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Phenolcarboxylic 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, phenolcarboxylic acids, gas romatography, 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 alilcyclic 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 posses 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 at 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 begining 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 dirspersed 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% 0V-17 on Chromasorb WHP 80-100 mesh;	200 °C
Injector	220 °C
Detector FID	240 °C
Time of analysis about	8 min
Carrier gas - Nitrogen at flow rate	20 ml/min

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_{254}
Mobile phase:	Chloroform, Aceton, Formic acid (10:8:2)
Detection:	UV 254
Evalution (with standard)	$R_f = 0.19$

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 synteses in *Echium vulgare* L.

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

Phenol-carbonic acids	**Standard**			**Sample**		
	Retention time	Areas	%	Retention time	Areas	%
Cis-cinnamic acid	2.62	76.1534	31.06	2.74	38.3850	15.66
p-coumaric acid	4.53	1.1767	0.48	4.73	1.0302	0.42
Ferulic acid	6.01	167.6286	68.38	6.32	183.3045	74.77
Caffeic acid	8.54	0.1726	0.07	8.75	0.3342	0.14

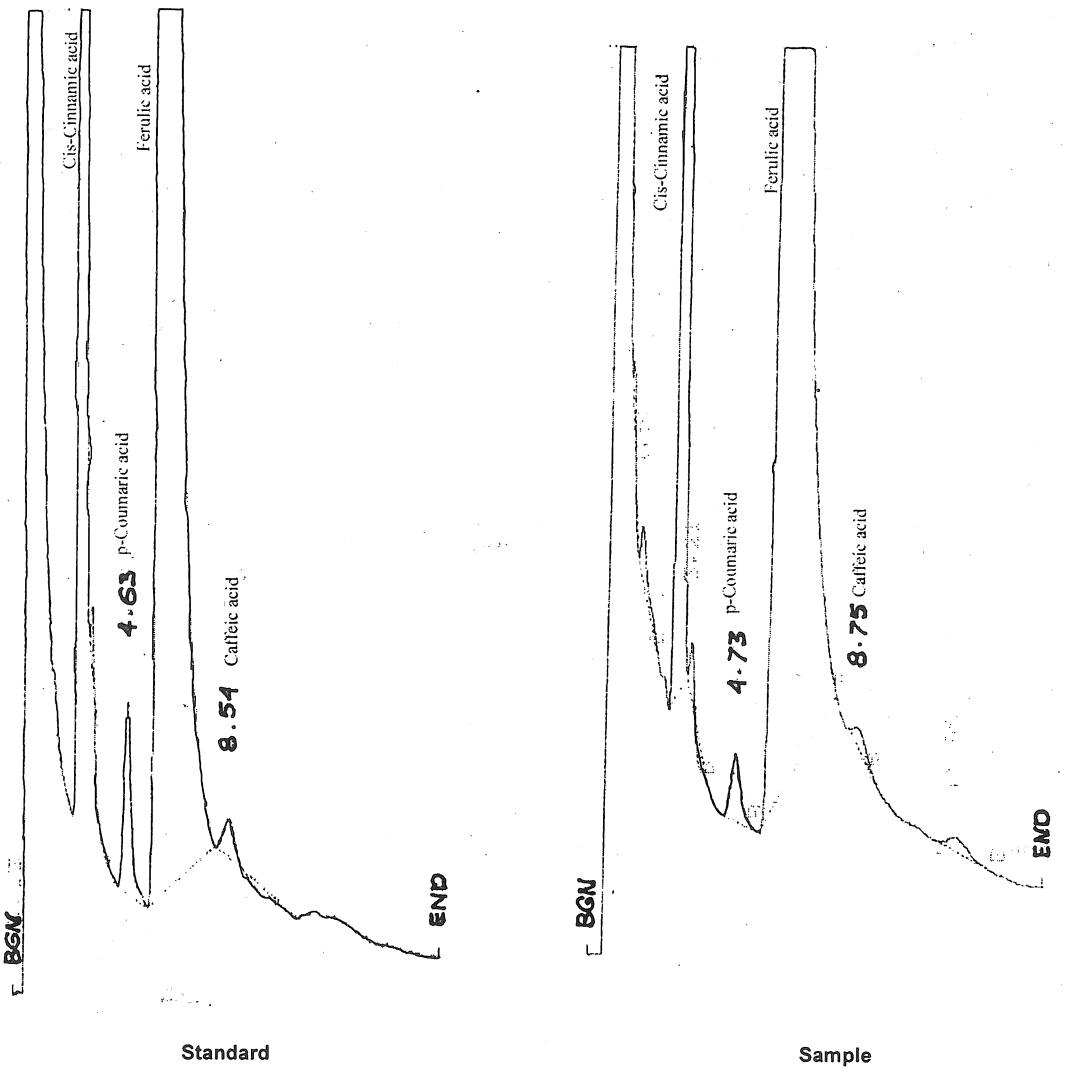

Fig.1 Gas chromatographic profile of methyllated phenolic acids

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