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RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

HPLC Analysis of Psoralen from *Psoralea acaulis* Stev. and *Psoralea bituminosa* L.*

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HPLC Analysis of Psoralen from Psoralea acaulis Stev. and Psoralea bituminosa L.

Summary : *A High Pressure Liquid Chromatographic technique was used to determine Psoralen, on a reversed phase C_{18} column, using methanol/ water (21:10) in the leafy branches, flowers, fruits and roots of Psoralea acaulis Stev. and Psoralea bituminosa L.*

In the present study, the amounts of Psoralen in the leafy branches, flowers, fruits, roots of P. acaulis were 0.2107 %, 0.3118 %, 0.1641 %, 0.1016 % and of P. bituminosa 0.0673 %, 0.1566 %,0.0708 %, 0.0771 % respectively.

Key words: *Psoralen, Psoralea acaulis Stev., Psoralea bituminosa L., RP- HPLC Analysis*

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Psoralea acaulis Stev. ve Psoralea bituminosa L.'daki Psoralen'in YBSK Analizi

Özet : *Psoralea acaulis Stev. ve Psoralea bituminosa L. bitkilerinin yapraklı dal, çiçek, meyve ve köklerinde bulunan psoralen'in kantitatif analizi ters faz YBSK ile C_{18} kolonunda metanol/ su (21:10) mobil fazı kullanılarak yapılmıştır.*

P. acaulis'in yapraklı dallarında % 0.2107, çiçeklerinde % 0.3118, meyvelerinde % 0.1641 ve köklerinde % 0.1016; P. bituminosa'nın yapraklı dallarında % 0.0673, çiçeklerinde % 0.1566, meyvelerinde % 0.0708 ve köklerinde % 0.0771 psoralen bulunmuştur.

Anahtar kelimeler: *Psoralen, Psoralea acaulis Stev., Psoralea bituminosa L., Ters Faz YBSK Analizi*

INTRODUCTION

The genus *Psoralea* (Fabaceae) is represented by 150 world-wide species[1].

The genus *Psoralea* is represented by 3 species, *P. bituminosa* L., *P. acaulis* Stev. and *P. jaubertina* Fenzl in the flora of Turkey[2].

Many phytochemical investigations were performed on the terpenic compounds, flavonoids and coumarins of the species of the genus *Psoralea*.

The linear furocoumarin Psoralen and its angular isomer Angelicin were isolated from the fruits and seeds of *Psoralea macrostachya* DC. Prod. and *Psoralea onobrychis* Nutt. by Cappeletti et al[3].

In the Plant kingdom, furocoumarins are the only compounds that have been reported to evoke photodermatitis. The naturally occurring furocoumarins are phototoxic compounds and act as primary irritants after activation[4].

A structure activity study on the naturally occurring furocoumarins demonstrated that Psoralen was the most active phototoxic agent[4].

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Psoralen and Angelicin obtained from the different vegetative organs of several cultivated *P. macrostachya, P. onobrychis, P. glandulosa* and *P. bituminosa* are widely used in skin diseases such as psoriasis, mycosis and fungoides[5].

Psoralea bituminosa L. is used as a tonic, emmenagogue and in the treatment of chronic diarrhoea in Indian folk medicine[6].

In our research, the amount of Psoralen was determined in the leafy branches, flowers, fruits and roots of the *Psoralea acaulis* and *Psoralea bituminosa* by RP-HPLC.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Jasco model Rheodyn injector, a PU-980 solvent pump and model UV- 975 detector equipped with a 300 nm filter.

Psoralen was quantitatively separated on a LiChrosorb RP 18-5 column (20 cmx 4.9 mm) with an elution of an isocratic mobile phase of methanol- water (21:10) (v/v). Psoralen was determined at a flow rate of 1 ml/min in all samples.

All solvents and samples were filtered through a 0.45 µm Milipore filter.

Chemical

The furocoumarin standard Psoralen was isolated from aerial parts of *Psoralea acaulis* and the structure of this compound was determined by Toru Okuyama from the Meiji College of Pharmacy, Tokyo, Japan.

Material

Research materials were collected from different regions of Turkey where the species are located. The locations are given in Table 1. The voucher specimens were deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF).

All samples were dried at room temperature, powdered and stored carefully until the experiments began.

Sample Preparation

Powdered samples were weighed into 2.0000 g lots and refluxed for 1 h with 100 ml methanol. Methanolic extracts were filtered. All extracts were filtered through a 0.45 µm Milipore filter and 10 µl of samples were injected.

Quantification

An external standard method based on peak area was used for quantitative determinations. The calibration curves were prepared by analysing four dilutions (n=4) of an authentic sample of Psoralen (0.2- 0.02 mg/ml). The linearity of Psoralen was confirmed by regression analysis. The correlation coefficients were found to be r^2= 0.9985.

Table 1. The Locations of Investigated *Psoralea* Species

Species	Location	Date
Leafy branches and flowers of *Psoralaea bituminosa*	Kastamonu: between İnebolu-Abana, slopes of Manastır bridge 50-60m (AEF 19205)	1.6.1995
Fruits and roots of *Psoralea bituminosa*	Kastamonu: between İnebolu-Abana, slopes of Manastır bridge 50-60 m (AEF 19494)	21.9.1995
Leafy branches and flowers of *Psoralea acaulis*	Trabzon : Çaykara, slopes of Uzungöl, 1100 m (AEF 19204)	27.5.1995
Fruits and roots of *Psoralea acaulis*	Trabzon : Çaykara, slopes of Uzungöl, 1100 m (AEF 19495)	28.9.1995

RESULTS AND DISCUSSION

Retention time (Rt) of the authentic sample of Psoralen was found to be 4.55 minutes (Fig.1).

HPLC analysis results of *Psoralen* contents in different organs of *P. acaulis* and *P. bituminosa* are given in Table 2.

Table 2. Psoralen Content of *P. acaulis* and *P. bituminosa* Samples

SAMPLE		% Psoralen±S.D
P. acaulis	Leafy branches	0.2107 ± 0.0001
	Flowers	0.3118 ± 0.0001
	Fruits	0.1641 ± 0.0001
	Roots	0.1016 ± 0.0003
P. bituminosa	Leafy branches	0.0673 ± 0.0006
	Flowers	0.1566 ± 0.0001
	Fruits	0.0708 ± 0.0001
	Roots	0.0771 ± 0.0003

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

The Psoralen contents of *P. acaulis* were found to be 0.2107 % for the leafy branches (Fig. 2), 0.3118 % for the flowers (Fig. 3), 01641 % for the fruits (Fig. 4) and 0.1016 % for the roots. For the other species, *P. bituminosa*, these values were 0.0673 %, 0.1566 % (Fig. 5), 0.0708 % and 0.0771 % respectively.

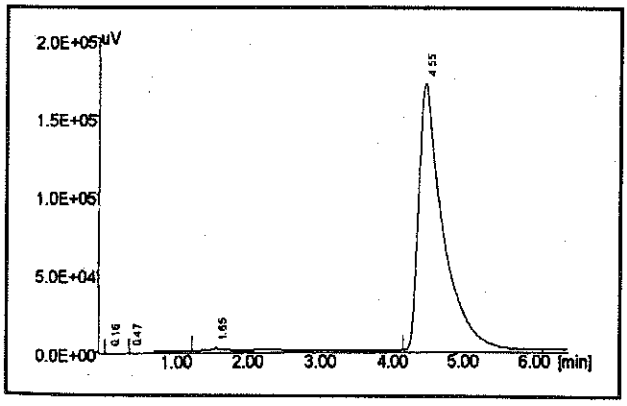

Fig.1 Standard Psoralen

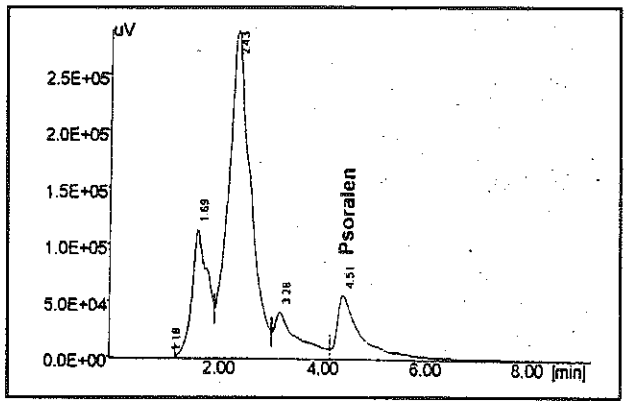

Fig.2 Analysis of psoralen in the leafy branches of *Psoralea acaulis* Stev.

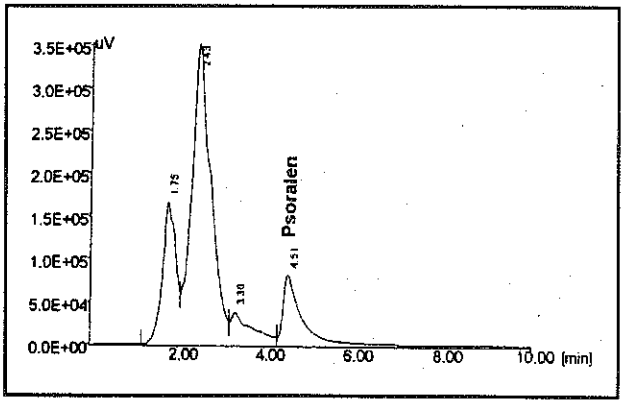

Fig.3 Analysis of psoralen in the flowers of *Psoralea acaulis* Stev.

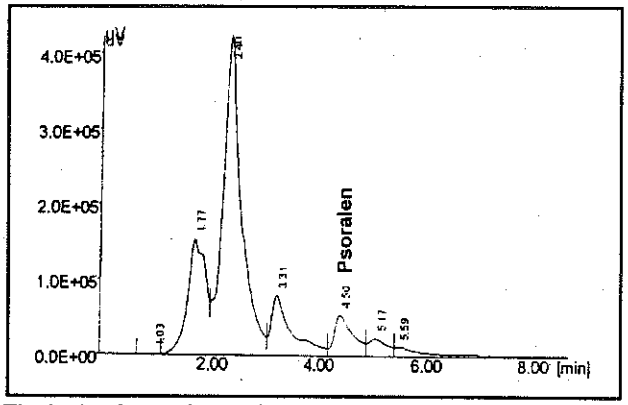

Fig.4 Analysis of psoralen in the fruits of *Psoralea acaulis* Stev.

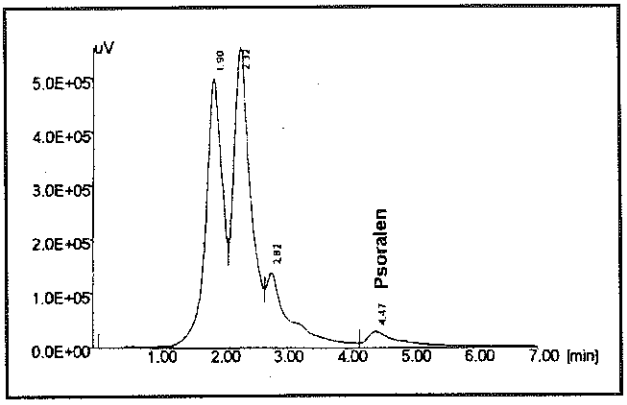

Fig.5 Analysis of psoralen in the flowers of *Psoralea bituminosa* L.

Psoralen content of the flowers of *P. acaulis* is higher than any other part of the plant. The quantity of the substance found in the leaf bearing branches and fruits was been found to be lower. This indicates that if we wish to use this plant as a source of Psoralen; paralleling with the plant's vegetative development; the flowering period can be considered as the most favourable period.

Psoralen content of *P. bituminosa* flowers is greater than *P. acaulis* roots and nearly equivalent to *P. acaulis* fruits. However since the overall Psoralen content of *P. bituminosa* is low, this plant can not be considered as a Psoralen source, in other words obtaining Psoralen from *P. bituminosa* can not be regarded as economic.

However, the fruits of different *Psoralea* species were examined by Innocenti et al. and the Psoralen amounts in the fruits were found to be 0.2443 % in the *P. corylifolia* and 0.2474 % in the *P. plumosa*. In the same study, Psoralen amounts in the fruits of *P. maritimi, P. pustulata, P. lachnostachys, P. cinera* and *P. leucantha* were determined to be between 0.016-0.034 %[7].

Thus, it was reported that the Psoralen amounts in *Psoralea* species were considerably different in the various *Psoralea* species[7].

As a result, *P. acaulis* can be considered as a good Psoralen source compared to *P. bituminosa*, whose Psoralen amount is very low, and it is not productive to obtain this compound from natural sources.

Acknowledgements

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