RESEARCH OF THE PRIMARY METABOLITES OF *ASTRAGALUS DASYANTHUS* HERB

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Introduction

Modern conditions, unfortunately, have led to a whole series of diseases associated with hypodynamia, the percentage of stress, bad habits and low-quality products.

The increase in the percentage of hypertensive diseases, which later leads to arterial hypertension (AH), is a reality of the 21st centur [1]. Over the past 30 years, the number of patients with hypertension on the planet has doubled to 1.28 billion. In Ukraine, more than 30% of the population - more than 12 million inhabitants - suffer from this pathology [1].

One of the directions of complex therapy of hypertension is the application of medicinal plant raw materials and preparations based on them. One of these plants is *Astragalus dasyanthus* Pall. *Fabaceae* legume family [2].

It is a perennial herbaceous plant. The raw material is grass. The chemical composition of raw materials is quite diverse: polysaccharides arabin and basorin, mucus, organic acids, triterpene saponins (cycloartane derivatives) [3,4], flavonoids (kaempferol, quercetin, isorhamnetin and their glycosides) [5,6], mineral compounds (compounds of iron, calcium, aluminum, phosphorus, magnesium, sodium, barium, silicon, strontium, molybdenum, vanadium and manganese, selenium) [3].

In the form of herbal infusions, this plant is used as a hypotensive, sedative, vasodilator, diuretic, antiedematous, hemostatic, antiemetic agent for hypertension, hypertension, angina pectoris, acute and chronic glomerulonephritis, rheumatism [3,7].

The plant is included in the Red Book of Ukraine, so it is grown for pharmaceutical purposes. Plants in the second year of life are the most productive. Propagated only by seeds. The expansion of the area of the introduced plant is inhibited due to the low productivity of the seeds, their low quality, and the difficulty of their collection [2]. In addition, the plant is affected by pests and diseases, for example, powdery mildew. At the Research Station of Medicinal Plants of the Institute of Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine, the variety *A. dasyanthus* "Favorite" was bred with a yield of up to 37 t/ha of air-dry raw material (content of triterpene glycosides up to 2.4%) [8].

The variety is undergoing State variety testing. The variety is suitable for mechanized harvesting. The regions with effective cultivation are Kharkiv, Luhansk, Donetsk and Dniprovsk. Today, the plant is non-pharmacopoeia in our country.

The aim of the work is to study the component composition of the primary metabolites of the herb *A. dasyanthus* grown in Ukraine.

Materials and methods

Grass was harvested from biennial cultivated plants in the phase of mass flowering in 2021 in the Kharkiv region. The determination of free monosaccharides in plant material was carried out by the GC/MS method [9,10]. Chromatographic separation was performed on an Agilent 6890N/5973inert gas chromatography-mass spectrometric system (Agilent technologies, USA). HP-5ms capillary column (30m×0.25mm×0.25mkm, Agilent technologies, USA). The evaporator temperature is 250 °C, the interface temperature is 280 °C. The separation was carried out in the temperature programming mode - the initial temperature of 160 °C was maintained for 8 min, then raised with a gradient of 5 °C/min to 240 °C. The final temperature was maintained for 6 minutes. A sample with a volume of 1 μl was injected in the mode of flow division 1:50. Detection was carried out in SCAN mode in the range (38-400 m/z). Plant raw materials were ground to a powdery state in a glass mortar. A weight of 500 mg of the drug was placed in a round-bottomed flask, a solution of 80% ethyl alcohol with an internal standard was added at the rate of 500 μg per sample. Extraction of free monosaccharides was carried out in a water bath at 100 °C using an inverted refrigerator for 2 hours. To obtain aldonitrile derivatives of monosaccharides, 2 ml of the extract was taken, evaporated to dryness on a rotary evaporator and 0.3 ml of derivatizing reagent (32 mg/ml of hydroxylamine hydrochloride in a mixture of pyridine/methanol (4:1 v/v)) was added. The dissolved extract was kept for 25 min. at 75 °C. For acetylation of aldonitrile derivatives of monosaccharides, 1 ml of acetic anhydride was added and kept for 15 min at 75 °C. 2 ml of dichloroethane was added to the reaction mixture, the excess of derivatization reagents was removed by double extraction with a 1N solution of hydrochloric acid and water. The dichloroethane layer was dried to dryness and dissolved in 300 μl of a mixture of heptane/ethyl acetate (1:1 v/v).Identification of monosaccharides of the studied mixture was carried out by comparing the retention times of standard monosaccharides and using the NIST 02 mass spectrum library. Quantitative analysis was carried out by adding a solution of the internal standard to the studied samples. Sorbitol solution was used as an internal standard. The mass of monosaccharide per 1 kg of raw material in mg was calculated according to the formula:

$$X=(Sx \times M \times 1000)/(C \times m),$$

where:
Sx is the peak area of the desired monosaccharide
M is the mass of the internal standard per sample
C is the peak area of the internal standard
m is the concentration of the drug.

Chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent technologies, USA). Zorbax AAA column 150 mm long, 4.6 mm internal diameter, 3 μm sorbent grain diameter. Mobile phase A - 40 mM Na2HPO4 pH 7.8; B - ACN:MeOH: water (45:45:10, v/v/v). The separation mode is gradient with a constant flow rate of 1.5 ml/min. The temperature of the column thermostat is 40 °C. Pre-column derivatization was performed in automatic programmed mode using FMOC

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reagent (Agilent 5061-3337) and OPA reagent (Agilent 5061-3335). Detection of derivatized amino acids was implemented using a fluorescent detector.

Common amino acids. A portion of the drug was placed in a vial, 2 ml of an aqueous solution of 6N hydrochloric acid was added and placed in a thermostat at 110 °C. Hydrolysis was carried out for 24 hours. 0.5 ml of the fumigated extract/hydrolyzate is evaporated on a rotary evaporator, washing three times with distilled water to remove hydrochloric acid. Resuspend in 0.5 ml of distilled water and filter through membrane filters made of regenerated cellulose with pores of 0.2 μm.

Obtaining fluorescent derivatives was carried out in automatic programmed mode before introducing the sample into the chromatographic column.

Identification of amino acids was performed by comparing retention times with a mixture of amino acid standards (Agilent 5061-3334). The content of bound amino acids was determined by subtracting the content of free amino acids from their total content [11-13].

Results and discussion

The results of the study of the carbohydrate composition of the herb *A. dasyanthus* are shown in Tables 1 and 2, and the chromatograms are shown in Figs. 1 and 2.

Free sugars are represented by *D*-mannose, *D*-glucose and sucrose. The content of sucrose (3.39±0.01 mg/g) is almost 10 times higher than the content of *D*-mannose (0.37±0.01 mg/g), *D*-glucose (1.19±0.02mg/g) - almost three times.

In addition, the polyols (+)-pinitol and myo-inositol were invented.

Tab. 1. Component composition of free carbohydrates of the herb *A. dasyanthus* by GC/MS method (n=5, mg/g)

Peak number	Retention time, s	Peak area	Library/ID	Sugar	Quantitative content, mg/g
1	12,1201	1,9338	2,3,4,5,6-penta-O-acetyl-D-mannonitrile	D-Man	0,37±0,01
2	12,3879	6,394	2,3,4,5,6-penta-O-acetyl-D-gluconitrile	D-Glu	1,19±0,02
3	13,1146	0,8236	Glucose propyl glucoside tetraacetate		0,16±0,01
4	14,1346	36,6456	(+)-Pinitol pentaacetate		6,86±0,01
5	15,5243	1,4331	Mio-inositol, hexaacetate		0,26±0,01
6	16,0088	30,0903	D-Sorbitol, hexaacetate	D-Sorbitol	внутрішній стандарт
7	16,3403	1,3095	1-Naphthalen-1-yl-ethylideneamine		0,25±0,01
8	18,7416	1,5172	2-Acetoamido-2-deoxy-d-gluconic acid		0,31±0,01
9	18,9923	1,7358	Glucose benzyloxime pentaacetate		0,32±0,01
10	32,7154	18,1171	Sucrose, oxatoacetate	Sucrose	3,39±0,01

Tab. 2. Component composition of total carbohydrates of *A. dasyanthus* herb by GC/MS method (n=5, mg/g)

Peak number	Retention time, s	Peak area	Library/ID	Sugar	Quantitative content, mg/g
1	5,218	1,9723	D-Rhamnonitrile, 2,3,4,5-tetraacetate	D-Rha	1,66±0,01
2	5,5665	2,4291	D-arabinonitrile, 2,3,4,5-tetraacetate	D-Ara	2,05±0,02
3	5,9023	1,7766	D-Xylonitrile, 2,3,4,5-tetraacetate	D-Xyl	1,50±0,01
4	12,0732	2,2324	2,3,4,5,6-penta-O-acetyl-D-mannonitrile	D-Man	1,88±0,01
5	12,4217	23,6717	2,3,4,5,6-penta-O-acetyl-gluconitrile	D-Glu	20,09±0,02
6	12,97	6,2764	2,3,4,5,6-penta-O-acetyl-galactonitrile	D-Gal	5,31±0,01
7	14,1472	30,3563	(+)-Pinitol, pentaacetate		25,73±0,01
8	15,5327	1,2316	Mio-inositol, hexaacetate		1,04±0,01
9	16,0214	27,6801	D-Sorbitol, hexaacetate	D- Sorbitol	internal standard
10	21,0322	2,3735	Sophorose peracetate		2,02±0,02

The bound carbohydrates of *A. dasyanthus* herb are represented by 6 monosaccharides, 2 polyols and 1 disaccharide. Significant amounts were found for *D*-glucose (20.09±0.02 mg/g) and (+)-pinitol (25.73±0.01 mg/g). Sophorose (β-*D*-glucopyranosyl-(1,2)-α-*D*-glucopyranose) was found in the bound state - a rather unusual disaccharide contained in representatives of the stevia genus *Stevia Cav.* and patented in the USA as a

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hypoglycemic and antidiabetic agent [14].

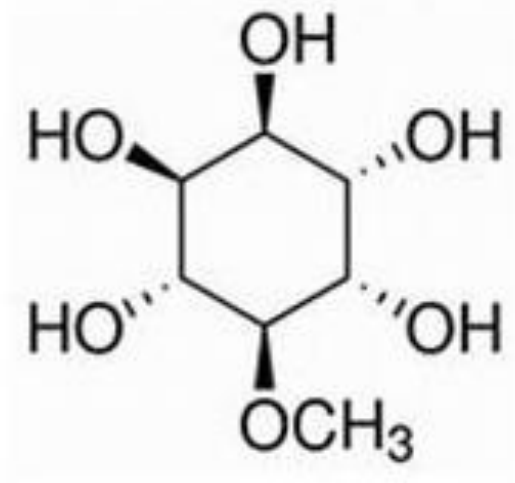

Fig. 1. (+)-Pinitol, (1S,2S,4S,5R)-methoxycyclohexane-1,2,3,4,5-pentol

In addition, pinitol has a preventive effect on cataracts, improves eyesight and the condition of the cardiovascular system, and has an anti-carcinogenic effect. Thus, considering the aspects of action and content of this compound, the herb *A. dasyanthus* may be a source of (+)-pinitol. The content of myo-inositol in the free state is 0.26±0.01 mg/g, in the bound state 1.04±0.01 mg/g. Myo-inositol (1,2,3,5-cis-4,6-cyclohexanhexol) (formula - Fig. 2), is one of the 9 stereoisomers of the 6-atom alcohol inositol, the so-called vitamin B_8, an insulin-sensitizing compound, increases fertility, that is, the functional state of the reproductive system (normalizes the hormonal background, increases potency, promotes healthy ovaries, normalizes menstruation), improves the state of the nervous system and brain, has a positive effect on lipid metabolism, is a component of cell membranes. It is a dietary supplement, produced by a number of acid companies.

In vegetable raw materials (legumes, wheat sprouts, rice bran, seeds, nuts) it is found in the form of physiologically low-active hexaphosphate, the so-called phytic acid.

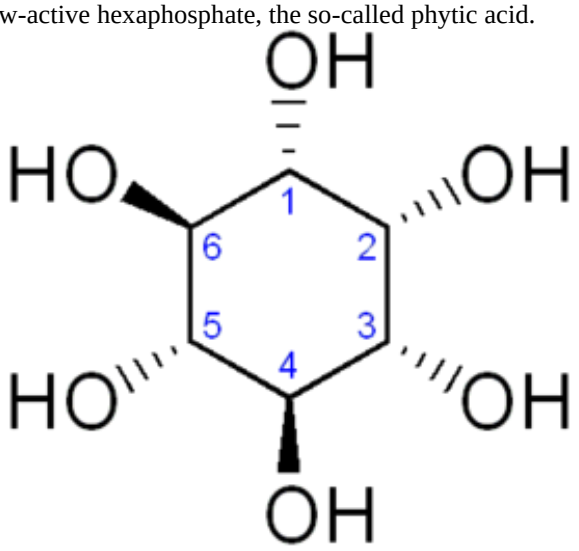

Fig. 2. Myo-inositol (1,2,3,5-cis-4,6-cyclohexanehexol)

The results of the study of the component composition of amino acids of the herb *A. dasyanthus* are shown in Fig. 3-4 and in table. 3.

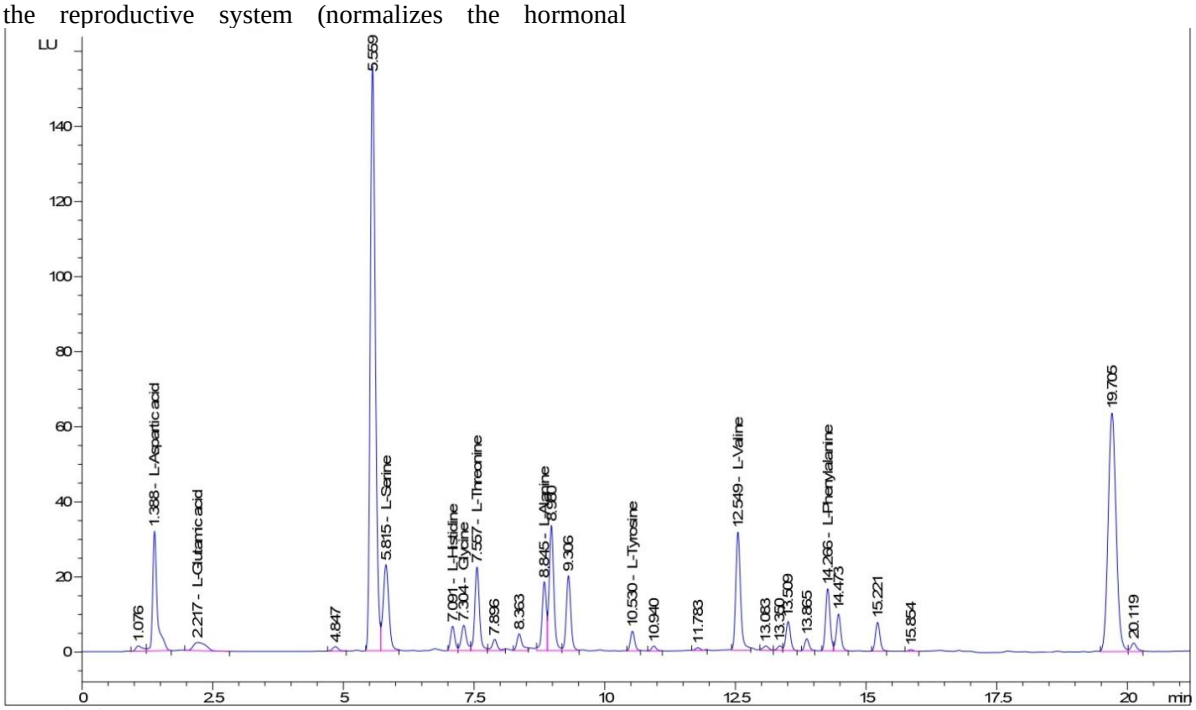

Fig. 3. GC chromatogram of free amino acids of A. dasyanthus grass

Free and bound amino acids are represented by 12 identical compounds. The content of *L*-aspartic acid, *L*-serine, *L*-histidine, *L*-threonine, *L*-valine, *L*-isoleucine and *L*-proline is higher in the free state, the rest in the bound state.

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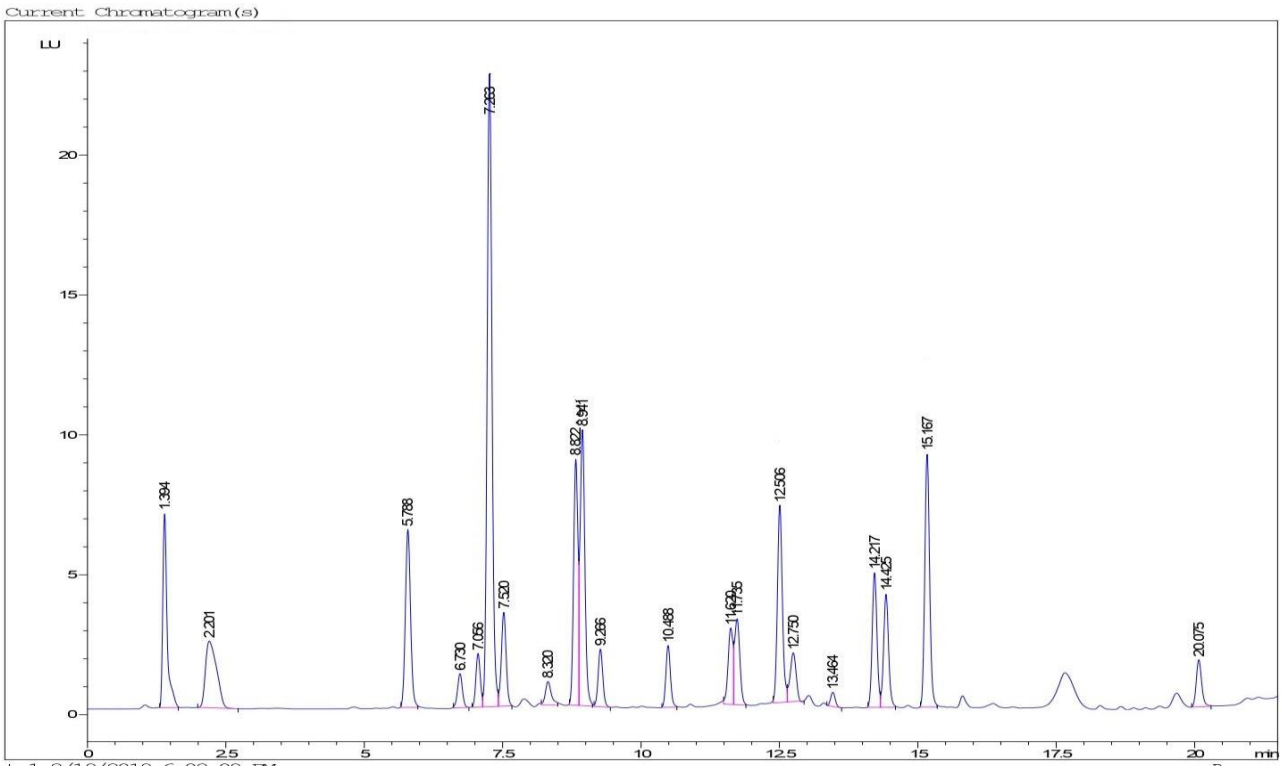

Fig. 4. GC chromatogram of bound amino acids of *A. dasyanthus* grass

Table 3. Amino acid composition of free and bound amino acids of *A. dasyanthus* herb (n=5, µg/mg)

Retention time, s	Amino acid name	Quantitative content, µg /mg		
		total	in the free state	in the bound state
1,394	*L*-Aspartic acid	3,193 ± 0,007	2,530 ± 0,005	0,663 ± 0,006
2,202	*L*-Glutamic acid	0,350 ± 0,004	0,072 ± 0,004	0,277 ± 0,003
5,788	*L*-Serine	2,233 ± 0,004	1,440 ± 0,003	0,793 ± 0,005
7,056	*L*-Histidine	2,090 ± 0,003	1,142 ± 0,003	0,948 ± 0,003
7,263	Glycine	5,080 ± 0,004	0,272 ± 0,002	4,808 ± 0,003
7,52	*L*-Threonine	1,250 ± 0,004	1,241 ± 0,004	0,009 ± 0,004
8,822	*L*-Arginine	0,000	0,000	0,000
8,941	*L*-Alanine	2,494 ± 0,006	0,678 ± 0,004	1,816 ± 0,002
10,488	*L*-Tyrosine	1,170 ± 0,004	0,007 ± 0,002	1,162 ± 0,004
12,506	*L*-Valine	2,151 ± 0,002	1,536 ± 0,002	0,615 ± 0,003
12,75	*L*-Methionine	0,000	0,000	0,000
14,217	*L*-Phenylalanine	0,000	0,000	0,000
14,425	*L*-Isoleucine	1,751 ± 0,004	1,039 ± 0,004	0,713 ± 0,004
15,167	*L*-Leucine	0,000	0,000	0,000
15,751	*L*-Lysine	0,000	0,000	0,000
20,075	*L*-Proline	1,498 ± 0,004	1,368 ± 0,004	0,130 ± 0,004

The highest content in the free state was established for *L*-aspartic acid (2.530 ± 0.005 µg/mg), in the bound state – glycine (4.808 ± 0.003 µg/mg). The lowest content in the free state was determined for *L*-glutamic acid (0.072 ± 0.004 µg/mg), in the bound state - *L*-threonine (0.009 ± 0.004 µg/mg). A significant content of glycine, even in a bound state, can cause a pronounced sedative effect.

Conclusions

1. The component composition of primary metabolites: free and bound carbohydrates, free and bound amino acids of the herb *A. dasyanthus* grown in Ukraine was studied.
2. It was established that in the free state it contains 2 monosaccharides, 1 disaccharide and 2 polyols, in the bound state - 2 monosaccharides, 1 disaccharide and 2 polyols. The high content of polyol: (+)-pinitol correlates with the use of this raw material as an antioxidant and metabolism normalizer.

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3. The amino acid composition is represented by 12 compounds both in free and bound state. *L*-Aspartic acid dominated in the free state, glycine in the bound state.
4. The obtained research results are a fragment of the systemic pharmacognostic study of the herb *A. dasyanthus*, grown in Ukraine, and testify to the appropriateness of creating preparations based on it.

Research of the primary metabolites of *Astragalus dasyanthus* herb

Olha Khvorost, Yehenia Zudova

Introduction. One of the directions of complex tepary of hypertension is the application of medicinal plant raw materials and preparations based on them. One of these plants is *Astragalus dasyanthus* Pall. *Fabaceae* legume family. It is a perennial herbaceous plant. The raw material is grass. The chemical composition of raw materials is quite diverse: polysaccharides arabin and basorin, mucus, organic acids, triterpene saponins (cycloartane derivatives), flavonoids (kaempferol, quercetin, isorhamnetin and their glycosides), mineral compounds (compounds of iron, calcium, aluminum, phosphorus, magnesium, sodium, barium, silicon, strontium, molybdenum, vanadium and manganese, selenium). **The aim** of the work is to study the component composition of the primary metabolites of the herb *A. dasyanthus* grown in Ukraine. **Materials and methods.** Grass was harvested from biennial cultivated plants in the phase of mass flowering in 2021 in the Kharkiv region.The determination of free monosaccharides in plant material was carried out by the GC/MS method. Chromatographic separation was performed on an Agilent 6890N/5973inert gas chromatography-mass spectrometric system (Agilent technologies, USA). HP-5ms capillary column (30m×0.25mm×0.25mkm, Agilent technologies, USA). The evaporator temperature is 250 °C, the interface temperature is 280 °C. The separation was carried out in the temperature programming mode - the initial temperature of 160 °C was maintained for 8 min, then raised with a gradient of 5 °C/min to 240 °C. The final temperature was maintained for 6 minutes. A sample with a volume of 1 μl was injected in the mode of flow division 1:50. Detection was carried out in SCAN mode in the range (38-400 m/z). Identification of monosaccharides of the studied mixture was carried out by comparing the retention times of standard monosaccharides and using the NIST 02 mass spectrum library. Quantitative analysis was carried out by adding a solution of the internal standard to the studied samples. Sorbitol solution was used as an internal standard. Chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent technologies, USA). Zorbax AAA column 150 mm long, 4.6 mm internal diameter, 3 μm sorbent grain diameter. Mobile phase A - 40 mM Na2HPO4 pH 7.8; B - ACN:MeOH: water (45:45:10, v/v/v). **Results and discussion.** Free sugars are represented by *D*-mannose, *D*-glucose and sucrose. The content of sucrose (3.39±0.01 mg/g) is almost 10 times higher than the content of *D*-mannose (0.37±0.01 mg/g), *D*-glucose (1.19±0.02mg/g) - almost three times. In addition, the polyols (+)-pinitol and myo-inositol were invented. The bound carbohydrates of *A. dasyanthus* herb are represented by 6 monosaccharides, 2 polyols and 1 disaccharide. Significant amounts were found for *D*-glucose (20.09±0.02 mg/g) and (+)-pinitol (25.73±0.01 mg/g). Sophorose (β-*D*-glucopyranosyl-(1,2)-α-*D*-glucopyranose). Free and bound amino acids are represented by 12 identical compounds. The content of *L*-aspartic acid, *L*-serine, *L*-histidine, *L*-threonine, *L*-valine, *L*-isoleucine and *L*-proline is higher in the free state, the rest in the bound state. **Conclusions.** 1. The component composition of primary metabolites: free and bound carbohydrates, free and bound amino acids of the herb *A. dasyanthus* grown in Ukraine was studied. 2. It was established that in the free state it contains 2 monosaccharides, 1 disaccharide and 2 polyols, in the bound state - 2 monosaccharides, 1 disaccharide and 2 polyols. The high content of polyol: (+)-pinitol correlates with the use of this raw material as an antioxidant and metabolism normalizer. 3. The amino acid composition is represented by 12 compounds both in free and bound state. *L*-Aspartic acid dominated in the free state, glycine in the bound state. 4. The obtained research results are a fragment of the systemic pharmacognostic study of the herb *A. dasyanthus*, grown in Ukraine, and testify to the appropriateness of creating preparations based on it.

Keywords: *A. dasyanthus*, herb, primary metabolites, free and bound carbohydrates, free and bound amino acids

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