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STUDY OF MONOSACCHARIDE COMPOSITION OF MEADOW PASQUEFLOWER

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Summary. The manuscript presents the results of monosaccharide composition analysis of the *Pulsatilla pratensis (L.) Mill* herb. Determination of free and general monosaccharides in herbal raw material was performed by the gas chromatography-mass spectrometry (GC/MS). According to the experimental results 13 carbohydrate compounds, 6 of them were monosaccharide and 2 of them were sugar alcohols. Among the determined monosaccharides Xylose (13.89 mg/g), Glucose (4.79 mg/g), Arabinose (3.01 mg/g) and Galactose (2.99 mg/g) were found in higher quantity.
Keywords: *Pulsatilla pratensis (L.) Mill.,* herb, monosaccharide composition, gas chromatography-mass spectrometry

Problem statement. In recent years herbal medicines have become increasingly popular for the pharmacotherapy of many diseases. According to the statistics of the World Health Organization near 80 % of world population give preference to herbal medicines. Interest in usage of medicinal plants and medicines produced from these plants stems from the fact that on condition of correct dosage these medicines are practically non-toxic, harmless, relatively available, effective and sometimes they do not have competitors due to complex action [4, p. 77].

Herbal medicines have a variety of advantages over chemotherapeutical medicines. Medicinal plants usually contain natural compounds necessary for normal human life: vitamins, carbohydrates, macro- and microelements, enzymes, hormones etc. Complex of substances present in plants has polyvalent action stimulating different body systems or compensating their insufficient function.

Herbal medicines are represented by active substances, isolated from plants, purified complexes of natural compounds (in the form of substances) and big group of complex herbal medicines (tinctures, herb teas, teas, extracts etc.), and also they are represented by the herbal raw materials.

Extensive resources, availability of raw material, possibility of cultivation make herbal raw material a prospective target of research with the aim of development of new herbal medicines [4, p. 77].

Pulsatilla pratensis (L.) Mill. is a herbaceous perennial poison plant, it is a species of the ranunculaceous family (Ranunculaceae) which belongs to such advanced herbal raw material. This plant is distributed on the Balkans, it is native to central and eastern Europe. It grows throughout most of the territory in Ukraine: in forest, forest-steppe and steppe zones except western-most districts and Crimea [1 p. 214, 5 p. 301-302].

Meadow pasqueflower contains anemonin, ranunculin, protoanemonic,ethereal oil, tannin, vitamin C, organic acids, flavonoids, residues of alkaloids, tanning substances (near 4.5 %). Seeds contain fatty oil (17.4 %). Plant has hypotensive, sedative, hypnotic, antispasmatic, antifungal and anesthetic action, it slows down heart rhythm and stimulates breathing [1 p. 214].

Complex photochemical investigation of meadow pasqueflower as herbal raw material is important today taking into account widespread use

of this plant in official and traditional medicine.

Analysis of research and publications. (aim, base material, conclusions). The aim of this work was to determine monosaccharide composition of *Pulsatilla pratensis (L.) Mill.* which is a member of ranunculaceous family (*Ranunculaceae)*. Air-dried herb of *Pulsatilla pratensis (L.) Mill.*, prepared at the time of flowering was used for study (supplier «Svit trav», Ukraine).

Determination of free and general monosaccharides in herbal raw material was performed by the gas chromatography-mass spectrometry (GC/MS), this method is based on the extraction of free monosaccharides, acid hydrolysis at the determination of general monosaccharides and obtaining of their aldonitrile acetate derivatives with further analysis by the the gas-liquid-chromatography-mass spectrometry .

Equipment and conditions of chromatographic separations:

The chromatographic separation was performed on gas chromatograph/mass spectrometer system Agilent 6890N/5973inert (Agilent technologies, USA). All chromatographic separations were performed on capillary column HP-5ms (30m×0.25mm×0.25mkm, Agilent technologies, USA). Evaporator temperature was 250 ^{0}C, interface temperature was 280 ^{0}C.

Separation was performed in programmed temperature mode – program started at 160 ^{0}C for 8 min and changed to 240 ^{0}C at the rate of 5 ^{0}C/min. Final temperature was held for 6 min. Sample injection was 1 µl. Injector was operated in a split mode with a split ratio of 1:50. MS scanning was performed from m/z 38-400. Flow rate of gas-carrier was 1.2 mL/min.

Identification was carried out by the comparison of the retention times of standard monosaccharides and by the usage of mass spectral library NIST 02.

Assay was done by the internal standard addition to the test samples.

Sample preparation and analysis of herbal raw material:

a) Free monosaccharides

Herbal raw material was crushed to powder in a glass mortar. A weighed portion of herbal material was mixed in a glass vial with 80% ethanol with internal standard (it corresponds to 200 mkg of sample). Extraction of free monosaccharides was performed on the on ultrasonic bath for 3 hours at the 80°C. 0.6 mL of extract was taken and evaporated to dryness on the rotary evaporator.

b) General monosaccharides

2 mL of 2M trifluoroacetic acid was added to weighed portion of herbal material. Hydrolysis was carried out for 6 hours at the 100C^0. Hydrolyzate was evaporated and washed with water to remove trifluoroacetic acid completely. It was reconstituted by the addition of 2 mL of water. 0.6 mL of extract was taken and evaporated to dryness on the rotary evaporator.

c) Obtaining of aldonitrile acetate derivatives

To obtain aldonitrile acetate derivatives of monosaccharides 0.3 mL of derivatizing reagent ((32 mg/mL hydroxylamine hydrochloride, 40 mg/mL 4-dimethylaminopyridine in the mixture of pyridine/methanol (4:1 v/v)) was added. Dissolved extract was kept at the 80 °C for 25 min. To perform acetylation 0.3 mL of acetic anhydride was added and held for 25 min at the 80°C. 0.3 mL of dichloroethane was added to reaction mixture, excess of derivatized reagents were double extracted with 1N hydrochloric acid and water. Dichloroethane layer was separated and analyzed by the gas chromatography-mass spectrometry (GC/MS).

Identification of monosaccharides in the reaction mixture was carried out by the comparison of the retention times of standard monosaccharides and by the usage of mass spectral library NIST 02. Assay was done by the internal standard addition to the test sample. Sorbitol solution was used as internal standard [2, 6, 7].

The chromatogram of determined monosaccharides is given on the fig. 1. Based on the findings 13 carbohydrate compounds were found, 6 of them were monosaccharide and 2 of them were sugar alcohols.

Among the determined monosaccharides Xylose (13.89 mg/g), Glucose (4.79 mg/g), Arabinose (3.01 mg/g) and Galactose (2.99 mg/g) are found in higher quantity. Other identified monosaccharides are found in fewer quantities: Rhamnose (1.49 mg/g) and Mannose (0.74 mg/g).

Manitol (0.59 mg/g) and Dulcitol (0.69 mg/g) are also found in a small quantity. In the human body D-xylose is a part of glycosaminoglycans of connective tissue.

D-xylose is contained in the composition of wood polysaccharides (particularly in wood gum), in different glycosides and oligosaccharides.

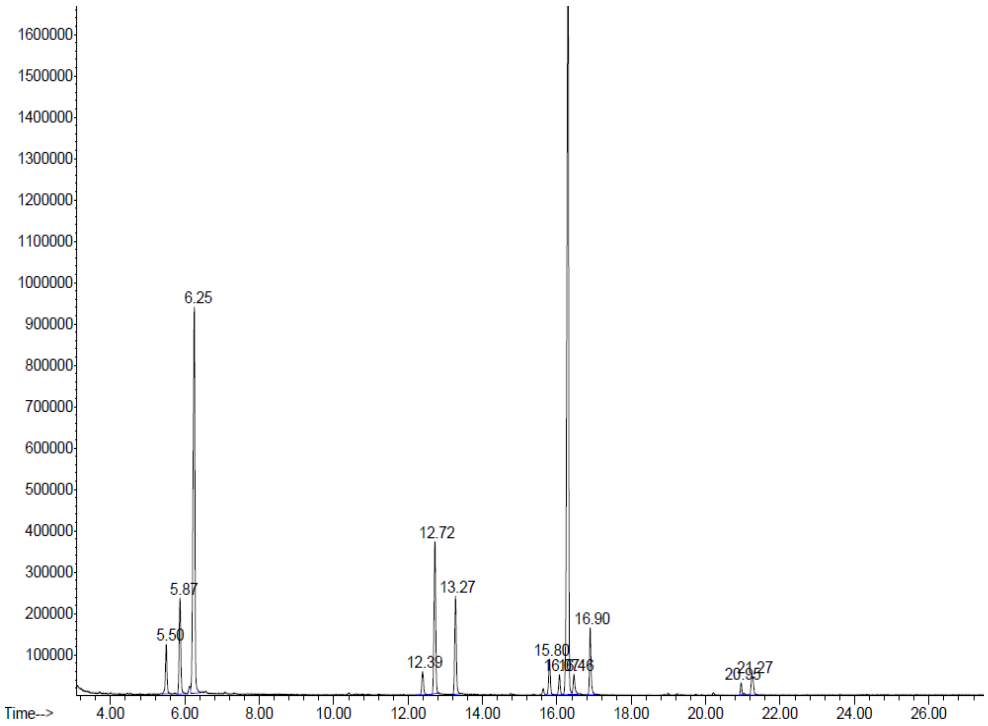

Fig. 1. HPLC-chromatogram of monosaccharides of *Pulsatilla pratensis (L.) Mill* herb.

Table 1.

Quantitative result of general monosaccharides in the herb *Pulsatilla pratensis (L.) Mill.*

Peak number	Retention time	Compound	Content, mg/g
1	5.4985	D-Rhamnonitrile, 2,3,4,5-tetraacetate (Rhamnose)	1.49
2	5.8683	D-Arabinononitrile, 2,3,4,5-tetraacetate (Arabinose)	3.01
3	6.255	D-Xylonitrile, 2,3,4,5-tetraacetate (Xylose)	13.89
4	12.392	2,3,4,5,6-Penta-O-acetyl-D-manonitrile (Mannose)	0.74
5	12.7193	2,3,4,5,6-Penta-O-acetyl-D-gluconitrile (Glucose)	4.79
6	13.276	2,3,4,5,6-Penta-O-acetyl-D-galactonitrile (Galactose)	2.99
7	15.8047	Myo-inositol, hexaacetate	1.10
8	16.0682	D-Mannitol, hexaacetate (Manitol)	0.59
9	16.2977	D-Sorbitol, hexaacetate (Sorbitol)	Internal standard
10	16.4592	D-Dulcitol, hexaacetate (Dulcitol)	0.69
11	16.897	.alpha.-d-Ribopyranoside, 2,3,4-tri-O-acetyl-.beta.-d-ribopyranosyl, triacetate	1.91
12	20.9472	Tetraacetyl-.alpha.-d-glucofurosyll benzenesulfonate	0.30
13	21.2702	Tetraacetyl-.alpha.-d-glucofurosyll benzenesulfonate	0.82

Glucose is of great physiological significance. Muscles work at the expense of energy released at the glucose oxidation. Cellulose and starch yields glucose after complete acid hydrolysis (in the presence of enzyme). Glucose is the key source of energy for the human body. Glucose-based medications and glucose are used to diagnose diabetes [3].

Therefore, the analysis results show that quantitative and qualitative composition in the herb of *Pulsatilla pratensis (L.) Mill* was determined. Among the determined monosaccharides *Xylose* (13.89 mg/g), Glucose (4.79 mg/g), Arabinose (3.01 mg/g) and Galactose (2.99 mg/g) are found in higher quantity. Obtained experimental findings give additional information about the chemical composition of herbal raw material in according to the applications in medicine and pharmacy. These findings are essential to technological and pharmocological studies performed during the development of *Pulsatilla pratensis (L.) Mill*-based phytotherapeutic drugs.

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