

## STANDARDIZATION OF HYDROPONIC AND WILD GROWING TEUCRIUM POLIUM L. BY HPLC Galstyan H.M<sup>1</sup>, Dumanyan K. H<sup>1</sup>., Tsaturyan A.O<sup>3</sup>., Gukasyan N.H<sup>1</sup>., Engibaryan A.A<sup>2</sup>., Chichoyan N. B<sup>1</sup>

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### Abstract

It was approved that flavonoids (luteoline, apigenin) and phenylpropanoid glycosides verbascoside best separation was implemented by using UM detector and isocratic isolation regime.

Depending on the growing conditions main influencing substance quantities fluctuate: in the wild growing plants were synthesized nearly 2 times more than in hydroponic growing plants. For the study were obtained 50<sup>0</sup> alcoholic extracts from hydroponic and wild growing *T. polium*. The studied samples before analysis were developed three-chloral-acetic-acid anhydride and centrifuged by 10 minutes 12000 rev/m speeds. The solution volume of each injected analysis was 10 mkl. Then some amount of standard verbascoside, luteolin and apigenin samples were solved in 1ml ethyl alcohol and were filled into the special test tubes for analysis. By *T. polium* chloroform-methanol and tower chromatography water fractions teupolozid, verbascoside and poliumozid phenylpropanoid glycosides were separated which Rf were equal to 0.25, 0.5, 0.37 in our researches in the past. These compounds composition was proved by complex spectroscopic methods. They had close structure to each other and verbascoside enclosed peaks in HPLC were supposed that belong to poliumozid and teupolozid and also was brought the above mentioned compounds quantities by calculating chromatography data.

**Keywords:** *Teucrium polium* L., wild and hydroponic, HPLC, standardization, verbascoside, apigenine, luteolin.

### Introduction

Every medicinal plant before becomes official, passes through some stage of the study. The standardization of medicinal plants and perfection of methods of the quality control of herbal medicines is one of the actual tasks of the Pharmacognosy.

*T. polium* L.(Lamiaceae) is a perennial herb, a member of Lamiaceae family. The height of the herb is 20-30cm; the stems are upright, tetrahedral and covered with dense villus. The flowers are white, with bilabiate corolla. The fruit consists of dark brown nuts. The herb has a pleasant distinctive smell with bitter taste.

*T. polium* L. has been used in empirical medicine for treating gynecological diseases, gastrointestinal tract disorders, and it also has analgesic, anti-inflammatory and antibacterial properties [Vardanyan S., 1990].

13 individual compounds have been isolated and identified by us in the overground part of wild *T. polium* L.: flavonoids- apigenin, luteolin, cirsiol, cirsimaritin, flavonoid glycosides 4-O-D-glukopiranozidapigenin, 7-O-rutinosidapigenin, 7-O-

D-glucopiranosidluteolin, 7-O-D-rutinosidluteolin, phenylpropanoid glycosides - verbascoside, poliumoside, teupolioside and two diterpene acids from clerodan type- tepolin A and B. [Galstyan H., Hovhannisyan G. et al., 1991; Galstyan H., 1992].

The study of the biological activity of individual fractions containing different classes of compounds it was found that alcohol extract of *T. polium* has pronounced antitumor, coronary vasodilating, anticonvulsant and antimicrobial activity [Chichoyan A., Galstyan H., et al., 1992].

With the aim of obtaining ecologically clean yield which contains programmed chemical compounds, *T. polium* has been introduced into the culture by us, whereby the suitability of growing the plant in hydroponics conditions has been determined [Galstyan H., 2010].

On the basis of toxicological studies it has been found [Galstyan H., Chavushyan V., 2011] that hydroponics *T. polium* occupies an intermediate position between the soil and wild variations (such correlation is also observed concerning the content of bioactive compounds).

Due to the low toxicity and high content of the biologically active substances (flavonoid and phenylpropanoid glycosides) the aqueous fraction of ethanol extract has been electrophysiologically studied on ovariectomized animals [Chavushyan V., et al., 2012]. I/m injection of T.polium indicates the anticholinesterase activity of T.polium and ability of bioactive compounds to modulate some neurotransmitter systems [Chavushyan V., et al., 2012].

As the study of new medicinal plants is completed with standardization of raw medicinal material, we have carried out microscopical analysis and have determined qualitative and quantitative analysis of main bioactive compounds of T.polium, by which estrogenic and antioxidant activity of T.polium is caused [Galstyan H., et al., 2010].

Fundamental stage of the whole raw material standardization is determination of authenticity with outward and microscopical characteristics [Markaryan A., 2004].

### Outward signs

Raw medicinal material is the overground part which is collected at the end of budding and at the beginning of blossoming. Ground raw material is pieces of leaves, stems and blossom clusters of different forms. The color of the stems and leaves is glaucous, the flowers are whitish, the smell is faint and the taste is bitter.

Each medicinal plant, when enters into life and is officially recognized, passes through some research phases. One of the main processes is plant standardization according to biological active compounds content. Many scientific publications face to the study of phytochemical and biological activity of the genus of Teucrium plant. Teucrium polium L. (family Lamiaceae) is a wild-growing flowering plant, found abundantly in South-Western Asia, Europe and North Africa. During the past 40 years, different classes of compounds have been isolated from various parts of T. polium of which the main groups are terpenoids and flavonoids. It has been found that these compounds possess a broad spectrum of pharmacological effects including anticancer, anti-inflammatory, hypoglycemic, hypolipidemic, antibacterial and antifungal (Bahramikia S1, Yazdanparast R. 2012).

Essential oil of T. Polium of Koohbanan suburb (Kerman, Iran) was extracted by hydro-distillation,

analysed using GC-MS coupled with multivariate curve resolution (MCR). The number of identified components increases from 74 using the direct method of analysis to 106 using the MCR method. It is shown that  $\alpha$ -pinene (8.93  $\mu\text{g/L}$ ), cis- verbenol (3.98  $\mu\text{g/L}$ ) and myrtenal (1.02  $\mu\text{g/L}$ ) are the most abundant components in the T. polium essential oil. (Nikpour H1, Mousavi M2, Asadollahzadeh H1)

According one of the research it was revealed the chemical constituents and antioxidant and cytotoxic activities of Teucrium pruinosum Boiss. essential oil. It was investigated the protective effects of Teucrium polium (T. polium) and vitamin C (Vit C) against carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity and nephrotoxicity in rats.(Rahmouni F1, Badraoui R1,2, Amri N1, Elleuch A3, El-Feki A4, Rebai T1, Saoudi M4).

From this point of view great interest presents Teucrium polium L wildy growing in Armenia and cultivated in hydroponic conditions.

Teucrium polium L. ( Lamiaceae) is classified among the above mentioned medicinal plants which is applied in the traditional medicine and has not still been formally recognized.

Chemical composition of Teucrium polium L. grown in Armenia is slightly different from Teucrium polium L. grown in Mediterranean and Eastern countries [1-3]. It turned out that Armenian T. polium general compounds are phenylpropanoids (teupolozide, verbascoside, poliumozid) and flavonoids (apigenin and luteolin) glycosides which quantities are around 6 and 3%, respectively [3, 4].

Phenylpropanoid glycosides are found in the polar fractions of plant alcoholic extract: chlorophorm-methanol and water.

Previously we confirmed that T. polium general compounds were separated during 50% ethyl alcohol extraction by maximum quantity when extractive substances output was 18-20% [5]: The aim of our research standardization of hydroponic and wild growing Teucrium polium L. by phenylpropanoid glycosides.

### MATERIAL AND METHOD

For the study were obtained 50<sup>0</sup> alcoholic extracts from hydroponic and wild growing T. polium by 1:5 ratio which were dried in a vacuum rotation apparatus until dry weight. Output of extractive substances was 20 $\pm$ 2%.

Then the obtained samples were solved in 1ml ethyl alcohol by the following contents:

1. Hydroponic *T. polium* 50% alcoholic dry extract was 0,417mg
2. Wild *T. polium* 50% alcoholic dry extract was 0,727mg.

The studied samples before analysis were tilled three-chloral-acetic-acid anhydride and centrifuged by 10 minutes 12000 rev/m speeds. The injected solution volume of each analysis was 10 mkl. Then some amount of standard verbascoside, luteolin and apigenin samples (table 1) were solved in 1ml ethyl alcohol and were filled into the special test tubes for analysis. Solutions were filtered by 0,45mkm filter for removing mechanical mixtures after which the obtained standard solutions were put into the chromatography in special section.

Analysis results were reflected on the computer screen in the form of chromatography and the program support allowed automatically integrate the peaks. HPLC was implemented by «Waters 2695 Separations Module» (USA) apparatus which had “Waters 2487” ultraviolet detector. “Nucleosil C<sup>18</sup>” mkm 250x4,6 mm stationary phase was implemented. Isolation was implemented by isocratic regime. As a portable phase was used:

1. 0,1% tri- fluorine-acetic acid (TTA + H<sub>2</sub>O)
2. Acetonitrile (M<sub>e</sub>CN).

Speed of raw material was 0.5ml/m. Detection was implemented under 350nm wave; the tower temperature was 25<sup>0</sup>C. Injection volume was 10ml.D

During the research by the methods HPLC were used phenylpropanoids (teupolozide, verbascoside, poliumozid) and flavonoids (apigenin and luteolin) glycosides standards (Sigma-Aldrich) which purity degree was greater than 99%.

## RESULTS AND DISCUSSIONS

Among the chromatographic analysis was confirmed that the above mentioned verbascoside, luteolin and apigenin best division was fulfilled by using UM detector and isocratic isolation regime.

Verbascoside, luteolin and apigenin standard solution chromatographs were brought in table 1. Identification was done by the help of retention period (fig. 1).

Table 1: Standards amount

	Name	Retention time	Conc. mg/ml
1.	Verbascoside	4.854	0.049
2.	Luteoline	6.142	0.034
3.	Apigenine	7.134	0.055

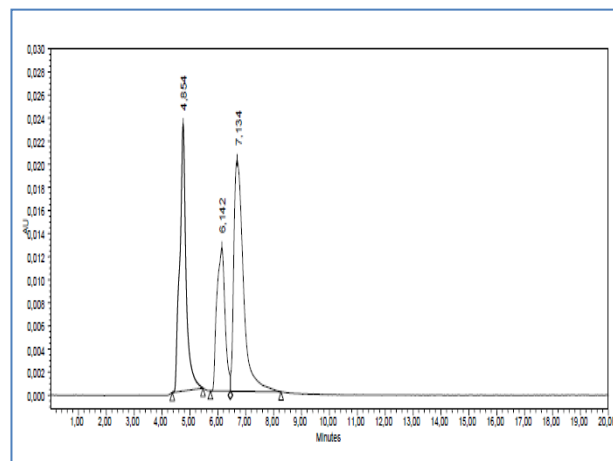


Figure 1: Standard solution mixture chromatography of verbascoside (1), luteolin (2) and apigenin (3)

On the base of obtained chromatographs were confirmed quantitative descriptions of phenylpropanoids and flavonoids glycosides in the 50% alcoholic extract of *T. polium* (table 2).

Table 2: Verbascoside, luteolin and apigenin quantitative description in *T. polium* 50% alcoholic extract

T. polium hydroponics			T. polium wild		
concentration mg/ml			concentration mg/ml		
verbascoside	luteolin	apigenin	verbascoside	luteolin	apigenin
0,0586	-	0,001	0,123	0,0063	0,0061

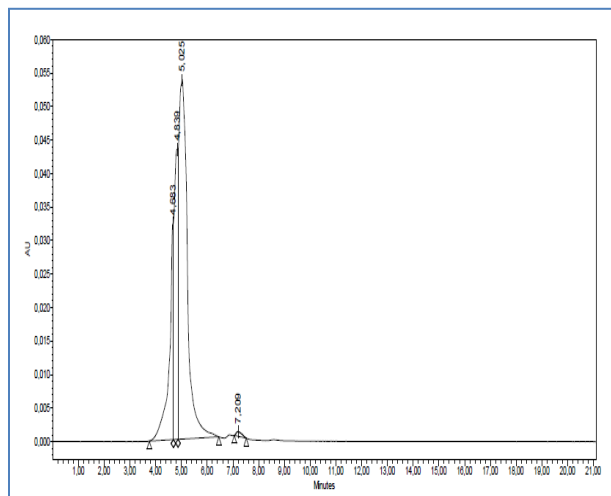
From the table 2 is seen that in hydroponic version; unlike the wild one; luteolin was not synthesized, apigenin was synthesized nearly 6 times lower and verbascoside was synthesized two times lower. In the raw material of hydroponic version verbascoside quantity was 1.17% and in wild version 2.46%.

In figures 2 and 3 were seen enclosed to verbascoside peak, more 2 peaks close to storage intervals, which values were equal to 4,667<sup>1</sup>(4,683<sup>2</sup>) and 5,025<sup>1</sup>(5,028<sup>2</sup>) accordingly.

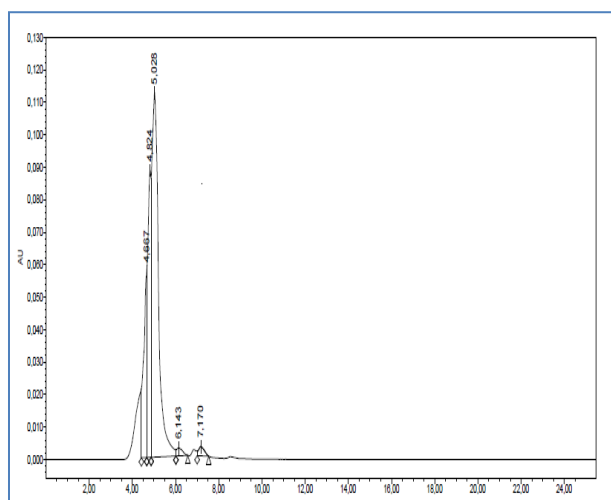
*T. polium* hydroponic and wild plant species 50% alcoholic extract of verbascoside, luteolin and apigenin chromatographs were brought in the figures 2, 3.

<sup>1</sup> Hydroponic *T. polium*

<sup>2</sup> Wild *T. polium*



**Figure 2:** Verbascoside (1), luteoline (2) and apigenin (3) chromatography in hydroponic *T. polium* 50% alcoholic extract



**Figure 3:** Verbascoside (1), luteoline (2) and apigenin (3) chromatography in wild *T. polium* 50% alcoholic extract

By *T. polium* chloroform-methanol (3:1) and column chromatography water fractions teupolizoid, verbascoside and poliumozid phenylpropanoid glycosides were separated which  $R_f$  were equal to 0.25, 0.5, 0.37 (EtoAc-MeOH-water 16:2:1 system) in our researches in the past [5]. These compounds composition was proved by complex spectroscopic methods (IR, UV, mass-spectrometer) [6]. They had close structure to each other (cinnamic acid derivatives) and verbascoside enclosed peaks in HPLC were supposed that belong to poliumozid and teupolizoid and also was brought the above mentioned compounds quantities by calculating chromatography data which were mentioned in table 3.

**Table 3:** Poliumozid and teupolizoid quantitative description of *T. polium* 50% alcoholic extract

T. polium hydroponic		T. polium wild	
concentration mg/ml		concentration mg/ml	
poliumozid	teupolizoid	poliumozid	teupolizoid
0.047	0.169	0.074	0.328

Poliumozid and teupolizoid quantities were 1,17%<sup>1</sup> (1,49<sup>2</sup>) and 4,22%<sup>1</sup> (6,56%<sup>2</sup>), accordingly, in the raw material.

### Conclusion

So, *T. polium* standardization according to verbascoside HPLC method was carried out by us for the first time.

It was approved that flavonoids (luteoline, apigenin) and phenylpropanoid glycosides verbascoside best separation was implemented by using UM detector and isocratic isolation regime.

Depending on the growing conditions main influencing substance quantities were different: in the wild growing plants were synthesized nearly 2 times more than in hydroponic growing plants.

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