

THE PHARMA INNOVATION - JOURNAL

Amino Acid Composition of the *Alchemilla* L. Genus Plants and *Nepeta cataria* L.

L.M. Grytsyk¹, N.I. Tuchak², T.G. Stasiv³, A.R. Grytsyk⁴

1. Cand. Sc. (Pharmacy). Organization and economy in pharmacy and drug technology department Ivano-Frankivsk National Medical University.
2. Master of Pharmacy, assistant of Pharmacy department Ivano-Frankivsk National Medical University. [E-mail: tuchak20@gmail.com; Tel: +3 (8099) 244-54-95]
3. Assistant of Pharmacy department Ivano-Frankivsk National Medical University.
4. D.Sc. in Pharmacy, professor. Department of Pharmacy Ivano-Frankivsk National Medical University.

The amino acid composition of the *Alchemilla* L. genus plants and *Nepeta cataria* L. was studied. In the grass of *Alchemilla flabelata* Bus., *Alchemilla subcrenata* Bus., *Alchemilla phegophila* Juz. and *Nepeta cataria* L. 17 amino acids were detected, including 8 essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine and lysine. It is found that, in quantitative terms, in the objects of study the aspartic and glutamic acid, glycine, serine and alanine are the dominated ones.

Keyword: Amino Acid Composition, *Alchemilla Flabelata* Bus., *Alchemilla Subcrenata* Bus., *Alchemilla Phegophila* Juz., *Nepeta cataria* L.

1. Introduction

Herbal medicines which contain complex amino acids and peptides are widely used in medical practice, and their number is growing^[1].

Amino acids are the sources of further synthesis of specific tissue proteins, enzymes, peptide hormones and some vitamins^[2].

Most amino acids are synthesized in the human body, but some of them get into the body with food. One of the potential sources of amino acids in the human body are herbs and phytomedications.

Amino acids play an important role in the functioning of various systems and organs of the human body and are characterized by distinct pharmacological properties. Some of them have a positive effect on the cardiovascular system, being involved in the processes of neural

regulation. Besides they maintain vascular tone. In particular, arginine and glutamic acid are characterized by antioxidant, hepatoprotective and membrane stabilizing properties. Alanine and glycine regulate blood sugar level and participate in tissue regeneration. Serine contributes to the accumulation of glycogen in the liver and muscles and influenced the metabolism of fats. Histidine forms a local hormone histamine. Tryptophan takes part in the synthesis of vitamin PP (niacin) being the intermediary of the neurotransmitter serotonin, which has a significant impact on the emotional state of the person. His lack is typical for depression. Lysine enhances nonspecific resistance has an influence on the tone of the arteries and makes blood cholesterol level lower. Methionine residue prevents the deposition of fat in the liver, protects

the cells from exposure of toxic substances and is involved in the synthesis of fosfatydolín. Amino acid "cystine" is a natural antioxidant^[3-5].

The aim was studying the amino acid composition of the *Alchemilla* L. genus species and *Nepeta cataria* L.

The grass of *Alchemilla* (A.) flabelata Bus., *A. subcrenata* Bus., *A. plegophila* Juz. and *Nepeta cataria* L., having been harvested in Ivano-Frankivsk region during the years of 2010 - 2012 are selected as the objects of study.

Analysis of literary sources suggests that the *Alchemilla* L. genus plants of Rosaceae family is a valuable source of biologically active substances. *Alchemilla* L. genus species are used in folk medicine as an anti-inflammatory, antiseptic, sedative, haemostatic, wound healing, expectorants, diuretic and astringent. *Alchemilla* L. genus plants are used in a medical diet for atherosclerosis, diabetes, obesity, and as a prophylactic measure for healthy nutrition^[6-9].

Genus *Nepeta* unites nearly 150 species, widespread in Ukraine. *Nepeta cataria* L., *Nepeta parviflora* M.B. grow in the western region of Ukraine. Analysis of the literature suggests that *Nepeta cataria* L. is a real source of biologically active compounds, such as terpenoids, flavonoids and vitamin C. The main material for *Nepeta cataria* L. is nepetolaktón, being a terpene with a content going up to 40 % in essential oil. It consists of two isoprene units with 10 carbon atoms.

In folk medicine *Nepeta cataria* L. is used against inflammatory diseases of the stomach, difficulty breathing, headache, as a diaphoretic for colds, coughs, to whet, externally, when itching^[9,10].

2. Materials and Methods

For identifying amino acids aqueous extracts of grass species were used: equal volumes (about 2 ml) of studied extract were mixed and 0.1 % freshly prepared solution of ninhydrin, the mixture was gently heated and during cooling the red-blue color, indicating the presence of the studied extracts amino acids was watched^[2,11,12].

This group of compounds, except for reaction identification was revealed by ascending paper chromatography in the solvent system *n*-butanol-

acetic acid-water (4 : 1 : 2). The analytical sample material was cut to the size of the particles that pass through a sieve with apertures of 2 mm, 20.0 g of powdered material was placed in a flask filled with 70 % ethanol (1 : 10) and insisted. Ethanol extract was evaporated to about 10 ml or so and applied to the chromatogram. For the comparison, a standard set of amino acids at a concentration of 0.1 % was used. Chromatograms were sprinkled with 0.1 % solution of ninhydrin in ethanol and heated in an oven for a few minutes at 80 - 100 °C. Amino acids were identified by color stains and determination of R_f in comparison with authentic samples^[2,11,12].

As a result of studying, it was found out about 15 amino acids, which depending on the type of material, but their clear separation was not observed and it is impossible to make accurate conclusions about the content of any of them. Therefore, to clarify the results of chromatographic studies were performed quantitative determination of amino acids.

The analysis was performed in comparison with the concentration of the standard amino acids hydrolysates according to State Standards of Ukraine ISO 13903 : 2005 on the amino acid analyzer AAA-T 339 M (Czech Republic). To determine the amino acid composition of the investigated sample material object 100 mg, dried at 60 °C, it was placed in a test tube for hydrolysis and added to 5 ml of purified water, stirred. It was also added an equal amount of concentrated hydrochloric acid. Hydrolysis was carried out at 120 °C for 15 min. Then the sample was neutralized with dry NaOH to pH = 11. Then it was transferred to a porcelain cup at one hour in order to accelerate evaporation of ammonia. A solution of hydrochloric acid to a pH = 2.2 was added to the sample, which was filtered, selected 0.1 - 0.5 ml liquid volume was adjusted to 2 ml of buffer solution with pH = 2.2^[13].

3. Results

The results of a qualitative study of amino acid composition of grass *A. flabelata* Bus., *A. subcrenata* Bus., *A. plegophila* Juz. and *Nepeta cataria* L. shown in Figure No 1.

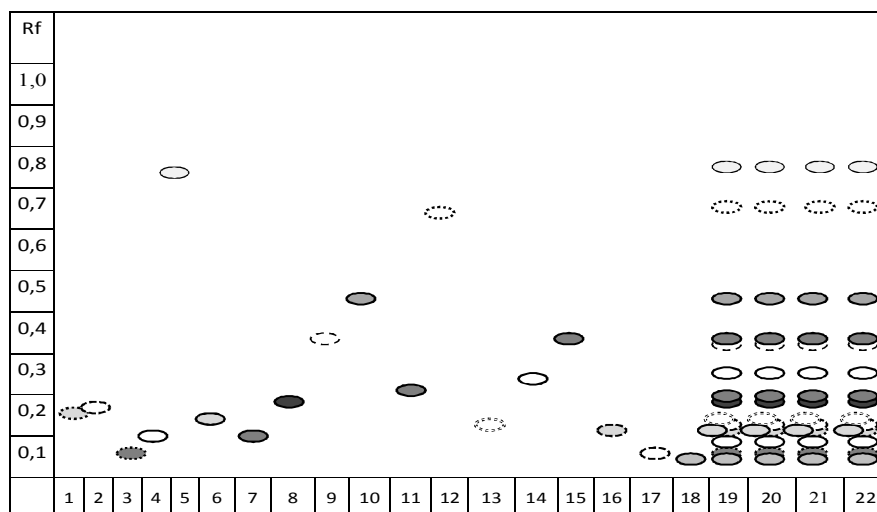


Fig 1: Figure chromatographic analysis of free amino acids in plant material

Note: Solvent system *n*-butanol-acetic acid-water (4:1:2): 1 - aspartic acid, 2 - threonine, 3 - arginine, 4 - histidine, 5 - isoleucine, 6 - glutamic acid, 7 - glutamine, 8 - alanine, 9 - phenylalanine, 10 - valine, 11 - glycine, 12 - leucine, 13 - serine, 14 - proline, 15 - methionine, 16 - asparagine, 17 - ornithine, 18 – lysine, 19 - 21 - water extracts of herbs *A. flabelata* Bus., *A. subcrenata* Bus., *A. phegophila* Juz. respectively, 22 - water extract of herb *Nepeta cataria* L.

As a result, chromatographic studies of the amino acid composition of grass *Alchemilla* L. and *Nepeta cataria* L. present (Figure No 1) it is established the presence of 15 amino acids, 8 of which are essential amino acids: threonine,

valine, methionine, isoleucine, leucine, phenylalanine, histidine and lysine.

The results of the quantitative determination of amino acids in the studied raw materials shown in Table. No 1.

Table 1: The Content of Free Amino acids in the Studied Sites

The study materials	The content of free amino acids, mg/100 g			
	<i>A. flabelata</i> Bus.	<i>A. subcrenata</i> Bus.	<i>A. phegophila</i> Juz.	<i>Nepeta cataria</i> L.
Aminoacid				
<i>Monoaminomonokarbons</i>				
Alanine	9,01	8,05	9,07	16,17
Valine	9,42	5,36	4,70	4,82
Glycine	11,51	16,39	16,80	12,04
Isoleucine	2,75	6,71	4,20	4,30
Leucine	2,25	7,45	8,40	7,22
Methionine	1,00	1,04	1,18	1,20
Serine	6,84	11,32	10,75	6,54
Tyrosine	1,58	0,89	1,01	1,03
Threonine	2,84	6,71	6,38	6,88
Phenylalanine	4,25	4,17	4,70	4,82
Cystine	1,42	1,34	1,51	1,55
<i>Monoaminodykarbons</i>				
Aspartic	14,51	13,41	13,61	15,48
Glutamic	11,93	4,47	5,04	5,16
<i>Dyaminomonokarbons</i>				

Arginine	13,34	2,98	2,52	2,58
Lysine	4,42	3,58	4,03	4,13
<i>Heterocyclics</i>				
Histidine	1,50	0,89	0,84	0,86
Proline	0,83	4,62	5,21	5,33

According to the experimental studies (Table No 1) it is revealed that in the grass of *A. flabelata* Bus., *A. subcrenata* Bus., *A. phegophila* Juz. and *Nepeta cataria* L. aspartic and glutamic acid, glycine, serine and alanine are the dominated ones.

4. Conclusions

1. For the first time in the grass of *A. flabelata* Bus., *A. subcrenata* Bus., *A. phegophila* Juz. and *Nepeta cataria* L. 17 amino acids were detected, including 8 essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine and lysine.
2. In quantitative terms, in the objects of study aspartic and glutamic acid, glycine, serine and alanine are the dominated ones.
3. The results make possible the arguing about the prospects of further phytochemical investigation grass of *A. flabelata* Bus., *A. subcrenata* Bus., *A. phegophila* Juz. and *Nepeta cataria* L.

5. References

1. Черкашина А.В., Ковальов С.В. Амінокислотний і мінеральний склад трави нуту звичайного. Фармаком 2009; № 2: С. 50 – 53.
2. Губський Ю.І. Біологічна хімія: Підручник. – Київ – Тернопіль: Укрмедкнига, 2000; 508 с.
3. Циряпін В.І. Гігієна харчування. – К.: Здоров'я, 1999; 541 с.
4. Злобін Ю.А. Курс фізіології і біохімії рослин. – Суми: Університетська книга, 2004; 463 с.
5. Ісюк М.В., Бензель І.Л., Бензель Л.В. Дослідження амінокислотного складу герані сибірської. Актуальні питання фармацевтичної і медичної науки та практики. 2012; № 3 (10). : С. 4 – 6.
6. Kurtto A., Frohner S., Lampinen R. (eds.). Rosaceae (*Alchemilla* and *Aphanes*). – Helsinki: The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, 2007; 200 p.
7. Перспективи використання рослин роду Приворотень у медицині та фармації: Методичні рекомендації / Укл.: А.Р. Грицик, Л.М. Грицик, Н.І. Тучак. — Львів : ДП МВС України «Львів - Інформ - Ресурси», 2011; 64 с.
8. Грицик Л.М., Тучак Н.І. Пошук нових фітозасобів для профілактики та лікування цукрового діабету. Матеріали за VIII міжнародна научна практична конференція [«Образование и наука на XXI век - 2012»]. София, 2012; Т. 37 : С. 12 – 15.
9. Лікарські рослини: Енциклопедичний довідник / Відп. ред. А.М. Гродзінський. – К.: Голов. ред. УРЕ, 1990; 544 с.
10. Растительные ресурсы СССР: Цветковые растения, их химический состав и использование; Семейства Carifoliaceae – Plantaginaceae. – Л.: Наука, 1990; 328 с.
11. Симонян А.В., Саламатов А.А., Покровская Ю.С., Аванесян А.А. Использование нингидриновой реакции для количественного определения α-аминокислот в различных объектах: Методические рекомендации. Волгоград, 2007; 106 с.
12. Ковальов С.В., Ковальов В.М., Безугла О.М. Амінокислотний та мінеральний склад деяких видів *Phaseolus* L. Вісник фармації. 2011; № 2 (66) : С. 41 – 44.
13. Ионоу И.А., Шаповалов С.О., Руденко Е.В., Долгая М.Н., Ахтырский А.В., Зозуля Ю.А. и др. Критерии и методы контроля метаболизма в организме животных и птиц. Х.: Институт животноводства НААН, 2011; 378 с.