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

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**INTRODUCTION**

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

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

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

The linear furcoumarin 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.

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.

A structure activity study on the naturally occurring furocoumarins demonstrated that Psoralen was the most active phototoxic agent.
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 fungoides⁵.

*Psoralea bituminosa* L. is used as a tonic, emmenagogue and in the treatment of chronic diarrhoea in Indian folk medicine⁶.

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 Rheodyne 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 cm x 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**

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leafy branches and flowers of</td>
<td>Kastamonu: between İnebolu-Abana, slopes of Manastır bridge 50-60 m</td>
<td>1.6.1995</td>
</tr>
<tr>
<td><em>Psoralea bituminosa</em></td>
<td>(AEF 19205)</td>
<td></td>
</tr>
<tr>
<td>Fruits and roots of <em>Psoralea</em></td>
<td>Kastamonu: between İnebolu-Abana, slopes of Manastır bridge 50-60 m</td>
<td>21.9.1995</td>
</tr>
<tr>
<td><em>bituminosa</em></td>
<td>(AEF 19494)</td>
<td></td>
</tr>
<tr>
<td>Leafy branches and flowers of</td>
<td>Trabzon : Çaykara, slopes of Uzungöl, 1100 m (AEF 19204)</td>
<td>27.5.1995</td>
</tr>
<tr>
<td><em>Psoralea acaulis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits and roots of <em>Psoralea</em></td>
<td>Trabzon : Çaykara, slopes of Uzungöl, 1100 m (AEF 19495)</td>
<td>28.9.1995</td>
</tr>
<tr>
<td><em>acaulis</em></td>
<td></td>
<td></td>
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</tbody>
</table>
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

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>% Psoralen±S.D</th>
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<tbody>
<tr>
<td>P. acaulis</td>
<td></td>
</tr>
<tr>
<td>Leafy branches</td>
<td>0.2107 ± 0.0001</td>
</tr>
<tr>
<td>Flowers</td>
<td>0.3118 ± 0.0001</td>
</tr>
<tr>
<td>Fruits</td>
<td>0.1641 ± 0.0001</td>
</tr>
<tr>
<td>Roots</td>
<td>0.1016 ± 0.0003</td>
</tr>
<tr>
<td>P. bituminosa</td>
<td></td>
</tr>
<tr>
<td>Leafy branches</td>
<td>0.0673 ± 0.0006</td>
</tr>
<tr>
<td>Flowers</td>
<td>0.1566 ± 0.0001</td>
</tr>
<tr>
<td>Fruits</td>
<td>0.0708 ± 0.0001</td>
</tr>
<tr>
<td>Roots</td>
<td>0.0771 ± 0.0003</td>
</tr>
</tbody>
</table>

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), 0.1641 % 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.
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 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 %.

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

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**

We are very grateful to Prof. Dr. Toru Okuyama for helping us to determine the structure of isolated Psoralen from the aerial parts of *Psoralea acaulis*.

**REFERENCES**