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Amino acids and monosaccharides composition of white dead-nettle (*Lamium album* l.) herb extract

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Abstract
Dead-nettle (*Lamium* L.) is a typical genus of the family *Lamiaceae*, represented more than 25 species in global flora. The aim of this research was to study the amino acids and monosaccharides composition of *Lamium album* L. herb extract. The object of the study was the extract, which have been obtained by 70% ethanol with white dead-nettle herb that was harvested in Kharkov region in summer 2011 at the flowering stage. By HPLC method the qualitative and quantitative analysis of 20 amino acids and 5 monosaccharides in the *Lamium album* L. herb extract was carried out. The observed amino acid (eg, a high content of glutamic and aspartic acid) and monosaccharide composition allows to predict to extract such types of pharmacological activity as sedative, antihypoxic, antitoxic, hepatoprotective and hypoammoniemic.

Keywords: HPLC, White dead-nettle (*Lamium album* L.), amino acids, monosaccharides.

1. Introduction
Dead-nettle (*Lamium* L.) is a typical genus of the family *Lamiaceae*, represented more than 25 species in global flora, there are 7 species in the flora of Ukraine [9].
The most common species in the genus is white dead-nettle (*Lamium album* L.), which has been used in folk medicine in many countries as an expectorant, anti-inflammatory, tonic, antispasmodic, diuretic, hemostatic and sedative remedy. The extracts of L. album L. herb demonstrated cytostatic, antiproliferative and antioxidant activity in the experiment [5, 10, 11].
According to scientific literature the main biologically active substances (BAS) of white dead-nettle are iridoids (about 2%, there are C-10 iridoids of loganin and asperuloside groups and secoiridoids); flavonoids (up to 0.52%, mainly glycosides of quercetin and kaempferol); phenolcarbonic acid (up to 3.4%); essential oil (up to 0.46%) [6, 11].
We have investigated the component composition of essential oil, lipophilic and phenolic compounds of *Lamium album* L. herb in previous studies [1-4]. Continuing the study we obtained the white dead-nettle herb dry extract, for which sedative effect was experimentally established. There was a need to investigate the chemical composition of the extract.
The aim of this research was to study the amino acids and monosaccharides composition of *Lamium album* L. herb extract.
The object of the study was the extract, which have been obtained by 70% ethanol with white dead-nettle herb that was harvested in Kharkov region in summer 2011 at the flowering stage.

2. Materials and Methods
Previous chromatographic study of the amino acids qualitative composition in the extract of white dead-nettle herb was performed by ascending paper chromatography using chromatographic paper "Filtrak № 4" in the solvent system *n*-butanol – acetic acid – water (4:1:2). Chromatograms were treated with 0.2% solution of ninhydrin in acetone and dried in drying oven at temperature of 60-80 °C. Amino acids were identified by comparing the RF values with reliable samples in parallel chromatography. 6 amino acids were founded, namely aspartic and glutamic acid, serine, arginine, phenylalanine, isoleucine (Table. 1).
Qualitative and quantitative analysis of free and bounded amino acids in the extract of white dead-nettle herb were performed using high performance liquid chromatograph Agilent Technologies (model 1100). We placed 1.0 g of extract (exact weight) in a 10 ml vial and added 10 ml of 3% solution of hydrochloric acid, then vials were sealed and kept for 45 minutes in the ultrasonic bath at room temperature. Then vial content was centrifuged and filtered through the Teflon membrane filter with a pore size of 0.45 microns in the vial for

analysis. For chromatography we used column AA 200 × 2.1 mm and protective precolumn; as mobile phase was used solution A (20 mM sodium acetate and 0.018% triethylamine, adjusted to pH 7.2 by 1-2% acetic acid), with the addition of 0.3% tetrahydrofuran, and solution B(40% CH_3CN, 40% MeOH and 20% 100 mM sodium acetate, adjusted to pH 7.2 by 1-2% acetic acid), volumetric flow rate – 0.450 ml/min, the compressibility of the solution A – 50×10^{-6} bar, B – 115×10^{-6} bar, column temperature of 40 °C; detection was performed using a UV detector [7-8].

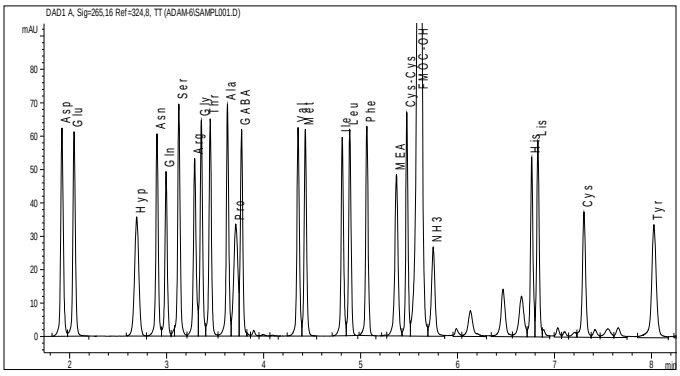

Fig 1: Chromatograms of amino acids standards

To determine the total content of amino acid hydrolyzed extract we placed 0.3 g (exact weight) of extract in a 10 ml vial and added 5 ml of 6 N hydrochloric acid solution. Then vial was sealed and kept for 24 hours at 100 ^{0}C in heat chamber. After cooling vial content was centrifuged, filtered and chromatographed under the conditions specified above. It should take into account that during the acidic hydrolysis asparagine and glutamine almost quantitatively converted to aspartic and glutamic acid, respectively, and cystine partially or completely decomposed into cysteine and cysteine acid [7-8].

Preliminary identification of monosaccharides was performed by ascending paper chromatography in the system *n*-butanol – acetic acid – water (4:1:5) compared to reliable samples of neutral monosaccharides in parallel chromatography. Chromatogram was developed by aniline phthalate solution. In the extract glucose, galactose, rhamnose and arabinose were identified before and after hydrolysis.

Analysis of monosaccharides was performed by HPLC method using chromatograph Agilent Technologies (model 1100), which is completed by a flow vacuum degasser G1379A, 4-channel low pressure gradient pump G13111A, automatic injector G1313A, column thermostat G13116A and refractometric detector G1362A. For the analysis carbohydrate chromatographic column size 7,8 × 300 mm «Supelcogel-C610H» was used. 1.0 g of extract (exact weight) was placed in a 10 ml vial 10 ml of 3% solution of hydrochloric acid was added, then vial was sealed and kept for 45 minutes in the ultrasonic bath at room temperature. Vial content was centrifuged and filtered through the Teflon membrane filter with a pore size of 0.45 microns to vial for analysis. Mode of chromatography: feed rate of the mobile phase – 0.5 ml/min, eluent – 0.1% aqueous solution of phosphoric acid, eluent working pressure – 33-36 kPa, column thermostat temperature – 30 °C, sample volume – 5 ul. Refractometric detection parameters: scale measuring 1.0, scan time of 0.5 s. The identification of mono sugars was carried out according to retention time of standards.

For the analysis of bounded sugars acidic hydrolysis was carried out, for which into the glass vial of 5 ml were placed 400 mg of extract (exact weight) and were added 5 ml of 6 N hydrochloric acid solution, then vial was sealed and kept for 24 hours at 100 ° strove S. After cooling vial contents were centrifuged and filtered through a teflon membrane filter with a pore size of 0.45 microns into the vial for analysis.

3. Results and Discussion

Chromatograms that were obtained under the process of amino acids content definition (before hydrolysis and thereafter), are shown in Fig. 2 and 3, respectively.

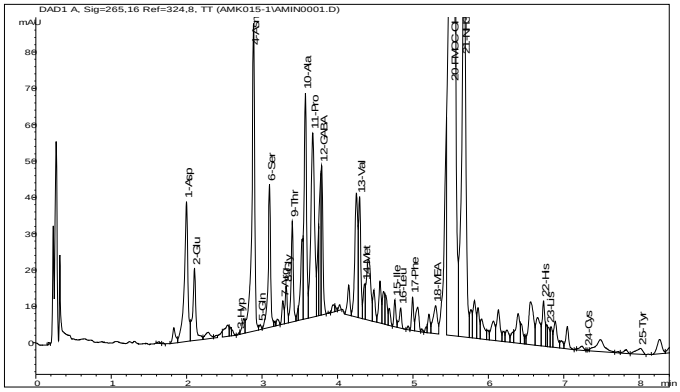

Fig 2: Chromatogram of free amino acids of white dead-nettle herb extract

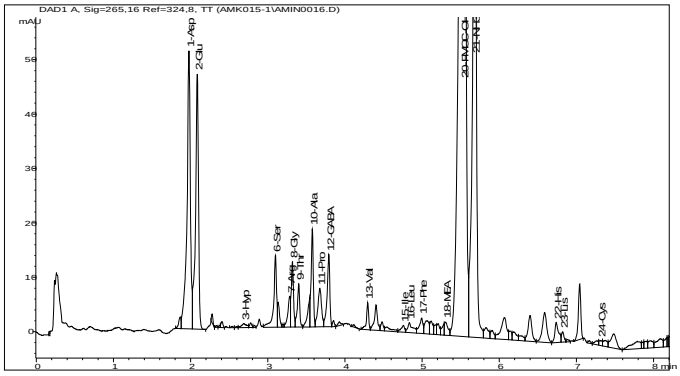

Fig 3: Chromatogram of total amino acid content of dead-nettle herb extract

The results of determination of the amino acid content of the white dead-nettle herb extract before and after hydrolysis are shown in Table. 1.

20 amino acids, including before hydrolysis – 19, after hydrolysis - 18 amino acids were identified and set contents as a result of research of the extract of white dead-nettle. The content of free amino acids was 870.1 mg/100 g (0.87%). Asparagine, aspartic acid and proline dominated among them. After hydrolysis the content of amino acids was 3391.8 mg/100 g (3.39%) glutamic acid (with glutamine) and aspartic acid (with asparagine) were dominated – 1003.7 mg/100 g and 942.0 mg/100 g respectively.

Chromatograms obtained while defining of the monosaccharides content before and after hydrolysis are shown in Fig. 4 and 5 respectively.

Table 1: Amino acid composition of *Lamium album* L. herb dry extract

Amino acid	Retention time, min	Amino acids content (mg/100 g)	
		Free amino acids content	Total amino acids content
Aspartic acid	1.97	89.0	942,0
Glutamic acid	2.15	53.9	1003,7
4-Hydroxyproline	2.68	0.7	23,8
Asparagine	2.82	208.3	-
Glutamine	2.94	2.4	-
Serine	3.08	54.0	175,0
Glycine	3.29	10.1	106,3
Arginine	3.36	12.0	112,2
Threonine	3.40	46.5	121,8
Alanine	3.53	74.5	162,8
Proline	3.67	147.9	171,4
γ-Aminobutyric acid	3.78	47.2	197,6
Valine	4.28	44.4	55,2
Isoleucine	4.78	10.9	28,2
Leucine	4.86	12.5	73,1
Phenylalanine	5.01	18.4	88,6
Monoethanolamine	5.24	10.9	24,4
Histidine	6.72	20.8	73,1
Lysine	6.92	5.7	20,4
Cysteine	7.25	-	12,1
Total		870.1	3391.8

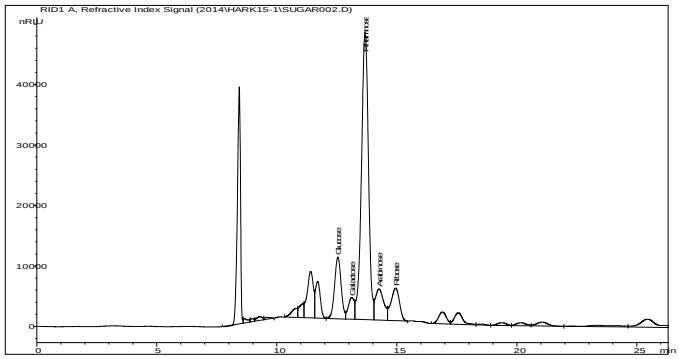

Fig 4: Chromatogram of white dead-nettle herb extract monosaccharides before hydrolysis

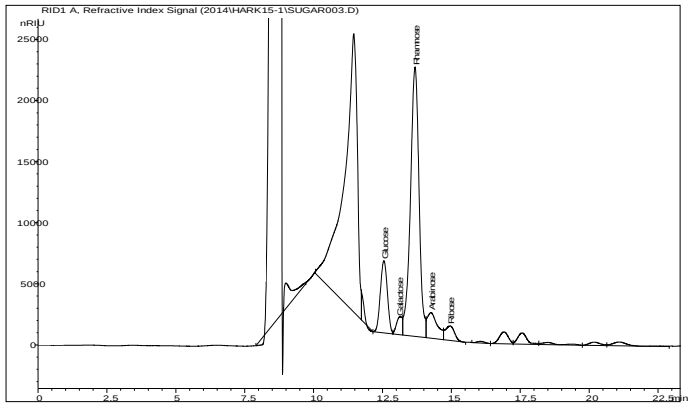

Fig 5: Chromatogram of white dead-nettle herb extract monosaccharides after hydrolysis

The results of qualitative and quantitative analysis of white dead-nettle herb extract monosaccharides are shown in Table. 2.

Table 2: Monosaccharides composition of *Lamium album* L. herb dry extract

Monosaccharide	Retention time, min	Monosaccharides content (g/100 g)	
		Before hydrolysis	After hydrolysis
Glucose	12.54	2.75	3,29
Galactose	13.12	0.71	0,93
Rhamnose	13.68	17.85	19,69
Arabinose	14.26	1.78	1,85
Ribose	14.95	0.93	2,06
Total		24.02	27.82

The study of mono sugar composition of white dead-nettle herb extract identified and established quantitative value of 5 compounds. The total content of free monosaccharides in the extract of the test is 24.02%, and after hydrolysis increased to 27.82%. Rhamnose is the dominant monosaccharide in the extract.

The observed amino acid (e.g., a high content of glutamic and aspartic acid) and mono sugars composition allows to predict to extract such types of pharmacological activity as sedative, antihypoxic, antitoxic, hepatoprotective and hypoammoniemic.

4. References

1. Гончаров АВ. Терпеноиды эфирных масел листьев и цветков *Lamium album* / А. В. Гончаров, А. М. Ковалева, А. Б. Седова, О. Н. Кошевой // Вестник Пермской государственной фармацевтической академии. Материалы научно-практической конференции с международным участием (21 – 23 ноября 2013 г.). Научно-практический журнал 2013; 11:С40-42.
2. Гончаров ОВ. Фенолкарбонові та гідроксикоричні кислоти трави яснотки білої / Гончаров О. В., Ковальова А. М., Ільїна Т. В. // Актуальні питання створення нових лікарських засобів: матер. всеукр. наук.-практ. конф. студ. та мол. вчених (19-20 квітня 2012р.) – Х.: НФаУ 2012; 724с:С. 58.
3. Ковальова АМ. Дослідження компонентного складу ефірної олії квіток *Lamium album* / А.М. Ковальова, Я.С.Колісник, О.В. Гончаров, Т.В. Ільїна // Запорожский медицинский журнал 2012; 3(72):С74-75.
4. Ковальова АМ. Порівняльне хромато-мас-спектрометричне дослідження жирних кислот в листі та квітках *Lamium album* L. / А.М. Ковальова, А.П. Осьмачко, О.В. Гончаров, О.В. Очкур // Збірник наукових праць співробітників НМАПО ім. П.Л. Шупика 2012; 21(4):296-301.
5. Bubueanu C. Antioxidant activity of butanolic extracts of Romanian native species – *Lamium album* and *Lamium purpureum* / C. Bubueanu, C. Gheorghe, L. Pirvu, G. Bubueanu // Romanian Biotechnological Letters 2013; 18(6):8855-8862.
6. Cui Shuya, Chen Xingguo, Hu Zhide. Identification and determination of ecdysone and phenylpropanoid glucoside and flavonoids in *Lamium maculatum* by capillary zone electrophoresis // Biomedical Chromatography 2003; 17(7):477-482.
7. Jámbor A. Quantitation of amino acids in plasma by high performance liquid chromatography: Simultaneous deproteinization and derivatization with 9-fluorenylmethyloxycarbonyl chloride / A. Jámbor, I. Molnár-Perl // Journal of Chromatography A 2009;

1216:6218-6223.
8. Jámbor A. Amino acid analysis by high-performance liquid chromatography after derivatization with 9-fluorenylmethyloxycarbonyl chloride. Literature overview and further study / A. Jámbor, I. Molnár-Perl // Journal of Chromatography A 2009; 1216:3064-3077.
9. *Lamium* // The Plant List Version 1.1, 2013.
10. Paduch R. *Lamium album* extracts express free radical scavenging and cytotoxic activities / R. Paduch, G. Matysik, M. Wójciak–Kosior // Polish J of Environ. Stud 2008; 17(4):569-580.
11. Yalçin FN. Ethnobotany, Pharmacology and Phytochemistry of the Genus Lamium (Lamiaceae) / F. N. Yalçin, D. Kaya // Fabad J Pharm Sci 2006; 31:43-52.