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Amino acid composition of *Galanthus woronowii* Losinsk. And *Galanthus nivalis* L. homoeopathic mother tinctures

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ABSTRACT

The amino acid composition of the homoeopathic mother tinctures, obtained from medicinal plants – *Galanthus woronowii* Losinsk. And *Galanthus nivalis* L, was studied. The 20 amino acids, including 8 essential amino acids, were found. Aspartate, glutamate, and arginine are presented in greatest amount. The amino acid composition of homoeopathic mother tinctures was identified for the first time.

KEY WORDS: Mother Tincture, *Galanthus woronowii*, *Galanthus nivalis*, Amino Acids.

1. INTRODUCTION

Amino acids are important natural low molecular weight organic compounds containing nitrogen and synthesized by plants. The functional role of amino acids is extremely diverse, they are characterized for a wide range of biological actions, they are the building blocks of proteins, enzymes and vitamins, and they are components involved in the biosynthesis of hormones and alkaloids. Plants synthesize all the amino acids required for their normal metabolism. Importantly, the medicinal herbs are often considered as a source of amino acids in easily digestible form. Amino acids are also involved in pharmacological safety ensuring, facilitate absorption of other biologically active substances (BAS), at the same time, potentiating their pharmacological effects (Meister A., 2012; Bonner, 2012; Berezov, 2004; Kretovich, 1986). Increasing level of quality requirements for crude herbal drugs (CHD) demands a quantitative determination of the main BAS groups. One of these groups is amino acids, which are very important for all living organism. Amino acid quantitative determination may be used in quality control of herbal homoeopathic mother tinctures and be useful for inclusion into pharmacopoeia monographs (Biber, 2005).

Homoeopathic mother tinctures (HMT) decimal dilution (D3-D5) of some snowdrop species are used in homeopathic pharmacy for the treatment of the nervous and cardiovascular system diseases. Voronov's snowdrop (*Galanthus woronowii* Losinsk.) and common snowdrop (*Galanthus nivalis* L.) have been used as sources of homeopathic crude herbal drugs (HCHD) in Russia and other countries (Bokov 2015, 2016). Amino acid composition data of two snowdrop species HMT is absent in the scientific literature. In this regard, it was of interest to study the qualitative and quantitative composition of amino acids contained in the HMTs prepared from *G. woronowii* and *G. nivalis*.

2. MATERIALS AND METHODS

Objects of study were *G. woronowii* and *G. nivalis* HMTs obtained from fresh flowering plants (all parts of the plant were used, *Planta tota*) according to the general pharmacopoeial monograph of State Pharmacopoeia of Russian Federation "Homoeopathic mother tinctures", method 3 a (G. Pharm. monograph "HMT"). The CHDs were harvested in the Botanical Garden of I.M. Sechenov First Moscow State Medical University in March-April 2016 at the flowering period.

For the analysis 500 µl of snowdrop HMT was taken and evaporated to dryness by a centrifugal vacuum evaporator «Savant» (USA). Then 200 µl of 0.1 M hydrochloric acid solution was added to the resulting residue, heating was performed in a water bath at 60 °C for 15 min. The hydrolyzate was cooled to room temperature, stirred and centrifuged for 3 min at 4000 rpm. For further analysis 50 µl of the hydrolyzate obtained after purification was taken.

Determination of amino acid composition and content in the HMT soluble fractions was performed by a high-speed amino acid analyzer «Hitachi» company (Model 835, Japan). Column with an inner diameter of 40 mm, length 150 mm, was used as a stationary phase. It was filled with the cation exchange resin Hitachi Custom ion-Exchange Resin № 2619 brand. Sodium citrate buffer solutions (I - 0, 18 N (pH. = 3, 25), II - 0, 3 N (pH 3, 9), III - 1, 6 N (pH = 4.75)) were used as the mobile phase.

Ninhydrin reagent (a solution of ninhydrin) was prepared on the basis of ethylene glycol monomethyl ether. Flow rate program of citrate buffers was standard (flow rate – 32 ml/hour). Ninhydrin reagent feed rate was 20 ml/hour. After passing through the analytical column separated amino acids are sequentially shifted into the mixing unit, where they were mixed with ninhydrin reagent in a ratio of 2:1. The formation of colored reaction products of amino acids with ninhydrin was performed for 4 minutes at 100°C reaction bath. Colorimetric detection of the colored complexes which are formed during the reaction with ninhydrin was performed simultaneously at two

wavelengths in a continuous mode. Primary amine complexes with ninhydrin were colored in purple (analytical wavelength – 570 nm), and the complexes of secondary amines (proline and hydroxyl proline) had a yellow color (analytical wavelength – 440 nm).

3. RESULTS AND DISCUSSION

Amino acids chromatograms of *G.woronowii* and *G.nivalis* HMT are shown at Figures 1 and 2.

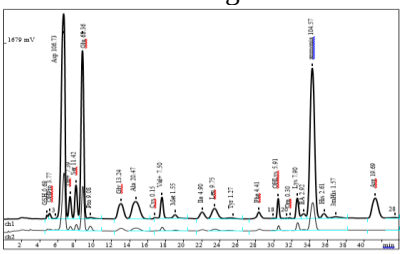

Figure.1. Amino acids chromatogramm of *G.woronowii* HMT.

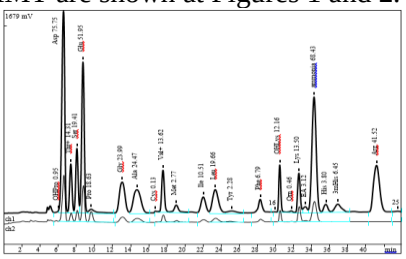

Figure.2. Amino acids chromatogramm of *G.nivalis* HMT.

The content of amino acids of *G. woronowii* and *G. nivalis* HMT is shown at the Table 1 and Figure 3.

Table.1. The amino acid composition and content of *G. woronowii* and *G. nivalis* HMT.

No.	Amino acid	Symbol	Chemical name	Content in HMT, %	
				G. woronowii	*G. nivalis*
Aliphatic monoamine monocarboxylic acids					
1.	Glycine	Gly G	aminoethanoic	0.0050	0.0078
2.	Alanine	Ala A	2-aminopropanoic	0.0091	0.0095
3.	Valine*	Val V	2-amino-3-methylbutanoic	0.0044	0.0069
4.	Isoleucine *	Ile I	2-amino-3-methylpentanoic	0.0032	0.0060
5.	Leucine *	Leu L	2-amino-4-methylpentanoic	0.0064	0.0112
6.	Serin	Ser S	2-amino-3-hydroxypropanoic	0.0060	0.0090
7.	Threonine *	Thr T	2-amino-3-hydroxybutanoic	0.0044	0.0076
Aliphatic monoamine dicarboxylic acids					
8.	Aspartate	Asp D	2-aminobutanedioic	0.0710	0.0442
9.	Glutamate	Glu E	2-aminopentanedioic	0.0503	0.0334
Aliphatic diamine monocarboxylic acids					
10.	Lysine *	Lys K	2,6-diaminohexanoic	0.0058	0.0086
11.	Hydroxylysine*	OH-Lys	2,6-diamino-5-hydroxyhexanoic	0.0048	0.0086
12.	Arginine	Arg R	2-amino-5-guanidinopentanoic	0.0172	0.0314
13.	Ornithine	Orn	2,5-diaminopentanoic	0.0003	0.0003
Aliphatic sulfur-containing acids					
14.	Cysteine	Cys C	2-amino-3-sulfhydrylpropanoic	0.0002	0.0001
15.	Methionine*	Met M	2-amino-4-(methylthio)butanoic	0.0012	0.0018
Aromatic acids					
16.	Phenylalanine *	Phe, F	2-amino-3-phenylpropanoic	0.0036	0.0049
17.	Tyrosine	Tyr Y	2-amino-3-(4-hydroxyphenyl)propanoic	0.0012	0.0018
Heterocyclic acids					
18.	Histidine	His H	2-amino-3-(1H-imidazol-4-yl)propanoic	0.0020	0.0026
19.	Proline	Pro P	pyrrolidine-2-carboxylic	0.0052	0.0100
20.	Hydroxyproline	OH-Pro	4-hydroxypyrrolidine-2-carboxylic	0.0025	0.0005
Amino alcohols					
21.	Ethanolamine	EA	2-aminoethan-1-ol	0.0007	0.0006
Total				0.2043	0.2069

* - essential amino acids

Twenty amino acids were found in *G. woronowii* and *G. nivalis* HMTs, eight amino acids were essential (№3 Val, №4 Ile, №5 Leu, №7 Thr, № 10 Lys, №11 OH-Lys, №15 Met, №16 Phe) and 12 – non-essential (№ 1 Gly, № 2 Ala, № 6 Ser, № 7 Thr, № 8 Asp, № 9 Glu, № 12 Arg, № 13 Orn, № 14 Cys, № 17 Tyr, № 18 His, № 19 Pro, № 20 OH-Pro).

Amino acids can be arranged as follows by the content (descending): in *G. woronowii* HMT – Asp> Glu> Arg> Ala> Leu> Ser> Lys> Pro> Gly> OH-Lys> Thr> Val> Phe> Ile> OH- Pro> His> Met> Tyr> Orn> Cys; in *G. nivalis* HMT – Asp> Glu> Arg> Leu> Pro> Ala> Ser> Lys> OH-Lys> Gly> Thr> Val> Ile> Phe> His> Met> Tyr>

OH-Pro> Orn> Cys. Three nonessential amino acids – aspartate, glutamate, and arginine – are major amino acids in two snowdrop species HMTs.

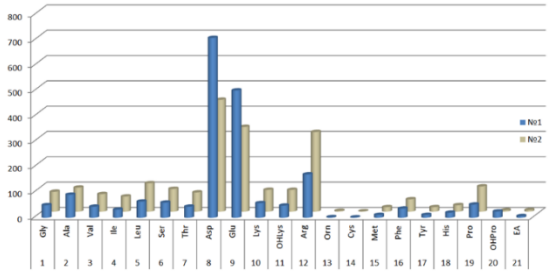

Figure.3. Content of amino acids in *G. woronowii* and *G. nivalis* HMT (%·10^4): *G. woronowii* HMT, *G. nivalis* HMT.

According to Fig.4, the percentage of essential amino acids to the total amount of amino acids in *G. woronowii* HMT (16.53%) and *G. nivalis* HMT (26.86%) is significantly lower than non-essential – 83.47% and 73.14%, respectively. It should be noted that the percentage of essential amino acids in *G. nivalis* HMT is 10.33% more than in *G. woronowii* HMT.

Figure.4. The essential and nonessential amino acids in *G. woronowii* (right) and *G. nivalis* HMT (left). Essential amino acids are presented in the nominated sectors diagrams.

4. CONCLUSION

Amino acid composition of homoeopathic mother tinctures prepared from two snowdrop species – Galanthus *woronowii* and *Galanthus nivalis* – is determined. There different types of amino acids occurred in snowdrop HMT: aliphatic monoamine monocarboxylic (glycine, alanine, valine, isoleucine, leucine, serine, threonine), aliphatic monoamine dicarboxylic (aspartate, glutamate), aliphatic diamine monocarboxylic (lysine, hydroxylysine, arginine, ornithine), aliphatic sulfur-containing (cysteine methionine), aromatic (phenylalanine, tyrosine), heterocyclic (histidine, proline, hydroxyproline). The non-essential amino acids – aspartate, glutamate, and arginine – are major components of HMT.

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