Phenolicarboxylic acids in *Echium vulgare* L.

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**Abstract**

Gas chromatographic (GC) method has been developed for determination of cinnamic acids, p-coumaric and ferulic acids. The method is based on GC separation of their methyl esters. The presence of chlorogenic acids is proved with the help of TLC. The results obtained confirm the path of biosynthesis of phenolic acids in *Echium vulgare* L.

**Key words:** *Echium vulgare* L, phenolicarboxylic acids, gas chromatography, biosynthesis

**Introduction**

*Echium vulgare* L. is popular in Bulgarian folk medicine in form of extracts for cleaning up the blood and healing wounds. Phytochemical research of the plant showed a number of

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biologically active substances: glucofructosanes, glucose, fructose, naphtoquinones, anthocyanines, flavonoids, allantoin, saturated and unsaturated dicarboxylic acids, hydroxydi and hydroxypolycarboxylic acids, octadecatetraenic and phenolcarboxylic acids. It is well known that the latter take significant part in photosynthesis and act as natural stimulators. In most cases phenolic acids are found in the form of esters of alicyclic acids, alkaloids, flavonoids and carbohydrates. They can also be bound to lignins.

Phenolcarboxylic acids belong to the group of natural phenolic compounds. They play an important vital part in increasing the stability of the plants in cases of unfavourable agents. Phenolcarboxylic acids possesses bacteriostatic properties which exhibit anti-virus action and suppress the activity of oxidizing ferments.

In the present study it was aimed to identity and determine the contents of phenolic acids in *Echium vulgare* L.

Different analytical techniques have been proposed for phenolic acids determination as: thin layer chromatography (TLC) (Slacanin et al., 1991), liquid chromatography (LC) (Akasbi et al., 1993; Hertog et al., 1992a; Obreshkova, 1994) and gas chromatography (GC) (Glaessen et al., 1992; Kiehne et al., 1996; Obreshkova et al., 2001) after preliminary derivatization.

Practically GC has a significant experience in analysis of cinnamic acid derivaties, but as far as plants are concerned, there are some difficulties due to their relatively low quantity. In this case the routine column purification would not produce any result.

**Materials and Methods**

Reagents. All solvents and reagents were of analytical grade quality. Plant material: Dried overground part of *Echium vulgare* L., collected at the beginning of July 2000 at the foot of Vitosha mountain. The plant was authenticated at the Bulgarian Academy of Siences - Institute
of Plants Physiology, Herbarium by Lilyana Vasileva and at the Dept. of Botany, Faculty of Biology, University of Sofia “St. Kliment Ohridski” from Assoc. Prof. D. Dimitrov. Voucher specimen was deposited and kept at the Herbarium of the Faculty of Biology (SO-101541).

**Sample preparation and derivatization.**

10 g of air dried plant was dispersed in 50ml water, adjusted to pH 3 with sulphuric acid. After 24 hours the suspension was filtrated and then extracted three times with diethylether. The combined ether extracts were dried with anhydrous sodium sulphate and evaporated under vacuum. Part of the obtained residue was methylated with diazomethane.

Mixed standard of cis-cinnamic acid (x 140 μg/kg); p-coumaric acid (x 100 μg/kg), ferulic acid (x 110 μg/kg) and caffeic acid (x 120 μg/kg) were methylated in the same way.

The standard substances used were from Carl Roth (Germany) GC and LC grade quality.

**GC Procedure**

Column 3% OV-17 on Chromasorb WHP 80-100 mesh;  
Injector  
Detector FID  
Time of analysis about  
Carrier gas - Nitrogen at flow rate  
The sensitivity of the method is 1 mg/kg

The other part of the residue was used for TLC identification of chlorogenic acids.

**Coating substance:** Silicagel F<sub>254</sub>  
**Mobile phase:** Chloroform, Aceton, Formic acid (10:8:2)  
**Detection:** UV 254  
**Evaluation (with standard)**  
R<sub>f</sub> = 0.19
Results and discussion

The gas chromatograms obtained in the analysis of mixed standard and *Echium vulgare* are shown on the Figure 1.

The obtained results are given in the Table 1.

It is known that cinnamic acids are intermediate products in biosynthesis of p-coumaric, caffeic, ferulic and chlorogenic acids. It can be concluded that the presence of the named phenolcarboxylic acids also confirm the path of their syntheses in *Echium vulgare* L.

Table 1. Retention times and percentages of the phenolcarboxylic acids of Mixed Standard and of extract from *Echium vulgare*, L.

<table>
<thead>
<tr>
<th>Phenol-carboxylic acids</th>
<th>Standard</th>
<th></th>
<th>Sample</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention</td>
<td>Areas</td>
<td>%</td>
<td>Retention</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td></td>
<td></td>
<td>time</td>
</tr>
<tr>
<td>Cis-cinnamic acid</td>
<td>2.62</td>
<td>76.1534</td>
<td>31.06</td>
<td>2.74</td>
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<tr>
<td>p-coumaric acid</td>
<td>4.53</td>
<td>1.1767</td>
<td>0.48</td>
<td>4.73</td>
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<tr>
<td>Ferulic acid</td>
<td>6.01</td>
<td>167.6286</td>
<td>68.38</td>
<td>6.32</td>
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<tr>
<td>Caffeic acid</td>
<td>8.54</td>
<td>0.1726</td>
<td>0.07</td>
<td>8.75</td>
</tr>
</tbody>
</table>
Fig. 1  Gas chromatographic profile of methylated phenolic acids
References


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