Determination of Carbohydrates in the Herb Erigeron Annuus

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The purpose of the article is to provide an assessment of phytochemical studies of the monosaccharide composition of an annual grass of the asteraceae family, common in Ukraine. The wide geographical distribution of Erigeron species from North America to Japan makes it promising to study the depth of the monosaccharide composition of Erigeron annuus. The carbohydrates were separated by gas chromatography-mass spectrometry after conversion into volatile aldononitrile acetate derivatives. The monomeric composition of polysaccharides was studied after their hydrolysis to form monosaccharides and polyalcohols. Quantitative analyses of free carbohydrates showed that the predominant sugars were fructose, glucose and disaccharide – sucrose. The chromatographic study revealed a number of polyalcohols that are important for the treatment and prevention of progression of diabetes mellitus and its complications, namely, mannitol.

Introduction

Erigeron annuus relates to *Erigeron* genus, *Asteraceae* family and includes about 150 species [1]. For the flora of Ukraine are character 8 species, the widely distributed are: *Erigeron acris* – erigeron or bitter fleabane, *Erigeron Canadensis – Canadian fleabane, Erigeron annuus* – erigeron or annual fleabane [2]. *Erigeron annuus* – is an annual plant, sometimes biennial, tall and strong plant. Its height is 20-90 cm, stem is rather dense hairy form with long branchy hairs, has numerous leaves; radical blades are mid-hairy, large, coarsely dentate, elliptical, widely ovate or suborbular, up to 10 cm. The acroscopik leaves are usually shorter and accumbent. Flowers: anthodes are 6-10 mm in size, collected in cymose inflorescence; petals are 3-5 mm, blueviolet, they are of changeable width, acuminate or damped, finely grandular or little villous, with long flattened transparent hairs. *Erigeron annuus* is an invasive species, that had been imported from North America, and then was widely distributed throughout the continent. It is most familiar as a weed, it grows

mainly in meadows, pasturelands, well-lit glades. *Erigeron* is a character steppe plant, so it's often suitable for animals to eat.

In traditional medicine, the infusion of *Erigeron* herb is used as an antidiarrhoeal medicine, as well as for hemorrhoid. In China and Korea, *Erigeron* herb is used for hypoglycemia, hematuria, hepatitis, enterocolitis [9]. *Erigeron* herb has been studied mainly by the foreign scientists - and there have been discovered antiproliferative activity [3], neuroprotective [5], antioxidant [6], antisclerotic activity [4]. The chemical composition of *Erigeron annuus* raw materials includes various groups of biologically active substances: phenolic compounds [7], mono- and sesquiterpenoids [8], polyacetylene compounds [10], sterols, derivatives of γ-pyrones [11].

At this time the herb of *Erigeron annuus* is understudied, which sets the stage for its in-depth pharmacognostic study, particularly carbohydrates.

Carbohydrates are one of the three macronutrients – by the side of proteins and fats, which is necessary for our body every day [13]. Carbohydrates are the main source of energy for the human body. They help to get energy the brain, kidneys, cardiac muscles and central nervous system. For example, fiber – is a carbohydrate, that helps digestion, helps you feel full and supports blood cholesterol level. The human body can hold over more than necessary carbohydrates in muscles and liver for use, when the body does not get enough carbohydrates in its diet [14]. The diet with carbohydrate deficiency can cause headache, fatigue, weakness, difficulty to concentrate, feeling sick, bad breath, vitamin and mineral deficiencies. Carbohydrates can be obtained from a broad spectrum of healthy food such as bread, bean, milk, popcorn, potatoes, cookie, spaghetti, soft drink, corn [17, 18]. It's also well-known about a glycemic index. The glycemic index typifies the carbohydrate foods according to their potential to increase a blood sugar level. The diets based on the glycemic index usually recommend to limit the foods that outweigh the glycemic index. The foodstuffs with a higher-glycemic include potatoes and white bread, as well as less healthy foods such as snacks and desserts that contain refined flour. There are many healthy foods, such as whole grain, beans, vegetables, fruits, and low-fat fermented dairy foods, have a lower glycemic index level [16]. The most common forms are sugar, fiber and starch. The high-carbon foods are an important part of a healthy diet [15]. Carbohydrates ensure the body with glucose, which is converted into energy that is used to support body functions and physical activity. But, an important quality of carbohydrates is: some types of foods, untreated or with minimum processing of whole grains, vegetables, fruits and beans promote a good health through the delivery of vitamins, minerals, fiber and many important phytonutrients [12, 19, 20]. Erigeron annuus is widespread in Ukraine and has a large raw

material base, therefore there is an opportunity and prospect to research and study its monosaccharide composition. We previously reported on the isolation of carboxylic acids, terpenoids, amino acids, flavonoids and inulin from the herb Erigeron annuus [26, 28].

Experimental part

Plant materials:

The object of research was Erigeron annuus herb which was harvested in Kharkiv and Sumy regions in 2021. After harvesting, the raw materials were dried, crushed and stored according to the general GACP requirements. Herbarium specimens № 030601820-10061820 are stored in the pharmacognosy department (National University of Pharmacy, Kharkiv, Ukraine). The voucher specimens of herbal raw materials have been deposited in the departmental herbarium for future records.

Reagents:

The herb was dried by conventional methods, and then stored in paper bags in a dry place. Standards and reagents Polysaccharide standard including L-rhamnose, D-xylose, D-fucose, D-galactose, D-arabinose, D-sorbitol, D-sucrose, D-mannose, D-fructose, D-glucose, D-ribose, obtained from Sigma Aldrich, was of analytical grade (≥ 95% purity). All other reagents were of analytical grade.

Detection of carbohydrates:

Take 1.00 g of crushed raw material, place in a volumetric flask with a capacity of 50 ml, add 20 ml of water R. The flask was connected to a reflux condenser, boiled in a water bath for 30 minutes and filtered. To detect water-soluble polysaccharides, 30 ml of ethanol R 96 % was added to 10 ml of extract and infused. Free sugars were detected by adding to 1 ml of extract 1 ml of freshly prepared copper-tartrate reagent; heated in a water bath; observed the color change. The bulk of carbohydrates found in nature exist in the form of polysaccharides [27].

Derivatization:

The method is based on the extraction of free monosaccharides and obtaining acetates of their aldonitrile derivatives with further analysis by gas-liquid chromatography-mass spectrometry method. To obtain the aldononitrile monosaccharide derivatives, the dried hydrolysates of the extracts were taken and 0.3 mL of a derivatization reagent 32 mg mL^{-1} hydroxylamine hydrochloride in the mixture of pyridine/methanol (4:1, V/V)] was added. The samples were incubated in a preheated water bath shaker at 75°C for 25 min. Then, for acetylation of aldonitrile derivatives, 1 mL of acetic anhydride was subsequently added to the samples and incubated at 75°C for 15 min. To the resulting reaction mixture, 2 mL of dichloroethane was added. The excess of the derivatization reagents was removed by the double extraction with 1 mol L^{-1} HCl and water. The dichloroethane phase was dried and dissolved in 300 μL of the mixture of heptane/ethyl acetate (1:1, V/V).

Chromatographic separation equipment and conditions:

Chromatographic separation was performed on a gas chromatography-mass spectrometry system Agilent 6890N/5973 inert (Agilent technologies, USA). Capillary column was HP-5ms (30 m×0.25 mm×0.25 mkm, Agilent technologies, USA). Evaporator temperature was 250°C, interface temperature was 280°C. The separation was performed in the mode of temperature programming - the initial temperature of 160°C was maintained for 8 minutes, raised with a gradient of 5°C/min. up to 240°C. The final temperature was maintained for 6 minutes. The sample with a volume of 1 µl, was introduced in the mode of split ratio 1:50. Detection was performed in SCAN mode in the range (38-400 m/z). The flow rate of the carrier gas through the column was 1.2 ml/min. Identification was performed by the retention time of the monosaccharide standards and using the collection of mass spectra NIST 02. Quantitative analysis was performed by adding an internal standard solution to the study samples.

Sample preparation and analysis of herbal raw materials:

Herbal raw materials were triturated to a powder in a glass mortar. A sample weight 500 mg was placed in a round bottom flask, 80% ethyl alcohol internal standard solution was added at the rate of 500 µg on the sample. Extraction of free monosaccharides was conducted in a water bath at 100°C using a reflux for 2 hours. 2 ml of extract was taken to obtain aldonitrile derivatives of monosaccharides, then it was evaporated to dryness on a ratotory 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 maintained for 25 minutes at 75°C. For acetylating of aldonitrile derivatives of monosaccharides 1ml of acetyloxide was added and maintained for 15 minutes at 75°C. To the reaction mixture was added 2 ml of dichloroethane, the excess of derivatizating reagents was removed by a double extraction with 1N hydrochloric acid and water. The dichloroethanoic ball was dried to dryness and dissolved in 300 µl mixture of heptane/ethyl acetate (1:1 v/v). Identification of monosaccharides of the study mixture was performed by comparing the retention times of the standard monosaccharides and using the collection of mass spectra NIST 02. Quantitative analysis was performed by adding an internal standard solution to the study samples. Sorbitol solution was used as an internal standard [21-25]. The mass of monosaccharide per 1 kg of the raw material in mg was calculated by the formula:

$$X = \frac{\text{Sx} \times \text{Miss} \times 1000}{\text{Siss} \times m}$$

Where:

S_x – is the peak area of a monosaccharide;

M_{iss} – is the mass of the internal standard for the sample;

S_{iss} – is the peak area of the internal standard;

m – is the sample weight.

The first typical chromatogram are shown in figure 1 and the elution times and peak areas are tabulated (**Table 1**).

Table 1. GC data of monosaccharides in standard mixture as their derivatisation.

№	Monosaccharide	Peak	Retention time, (min)
1	Ribose (Rib)	1	7.94
2	Rhamnose (Rham)	2	8.03
3	Arabinose (Ara)	3	8.55
4	Xylose (Xyl)	4	8.89
5	Fucose (Fuc)	5	9.00
6	Manose (Man)	6	14.79
7	Glucose (Glu)	7	15.07
8	Galactose (Gala)	8	15.60
9	Sorbitol	9	18.44
10	Fructose (Fru)1	10	21.07
11	Fructose (Fru)2	11	21.33

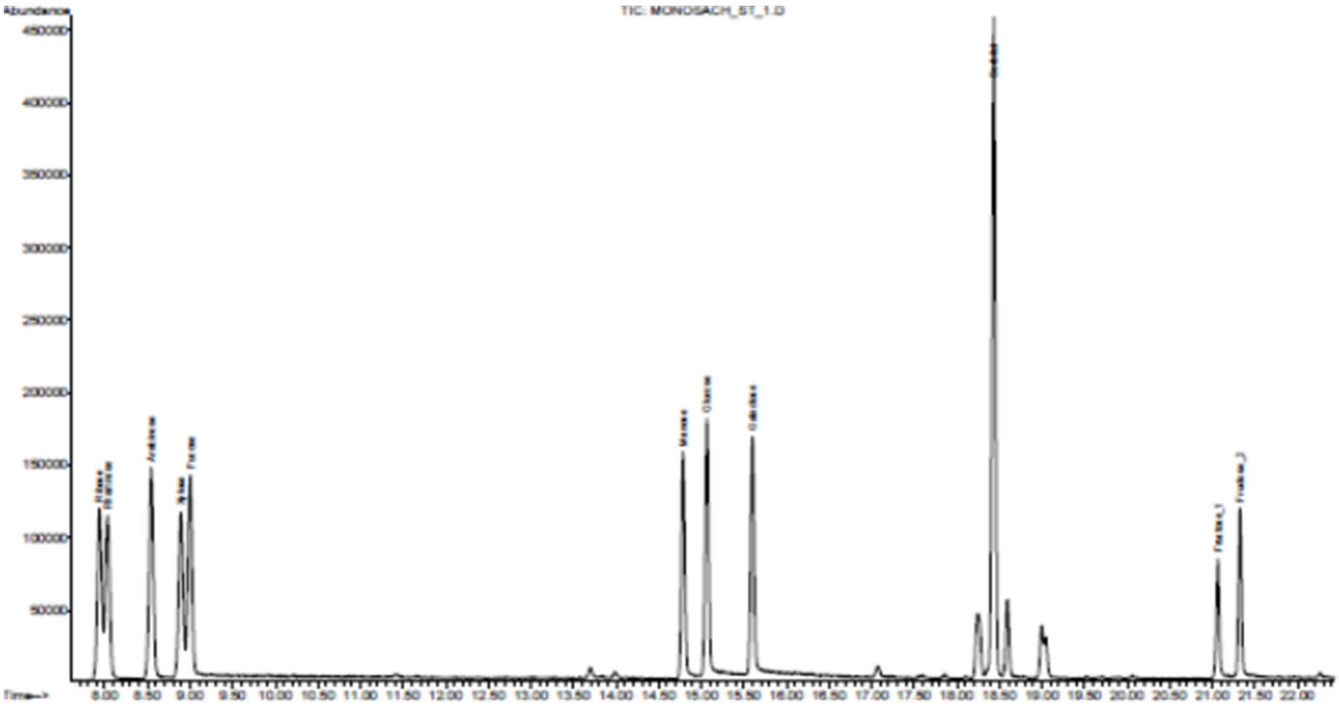

Figure. 1. GC chromatograms of standards monosaccharides.

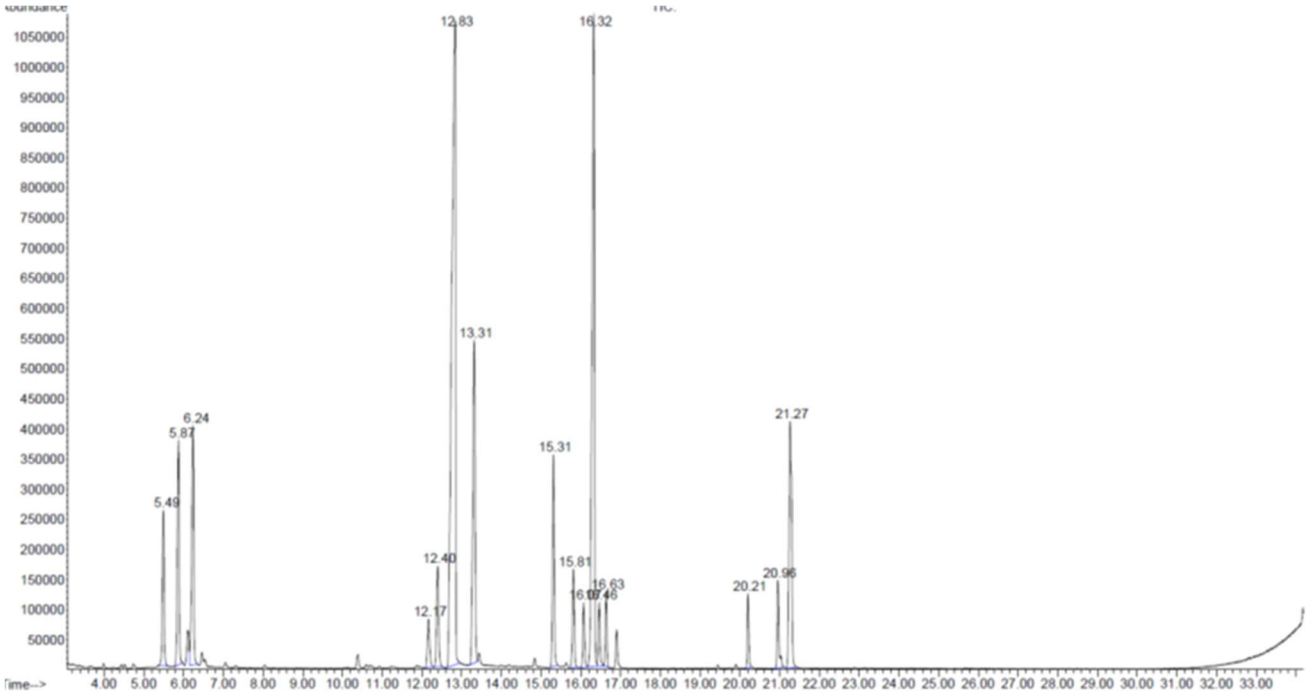

Figure. 2. The chromatogram GL-MS of the linked monosaccharides of *Erigeron annuus* herb.

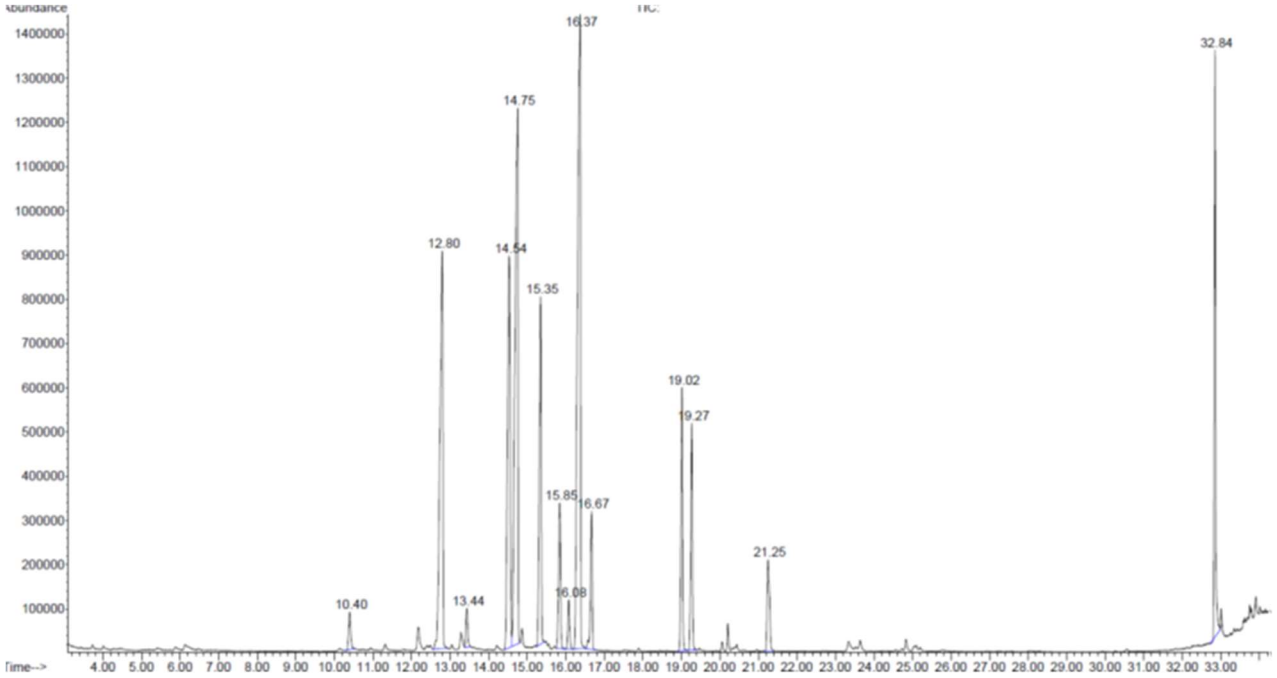

Figure. 3. The chromatogram GL-MS of free monosaccharides of *Erigeron annuus* herb.

Table 2. The linked monosaccharides of *Erigeron annuus*, mg/g.

Monosaccharide	Peak	Compound Library/ID	Retention time, min	Concentration of mg/g
Rhamnose	1	D-Rhamnonitrile, 2,3,4,5-tetraacetate	5.4901	4.87
	2	2,3,4,5,6-penta-O-acetyl-D-gluconitrile	5.8726	7.64
Arabinose	3	D-Arabinononitrile, 2,3,4,5-tetraacetate	6.2381	8.78
	4	Acetic acid, 4-acetoxy-2-[1,3,5]trioxan-2-yl-cyclopentyl ester	12.1668	1.96
	5	2,3,4,5,6-penta-O-acetyl-D-gluconitrile	12.3963	3.89
Glucose	6	2,3,4,5,6-penta-O-acetyl-D-gluconitrile	12.8341	44.95
Galactose	7	4-(7-hydroxy-2,2,7-trimethyltetrahy-dro[1,3]dioxolo[4,5-c]pyran-4-yl)-3-methylbut-2-enoic acid, methyl ester	13.3143	12.95
	8	Allo-Inositol, hexaacetate	15.3118	6.48
	9	Myo-inositol, hexaacetate	15.8133	3.24
Mannitol	10	D-Mannitol, hexaacetate	16.0683	2.00
Sorbitol	11	D-Sorbitol, hexaacetate	16.3233	Internal-standard
Dulcitol	12	D-Dulcitol, hexaacetate	16.4636	1.94
	13	Muco-Inositol, hexaacetate	16.6336	2.07
	14	Cellobioseoctaacetate	20.2078	1.99
	15	Tetraacetyl-α-D–glucofurosyllbenzenesul-fonate	20.9600	2.85
	16	Melibioseperacetate	21.2660	11.98

Table 3. Free monosaccharides of *Erigeron annuus*, mg/g.

Monosaccharide	Peak	Compound Library/ID	Retention time, min	Concentration of mg/g
	1	Iditol, hexaacetate	10.3989	0.57
Glucose	2	2,3,4,5,6-Penta-O-acetyl-D-gluconitrile	12.7959	8.23
	3	β-D-Mannopyranoside, methyl, tetraacetate	13.4376	0.52
	4	α-D-Glucopyranose, pentaacetate	14.5384	7.48
	5	D-Glucose, 2,3,4,5,6-pentaacetate	14.7509	11.45
	6	Allo-Inositol, hexaacetate	15.3501	5.01
	7	Myo-inositol, hexaacetate	15.8474	2.09
Mannitol	8	D-Mannitol, hexaacetate	16.0811	0.63
Sorbitol	9	D-Sorbitol, hexaacetate	16.3701	Internal-standard
Dulcitol	10	D-Dulcitol, hexaacetate	16.6718	1.80
Fructose	11	α-D-Glucofuranosidurono-6,3-lactone, phenyl-2,5-di-O-acetyl-1-thio	19.0178	3.05
Fructose	12	Glucosebenzyloximepentaacetate	19.2728	2.61
	13	Melibioseperacetate	21.2491	1.60
Sucrose	14	Sucrose Octaacetate	3.843	5.20

The chromatograms of the linked and free monosaccharides of *Erigeron annuus* herb are shown (**Figures 2, 3**).

The results of the research of receiving the linked and free monosaccharides of *Erigeron annuus* herb are shown (**Tables 2, 3**).

Results and discussion

Carbohydrates are an essential part of macronutrients, that perform many functions in the human body. They are now becoming increasingly important as prebiotics,

nutraceuticals, immunostimulants, antiproliferative agents and biodegradable polymers in the pharmaceutical and cosmetological industries. A wide range of biological resources including bacteria, plants, fungi, lichens, animals, algae allows the use of carbohydrates as means of energy storage.

Carbohydrates represent great structural and functional diversity, because the type of bond and the percentage of branching in their structure determine the functional properties of carbohydrates. Plants produce a wide range of carbohydrates that serve as energy reserves for structural functions. Only the human brain accounts for 20-25% of the main metabolic costs of an adult. In addition to the high energy needs of the brain, approximately 170 g of glucose per day are required for the normal functioning of the brain, renal medulla, red blood cells, and reproductive tissues. This glucose is mainly supplied by dietary carbohydrate intake, although it can also be derived from gluconeogenesis or from propionate absorbed as a result of intestinal fermentation of dietary carbohydrates. There was conducted a detailed study of the carbohydrates of *Erigeron annuus* herb by gas-liquid chromatography-mass spectrometry method for the first time ever. The results are presented in (**Tables 2, 3**) and in (**Figures 2, 3**). Under the present chromatographic condition, all derivatised monosaccharide were obvious baseline separation and the peaks for all monosaccharides were sharp and symmetrical in chromatogram. The retention times of Rib (ribose), Rham (rhamnose), Ara (arabinose), Xyl (xylose), Fuc (fucose), Man (mannose), Glu (glucose), Gal (galactose), Sorbitol, Fru 1 (fructose), Fru 2 (fructose) were 7.94, 8.03, 8.55, 8.99, 9.00, 14.79, 15.07, 15.60, 18.44, 21.07 and 21.33 min, respectively. In the results of the research in *Erigeron annuus* herb were found 28 compounds of carbohydrate nature, of them 13 were identified as free monosaccharides and 15 were identified as the linked monosaccharides. D-fructose was also found in the herb, the content of which was 3.05 mg/g. Glucose is an aldohexose that is polyhydroxy alcohol having an aldehyde group and is one of the most important carbohydrates in biology. A chromatographic study identified a number of sugar alcohols that are often used as sweeteners for diabetics because they are lower in calories and are poorly digested carbohydrates that are only partially absorbed from the small intestine and not metabolized. Mannitol is most often used as a sugar substitute due to its low calorie content (1.6 calories per gram) and our study showed that Erigeron annuus contain this polyol in free and bound forms (**Table 2, 3**). Among free monosaccharides dominate: sucroseoctaacetate – 5.20 mg/g; glucopyranose, pentaacetate – 7.48 mg/g; 2,3,4,5,6-Penta-O-acetyl-D-gluconitrile – 8.23 mg/g; α-D-D-glucose,2,3,4,5,6-pentaacetate – 11.45 mg/g. Among the linked monosaccharides dominate: 2,3,4,5,6-penta-O-

acetyl-D-gluconitrile – 7.64 mg/g; D-Arabinononitrile,2,3,4,5-tetraacetate – 8.78 mg/g; 2,3,4,5,6-penta-O-acetyl-D-gluconitrile – 44.95 mg/g; 4-(7-hydroxy-2,2,7-trimethyltetrahy-dro[1,3]dioxolo[4,5-c]pyran-4-yl)-3-methylbut-2-enoicacid, methylester – 12.95 mg/g; melibio-seperacetate – 11.98 mg/g; allo-Inositol, hexaacetate – 6.48 mg/g. In the raw materials were determined 28 compounds, where there were 13 free monosaccharides. The dominant free monosaccharides were: D-glucose, 2,3,4,5,6-pentaacetate - 11.45 mg/g; 2,3,4,5,6-penta-O-acetyl-D-gluconitrile - 8.23 mg/g; α-D-glucopyranose, pentaacetate - 7.48 mg/g; allo-Inositol, hexaacetate - 5.01 mg/g; α-D-glucofuranosidurono-6,3-lactone, phenyl-2,5-di-O-acetyl-1-thio - 3.05 mg/g; sucroseoctaacetate - 5.20 mg/g; glucosebenzyloximepentaacetate - 2.61 mg/g; among the linked monosaccharides the dominant were: 2,3,4,5,6-penta-O-acetyl-D-gluconitrile - 44.95 mg/g; 4-(7-hydroxy2,2,7-trimethyltetrahydro[1,3]dio-xolo[4,5-c]pyran-4-yl)-3-methylbut-2-enoicacid,methylester-12.95 mg/g; melibio-seperacetate - 11.98 mg/g; D-arabinononitrile, 2,3,4,5-tetraacetate - 8.78 mg/g; 2,3,4,5,6-penta-O-acetyl-D-gluconitrile - 7.64 mg/g. Increases in the incidence of obesity and diet-related metabolic diseases are related to profound environmental and behavioral changes, especially dietary behavior. The increase in the consumption of cereals and refined sugars, as well as dairy products, refined vegetable oils, and fatty meats, are at the root of the epidemic of nutrition-related chronic diseases. Diverse microbiota are synonymous with health, with dietary fiber having a major impact on the composition, diversity, and richness of the microbiome. Thus, accessible carbohydrates are beneficial for the well-being and growth of microorganisms and consequently for the host in this symbiotic relationship. Carbohydrates may have mood-enhancing properties, affecting mental and psychological well-being CHO-rich foods have a negative impact on mood categories, including alertness, tiredness, and higher rates of depression in the long term. Carbohydrates, when taken in an adequate amount and in the right balance in the diet, can sustain good mental health. Carbohydrate intake can affect the development and prognosis of metabolic disease, as an uncontrolled intake of refined carbohydrates puts individuals at risk of developing metabolic syndrome and subsequently developing metabolic disease. The nature of the carbohydrates rather than the amount is the key factor in reducing the risk of cardiovascular disease or in improving the cardiovascular risk markers. Complex carbohydrates may modulate insulin-like growth factor binding protein 3 blocking cell proliferation and tumoral growth. Fiber intake may modulate gut microbiota and, consequently, their short chain fatty acid production, reducing inflammation and having a positive effect on cancer development. Complex carbohydrates

and fiber may increase fecal excretion of carcinogens, improving their elimination and reducing their negative effects in organisms. Consumption of simple sugars as sweeteners in different sugary beverages is related to asthma due to malabsorption. Formation of pro-inflammatory products between unabsorbed fructose and some dietary proteins, after intestinal absorption, are associated with asthma. Nutrition guidelines stablished that carbohydrates make up 40% to 65% of total daily calories to fulfil body energy demands and to reduce the risk of some non-communicable diseases. Recommended daily intake of carbohydrates should be based on whole grains. Low glycemic index sources and fiber intake should be higher than 25–30 g per day [29].

The carbohydrates obtained from plants are very important active substances for the prevention and treatment of diabetes mellitus and diabetic angiopathies, because they have hypoglycemic, hypolipidemic, anticholesterolemic, antioxidant, anti-inflammatory and detoxifying activities.

Conclusions

1. The qualitative composition of carbohydrates in both free and bound form, as well as the quantitative content in the herb Erigeron annuus, used in folk medicine, was analyzed by the GC-MS method. Free carbohydrates and monomers after hydrolysis of bound carbohydrates, as well as a number of polyalcohols, were quantified.

2. Chromatographic analysis of carbohydrates of the Erigeron annuus herb presented it as a promising herbal preparation for the prevention and treatment of diabetes and its complications. The conducted studies allow us to predict its possible use to optimize antidiabetic pharmacotherapy with the feasibility of their further phytochemical and pharmacological studies.

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