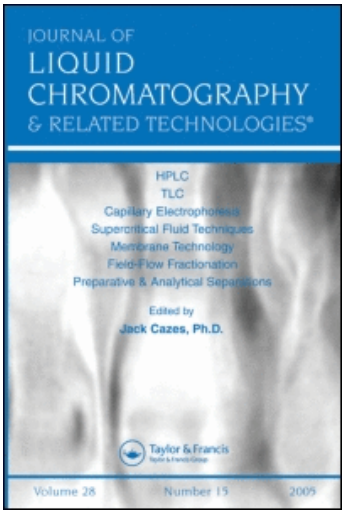

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF SYRINGIN IN *VISCUM ALBUM* L. SSP. *ALBUM* SAMPLES COLLECTED FROM DIFFERENT HOST PLANTS

D. Deliorman[a]; F. Ergun[a]
[a] Faculty of Pharmacy, Department of Pharmacognosy, Gazi University, Ankara, Turkey

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF SYRINGIN IN *VISCUM ALBUM* L. SSP. *ALBUM* SAMPLES COLLECTED FROM DIFFERENT HOST PLANTS

D. Deliorman, F. Ergun

Gazi University
Faculty of Pharmacy
Department of Pharmacognosy
06330 Hipodrom
Ankara, Turkey

ABSTRACT

V. album (Loranthaceae) has been utilized for the treatment of various diseases such as hypertension, arteriosclerosis, and cancer, either alone or in combination with other plant extracts in modern medicine. In this study, qualitative and quantitative determinations of syringin contents of *Viscum album* L. ssp. *album* samples, collected from nine different host plants, were carried out by using HPLC.

This analysis was performed on a LiChrospher RP-18e column, using an isocratic mobile phase (Methanol:water: 0.1 N sodium acetate 20:73.5:6.5).

INTRODUCTION

Viscum L. (Loranthaceae) is a semi-parasitic genus growing on various host plants, i.e. trees and shrubs. In Turkey, the genus is represented by one species, and three subspecies. They are, *V. album* L. ssp. *album*, *V. album* ssp.

abietis (Wiesb.) Abromeit, *V. album* ssp. *austriacum* (Wiesb.) Vollmann,[1] and all these plants are known as "Ökse Otu," which means "bird-lime herb."[2]

The European mistletoe, *V. album* L., is an evergreen parasitic plant widely distributed throughout Europe, except in northern areas.[3] The main areas of therapeutic applications are; cardiovascular illnesses, especially hypertension and arteriosclerosis, cancer, and arthrosis.

The flavonoids and phenol carboxylic acids, together with the phenylpropanes and lignans, as well as the amines, are postulated as possible agents of cardiovascular activity.[4]

The literature indicated that, butanol extracts of *V. album* collected from apple tree, spruce, and elm showed an inhibitory effect on the enzyme, cyclic adenosine monophosphate (cAMP)-phosphodiasterase (PDE). Phenylpropane and lignan derivatives were suggested to play a role in the inhibition of this enzyme, and a correlation was established between the in vitro inhibition of PDE and in vivo pharmacological activity.[5]

Syringin is the main phenylpropane constituent of *V .album* (Figure 1), and qualitative and quantitative determinations of this compound in different subspecies were previously reported by using High Performance Liquid Chromatography (HPLC).[6]

In this study, considering the different host plants, syringin content in nine different *V. album* ssp. *album* samples were studied quantitatively by using the same HPLC technique.

EXPERIMENTAL

Chemicals

The isolation procedure of syringin from *Viscum album* ssp. *album* (*Armeniaca vulgaris* L.) was reported in a previous study.[6] The structure of

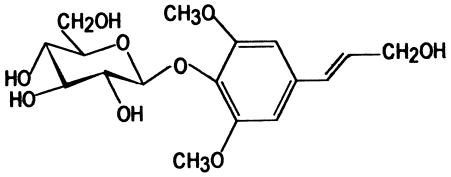

Figure 1. The structure of syringin.

syringin was elucidated by UV, IR, ^{1}H-NMR, ^{13}C-NMR, and MS.[7] HPLC grade solvents and bidistilled water were used for the chromatographic studies. All chromatographic experiments were performed at room temperature.

Plant Materials

Nine *V. album* L. ssp. *album* samples were collected from different regions of Turkey. All the specimens are deposited in The Herbarium of Ankara University, Faculty of Pharmacy (Ankara, Turkey). The materials, specimen numbers, and their collection sites are described in Table 1.

Instrumentation

A Hewlett-Packard HPLC system was used consisting of the following components: Model 1050 pump, equipped with a Rheodyne Model 7125 valve

Table 1

The Collection Sites of *Viscum album* ssp. *album* Samples

Host Plants	Herbarium No.	Locality
Armeniaca vulgaris Lam. (Apricot)	AEF 18953	Ankara,Baglum in orchard June 1995
Armeniaca vulgaris Lam (Wild apricot)	AEF 18940	Corum, İskilip in orchard June 1995
Cerasus avium (L.) Moench. (Cherry)	AEF 18962	Corum, İskilip in orchard June 1995
Cerasus vulgaris Miller (Sour cherry)	AEF 18963	Corum, İskilip in orchard June 1995
Cydonia oblonga Miller (Quince)	AEF 18957	Corum, İskilip in orchard June 1995
Prunus x domestica L. (Plum)	AEF 18945	Isparta, Aglasun April 1995
Pyrus communis L. ssp. *Communis* ssp. *sativa* (DC.) Hegi (Pear)	AEF 18944	Ankara, Baglum in orchard June 1995
Pyrus eleagnifolia Pallas ssp. *eleagnifolia* (Wild pear)	AEF 18941	Ankara, Kizilcahaman road April 1995
Robinia pseudoacacia L. (Acacia)	AEF 18961	Ankara, Baglum in orchard June 1995

fitted with a 20 μL loop, a model 1050 UV detector set at 264 nm, and an HP-3996 A integrator.

Sample Preparation

The air-dried and powdered leaves, stems, and twigs of *V. album* ssp. *album* samples, growing on nine different host plants (10 g), were extracted with methanol in a water bath (50°C) for three days. The process was followed by TLC (Kieselgel $60F_{254}$, 0.2 mm, Merck, Art.5554, $CHCl_3$:MeOH:H_2O 61:32:7).

Spots were detected by spraying with 1% vanillin in H_2SO_4, followed by heating at 110°C, for 5-10 min. After filtration, the methanolic extracts were evaporated to dryness.

The methanolic extracts of nine different *V. album* ssp. *album* samples were accurately weighed into 10 mL volumetric flasks and adjusted with methanol. Then, they were filtered through a Millipore HA (0.45 μm) membrane filter and, finally, degassed under vacuum before use.

Standard Preparation and Calibration Curve

For this analysis, the internal standard method was used.[4,5,6] Resorcinol (Merck, Art. 7590), reported in the literature, was used as an internal standard. Resorcinol (10 μL) was injected into the column and its retention time was determined as 7.589 min. (Figure 2). Syringin (0.04 mg) was accurately weighed into a 10 mL volumetric flask and dissolved in methanol to prepare a 0.004 mg/mL solution.

A standard resorcinol solution (0.095 mg), was also prepared in the same manner. This stock solution was used to prepare an 0.0095 mg/mL solution. The final dilution of resorcinol (1.5 mL), as an internal standard, was then added to syringin samples. The total volume was adjusted to 10 mL. The dilution ratios used for the calibration curve are given Table 2.

The calibration curve of syringin was constructed by triplicate injections of mixtures of resorcinol-syringin at concentrations ranging from 2 μg/mL to 8 μg/mL for syringin and by plotting the peak heights versus the concentrations.

At least four standard points were used for the curve, and standard linear regression was used to determine the slope and intercept. The regression equations and correlation coefficients determined for the standard were [y=0.6786x+0.1435] (r=0.9979).

Syringin is a major compound in all of the methanolic extracts.

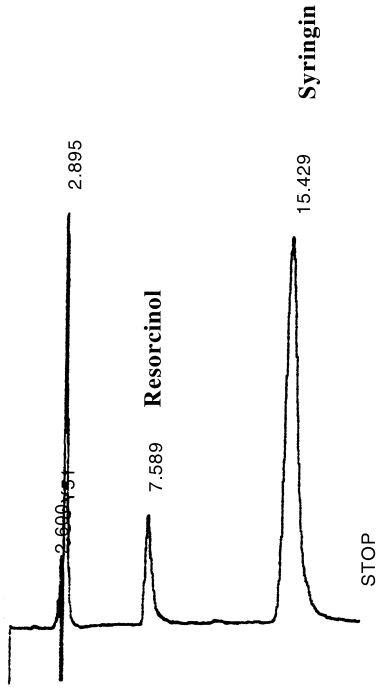

Figure 2. HPLC chromatograms of resorcinol and syringin.

Table 2

Syringin and Resorcinol Ratios in Dilutions Used for Calibration Curve

	Syringin (0.004 mg/mL)	Resorcinol (0.0095 mg/mL)	Total Volume
1. Dilution	0.50 mL	1.50 mL	10 mL
2. Dilution	1.00 mL	1.50 mL	10 mL
3. Dilution	1.50 mL	1.50 mL	10 mL
4. Dilution	2.00 mL	1.50 mL	10 mL

Validation Parameters

The precision of the analytical method was determined by assaying at the triplicate applications of each test sample. As can be seen in Table 3, the repeatability expressed by X, S.D., showed variations depending on the source of the material. The formula used to estimate S.D. is as follows:

$$\sqrt{\frac{n\sum x^2-\left(\sum x\right)^2}{n(n-1)}}$$

x: The peak height of syringin / the peak height of resorcinol

n: The number of nearest x values used to estimate S.D. (n:2)

Chromatographic Conditions

HPLC Column: LiChrospher RP-18e, 5 μm particle size, 4 mm i.d.x250 mm (Hewlett-Packard Chemical Industries, Ltd.); Mobil Phase: Water-methanol-0.1 N sodium acetate (73.5:20:6.5 v/v/v pH:6.60); Flow Rate: 0.8 mL/min; Detection: UV, 264 nm; Detection sensivity: 0.005 aufs; Column pressure: 180 barr; Chart speed: 0.3 cm/min

RESULTS

The isolation procedure of syringin from *V. album* ssp. *album* (*Armeniaca vulgaris* L.) was described in a previous study.[6] In this study, quantitative and qualitative analysis of syringin content of methanolic extracts belonging to nine different *V. album* ssp. *album* samples are performed by using HPLC.

For qualitative analysis, the retention times of eluted peaks of the test samples are compared with that of the authentic sample. Retention time of syringin is found to be 15.429 min. (Figure 2), and is observed as the major component of all test samples.

While the highest amount of syringin is found to be in the sample of ssp. *album* growing on Pear trees (Figure 3), the lowest in that sample is obtained from quince (Figure 3) (Table 3).

DISCUSSION

In this study, the amounts of syringin in *V. album* ssp. *album* samples collected from various host plants are determined to be between 0.5766-2.4257

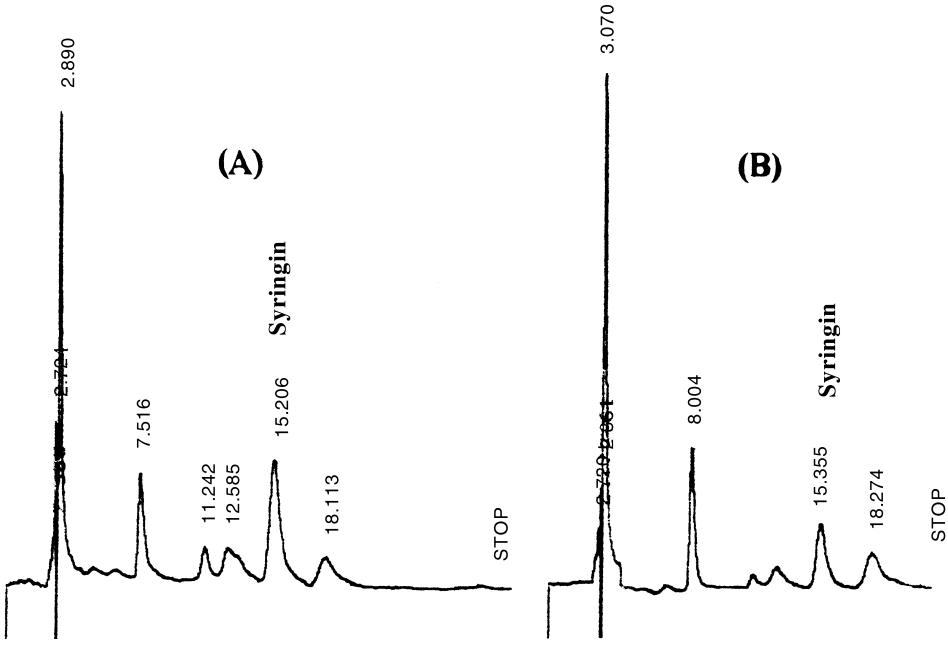

Figure 3. HPLC chromatograms of the methanolic extracts of two samples (A) pear, (B) quince.

w/w %. Syringin concentration in *V. album* ssp. *album* samples are found to be more than the other two subspecies (*V. album* ssp. *abietis,* w/w 0.4692 %; *V.album* ssp. *austriacum,* w/w 0.5208 %), which was reported previously.[6]

All literature surveys (Table 4) showed that the amounts of syringin in nine different *V. album* ssp. *album* samples collected from Turkey have been found to be the highest of all the other studies.[4, 5]

The results obtained in HPLC analysis showed that methanolic extracts belonging to *V. album* ssp. *album* samples collected from nine different host plants have been manifested to be potential sources of syringin. Syringin has been reported to have adaptogenic activity[8] and an inhibitory effect on cAMP-PDE.[9] Therefore, *V. album* ssp. *album* samples used in this study could be evaluated in this respect.

Generally, the subspecies of *V. album* samples were not taken into consideration in previous studies, except for in a few literatures.[6,10-13] *V. album* is rep-

Table 3

Syringin Percentages in *V. album* ssp. *album* Samples

Host Plants	w/w % ± S.D.
Armeniaca vulgaris Lam (Apricot)	$1.4140 \pm 1.0364 \times 10^{-6}$
Armeniaca vulgaris Lam. (Wild apricot)	$0.9371 \pm 4.4121 \times 10^{-6}$
Cerasus avium (L.) Moench. (Cherry)	$0.5936 \pm 1.1279 \times 10^{-6}$
Cerasus vulgaris Miller (Sour cherry)	$1.2680 \pm 1.6035 \times 10^{-6}$
*Cydonia oblonga*Miller (Quince)	$0.5766 \pm 4.0589 \times 10^{-4}$
Prunus x domestica L. (Plum)	$1.0471 \pm 2.6987 \times 10^{-6}$
Pyrus communis L. ssp. *communis* ssp. *sativa* (DC.) Hegi (Pear)	$2.4257 \pm 4.8704 \times 10^{-6}$
Pyrus eleagnifolia Pallas ssp. *eleagnifolia* (Wild pear)	$1.5265 \pm 4.3915 \times 10^{-5}$
Robinia pseudoacacia L. (Acacia)	$1.6692 \pm 8.4708 \times 10^{-6}$

Table 4

Syringin Percentages in *V. album* Samples[a] Reported in Literature[4,5]

Host Plants	Extract Alcoholic-Aqueous	n-BuOH	Syringin
Malus sp.	+	----	0.065
Tilia cordata			
Leaves	+	----	0.018
Stems	+	----	0.048
Berries	+	----	0.027
Ulmus glabra	+	----	0.043
Unidentified		+	0.03 - 0.07*

[a] w/w % + studied extracts. * Total amount of phenylpropanes (Syringin, syringin-4-O-[apiofuranosyl (1→2)]glucoside).

resented by three subspecies in the world. In many literatures, the samples belonging to this plant were called *V. album* without discriminating the subspecies.

Therefore, in many studies releated with the biological effects and the chemical contents of *V. album* samples have some deficiencies. Actually, the samples collected on different host plants, which are called *V. album,* belong to different subspecies (ssp.*album*, ssp. *abietis*, ssp. *austriacum*). Therefore, the subspecies of *V. album* samples, as well as the name of the host plant should be notified in the studies, in order to evaluate the biological effect of the plant.

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