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Amino acid composition of *Veronica teucrium* L. herb

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ABSTRACT

By HPLC method amino acid composition of V. teucrium L. herb had been studied, 23 amino acids have been identified and quantified, of which 21 are free and 19 are bound, 10 essential amino acids have been found – threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, tyrosine and arginine. The content of free amino acids is 0.48%, the total content of amino acids after hydrolysis – 2.37%, the content of bound amino acids – 2.03%. In V. teucrium L. herb quantitatively dominated (mg/100g) arginine, serine, glycine, proline, phenylalanine, leucine, threonine and histidine. Among free amino acids asparagine and aspartic acid, alanine, glutamic acid and γ-aminobutyric acid dominated. Among bound amino acids the dominant were serine, arginine, glycine, threonine, proline, leucine, phenylalanine, histidine.

Key words: *Veronica teucrium* L., amino acids, paper chromatography (PC), high performance liquid chromatography (HPLC).

INTRODUCTION

In the flora of Ukraine *Veronica* L. genus (*Plantaginaceae* family) comprises about 60 species and *Veronica teucrium* L. is one of the most common species [1, 2]. Infusions and tincture of *V. teucrium* L. are used in folk medicine of many countries for upper respiratory tract diseases, as an expectorant and anti-inflammatory, antispasmodic and choleretic for liver diseases, and externally for infected wounds as an antibacterial [3, 4, 5].

V. teucrium L. as well as other species of the genus are promising sources of biologically active substances (BAS) [6, 7] what is confirmed by our previous studies. In *V. teucrium* L. herb phenolcarboxylic acids, hydroxycinnamic acids, coumarins, flavonoids, tannic materials, terpenoids, iridoids, saponins, carboxylic acids, including ascorbic acid, macro- and microelements have been found.

In our previous study by chromatographic method (PC) in *V. teucrium* L. «Blaubart» herb 9 amino acids have been identified, namely: lysine, serine, *L*-arginine, glutamic acid, alanine, cysteine, methionine and phenyl-*β*-alanine and 1 amino acid was not identified [8].

Amino acids possess different pharmacological activities and significantly impact the bioavailability of BAS in extracts [9], and an aim of our research was to identify and quantify amino acids of *V. teucrium* L. herb by means of paper chromatography and high performance liquid chromatography.

MATERIALS AND METHODS

The objects of the present study were air-dried samples *V. teucrium* L. herb, harvested at the flowering stage in June-July, 2014 in Kharkiv region.

Chromatographic research (PC) of 70º alcohol extract from *V. teucrium* L. herb was carried out using paper «Filtrac» (FN-4). As reference solutions 0.1% alcohol solutions of authentic substances of *L*-arginine, *β*-alanine, methionine, phenyl-*β*-alanine, glutamic acid, serine, lysine and cysteine were used.

Chromatographic conditions: solvent system *n*-butanol – acetic acid – water (4:1:2), single run, temperature 20 °C, UV-light (λ=354 nm), developer – solution of 2% ninhydrin. Amino acids have been identified by the fluorescence in UV-light and R_f value comparing with those of authentic substances.

Identification and quantification of free and bound amino acids of *V. teucrium* L. herb were carried out using HPLC chromatographer Agilent Technologies (Model 1100) with G1322A vacuum degasser, G1312B high-pressure binary gradient pump, G1313A automatic injector, G1316A column thermostat, G1315B diode-matrix detector.

To study free amino acids (AA) 3 ml of 0.1 N aqueous solution of hydrochloric acid with 0.2 % *β*-mercaptoethanol were added to a vial with accurately weighed amount of herbal drug, vial was sealed and placed in an ultrasonic bath for 2 hours at 50 °C. To study total content (bound and free amino acids) of amino acids (BB): 3 ml of 6 N aqueous solution of hydrochloric acid with 0.4% *β*-mercaptoethanol were added to a vial with accurately weighed amount of herbal drug and kept at 110 °C for 24 hours. The contents of vials were centrifuged and filtered, then 100 µl of AA filtrate and 20 µl of BB filtrate were transferred to vials and kept in a vacuum desiccator at 40-45 °C and under the pressure not more than 1.5 mm Hg until complete removal of hydrochloric acid.

To vials 200 µl of 0.8 M borate buffer pH 9.0, 200 µl of 20 mM solution of 9-fluorene methoxycarbonyl chloride in acetonitrile were successively added via automatic dispenser, after 10 minutes 20 µl of 150 mM solution of amantadine hydrochloride in 50 % aqueous solution of acetonitrile were added. For quantification standard solutions of amino acids were used (TC 6-09-3147-83) [10].

Chromatographic conditions: column 2.1×150 mm; octadecylsilyl sorbent ZORBAX-XDB-C18 (3.5 mm), column thermostat temperature – 50 °C, λ=265 nm, flow rate – 0.25 ml/min, injection volume – 1 µl.

Mobile phases: 0.05 M aqueous solution of sodium acetate pH 6.5 (A), acetonitrile/water mixture (9:1 v/v) (B). Gradient mode: 0 min A 70% and B 15% – 10 min A 27% and B 40% – 15 min A 0% and B 55% – 22 min A 0% and B 100% – 26 min A 0% and B 100% – 26.10 min A 0% and B 15% – 30 min A 70% and B 15%.

Tests have been performed in triplicate. A chromatographic profile of free amino acids and the chromatographic profile of sum of bound and free amino acids (Fig. 1 and 2), the chromatographic profile of standards (Fig. 3) have been recorded. The identification of amino acids was performed by comparison of their retention times and spectral characteristics with those of standards. The calculation of the bound amino acids content was carried out by subtracting the content of free amino acids from their total content.

Results of identification and quantification of bound and free amino acids of *V. teucrium* L. herb are shown in table 1.

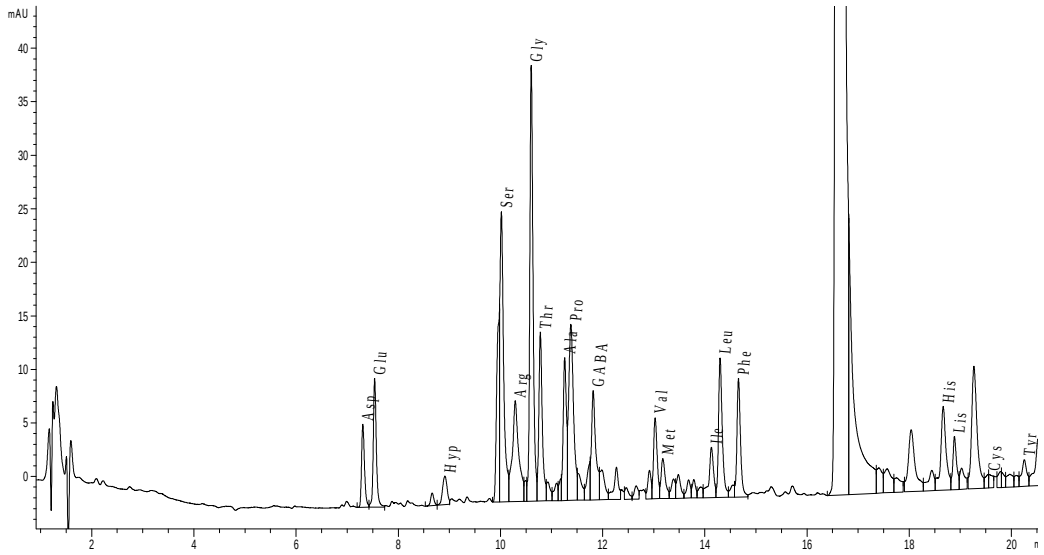

Fig.1. HPLC-chromatogram of sum of bound and free amino acids of *Veronica teucrium* L. herb, λ=265 nm

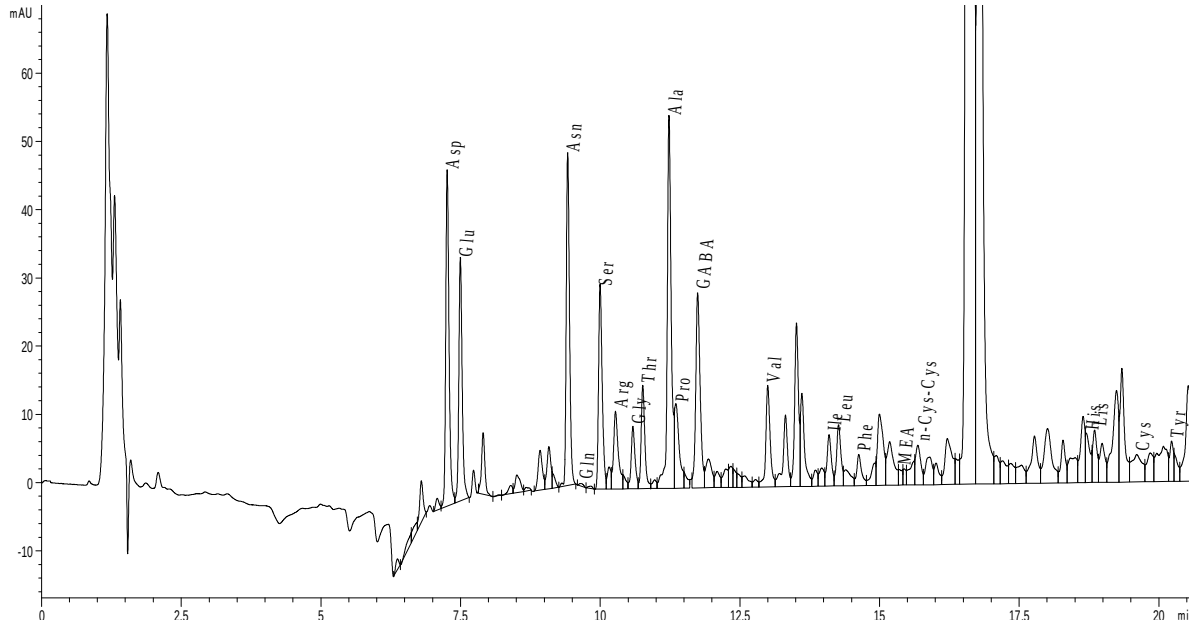

Fig.2. HPLC-chromatogram of free amino acids of *Veronica teucrium* L. herb, λ=265 nm

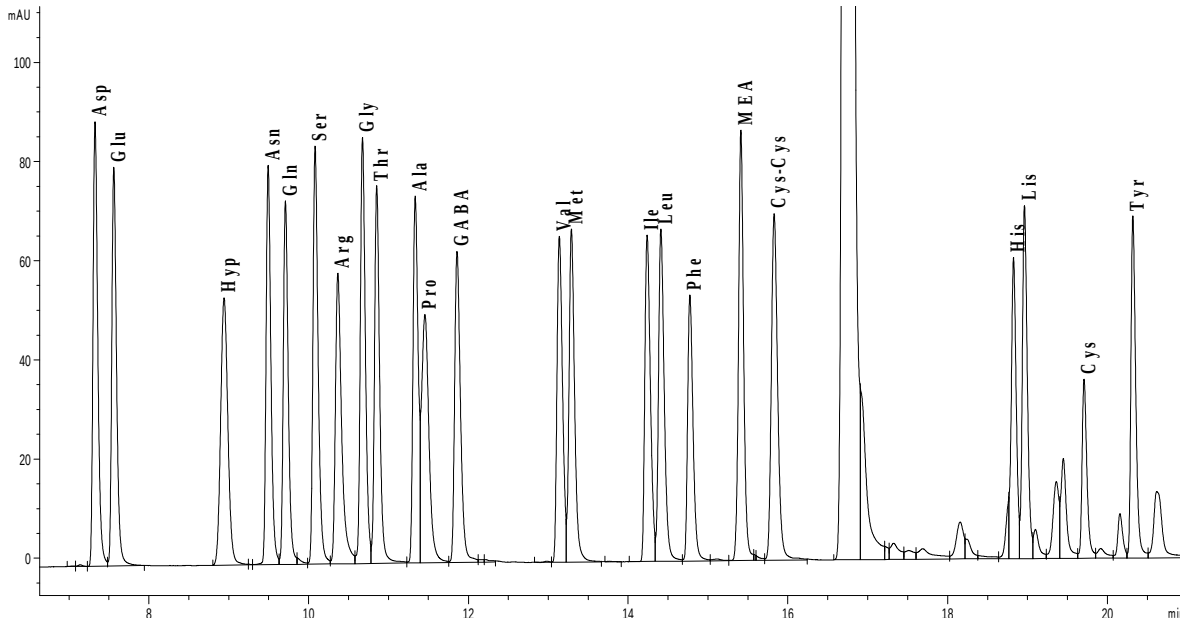

Fig.3. HPLC-chromatogram of amino acids standards, λ = 265 nm

RESULTS AND DISCUSSION

By the chromatographic research (PC) 8 compounds have been determined, 7 of which have been identified. According to R_f values and coloration of spots before and after development in daylight and fluorescence in UV-light in *V. teucrium* L. herb cysteine, lysine, *L*-arginine, serine, methionine, glutamic acid and phenyl-*β*-alanine have been identified.

In the result of HPLC analysis of amino acid composition of *V. teucrium* L. herb 23 amino acids have been quantified, of which 21 are free and 19 are bound. Essential amino acids are threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, tyrosine and arginine (table. 1).

Table 1 Sum of bound and free amino acids of *Veronica teucrium* L. Herb

№	Amino acid	Retention time, min	Free amino acids, mg/100g	Total, mg/100g	Bound amino acids, mg/100g
1	Asp	7.27	54.7	66.2	11.5+60.8
2	Glu	7.51	47.6	124.8	77.2+0.8
3	4-Hyp	8.91	-	37.1	37.1
4	Asn	9.41	60.8	-	-
5	Gln	9.62	0.8	-	-
6	Ser	10.03	30.2	236.1	205.9
7	Arg	10.31	27.9	268.8	240.9
8	Gly	10.63	7.3	231.1	223.8
9	Thr	10.81	18.1	151.0	132.9
10	Ala	11.30	55.8	94.0	38.2
11	Pro	11.42	19.1	224.5	205.4
12	γ-Abu	11.81	37.8	113.5	75.7
13	Val	13.11	23.4	84.8	61.4
14	Met	13.26	-	60.7	60.7
15	Ile	14.19	13.8	79.4	65.6
16	Leu	14.36	15.2	164.4	149.2
17	Phe	14.71	11.3	171.5	160.2
18	Monoethanolamine	15.35	1.6	-	-
19	$(Cys)_2$	15.68	9.4	-	-
20	His	18.78	15.2	169.1	153.9
21	Lys	18.92	7.3	35.9	28.6
22	Cys	19.58	18.5	19.2	0.7+9.4
23	Tyr	20.30	6.8	35.5	28.7
Total:			482.60	2367.60	2028.60

Note: «-» substance not found

The sum of amino acids after hydrolysis is 2.37 ±0.04%, the content of free amino acids is 0.48±0.04%, the content of bound amino acids – 2.03 ±0.02%.

The content of amino acids (mg/100g) in *V. teucrium* L. herb: arginine – 268.8; serine – 236.1; glycine – 231.1; proline – 224.5; phenylalanine – 171.5; leucine – 164.4; threonine – 151.0 and histidine – 169.1; these amino acids are dominant in *V. teucrium* L. herb. Among free amino acids the dominant are (mg/100g): asparagine – 60.8 and aspartic acid – 54.7; alanine – 55.8; glutamic acid – 47.6; γ-aminobutyric acid – 37.8. Among bound amino acids the dominant are (mg/100g): serine – 205.9; arginine – 240.9; glycine – 223.8; threonine – 132.9; proline – 205.4; leucine – 149.2; phenylalanine – 160.2 and histidine – 153.9.

Traces of monoethanolamine have been found: this compound is involved in the synthesis of choline or is a glycine degradation product produced during hydrolysis.

Free amino acids are in interest in terms of the pharmacological properties because these compounds are involved in plant primary metabolism and have the pronounced pharmacological activity.

Sufficiently high content of γ-aminobutyric acid and glycine possessing neuroprotective activity have been identified in *V. teucrium* L. herb, therefore, a development of phytopreparation from *V. teucrium* L. herb with target pharmacological activity can be considered possible.

CONCLUSION

By HPLC method amino acid composition of *V. teucrium* L. herb had been studied; 23 amino acids have been identified, of which 21 are free and 19 are bound, 10 essential amino acids were found.

The content of free amino acids is 0.48%, the total content of amino acids –2.37%, the content of bound amino acids – 2.03%. Arginine, serine, glycine, histidine, phenylalanine, proline, threonine, leucine, asparagine and aspartic acid, alanine, glutamic acid, γ-aminobutyric acids are dominant amino acids in *V. teucrium* L. herb.

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