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Study and determination of fructan-type polysaccharide content in *Erigeron annuus* L

Sergey Kovalev[1*], A. Golovach[1], Vladimir Kovalev[1], Ewa Poleszak[2*], Elshan Akhmedov[3], Olga Bobrytska[4]

[1] Department of Pharmacognosy of the National University of Pharmacy, Kharkov, Ukraine
[2] Chair and Department of Applied and Social Pharmacy, Medical University of Lublin, Poland
[3] Department of Analytical Chemistry and Analytical Toxicology, National University of Pharmacy, Kharkov, Ukraine
[4] Kharkiv State Zooveterinary Academy, Kharkov, Ukraine

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ABSTRACT

Fructan-type polysaccharidescomes from natural sources and occur in a large variety of plants, where they play important biological roles as reserve carbohydrate. One of the most commonly distributed compound from this group – inulin has been part of human daily diet for hundreds of years, as it is found in many fruits and vegetables, among others, bananas, onions and wheat. The inulin-type fructans: inulin and fructooligosaccharides (FOS) are considered to be functional food elements, the consumption of which brings about health benefits. Indeed, inulin can be consumed to increase the dietary fiber content. Fructan compounds, inulin and fructooligosaccharides have a strong bifidogenic effect, and have a positive action on the gut microbiota. In this work, we preformed gas-chromatography-mass spectrometry analysis of *Erigeron annuus* L. herb. The GC-MS analysis of carbohydrate composition confirmed the presence of free (arabinose, glucose, fructose 1, fructose 2) and fermented (arabinose, glucose, fructose 1, fructose 2, sucrose) carbohydrates at the quantity of 69.83 and 91.70 mg/g d.w., respectively.

INTRODUCTION

The genus *Erigeron* L. belonging to the family Asteraceae, involves about 150 species occurring in the Northern Hemisphere zone, mainly in North America [1], however, some were introduced to Europe [2]. There are several species of Erigeron in Ukraine, including *E. annuus*, the most common [1]. *E. annuus* (daisy flea) is an annual plant, and reaches a height of 150 cm. This species has an erect, branched stem, finished with inflorescences. It often found at roadsides and in wastelands [3]. *E. annuus* has been used in Chinese folk medicine for the treatment of indigestion, enteritis, epidemic hepatitis and haematuria [4]. Some studies indicate that chloroform and n-hexane extracts from the roots of *E. annuus* show moderate antiproliferative effectiveness against MCF7 cells [5]. Moreover, the constituents of the aerial part of *E. annuus* have been reported to contain γ-pyranone derivatives, flavonoids, phenolic acids and their derivatives, sesquiterpenoids, and cyclopentenone derivatives [6]. The chemical composition of *E. annuus* in Ukraine has, however, hardly been studied. Based on the collected information, the authors of the manuscript found it interesting to determine the composition of polysaccharides in the plant. One of the widely distributed component from this group that occurs in chicory, Jerusalem artichoke, garlic, leek, onion and in the representatives of Asteraceae – is inulin.

Inulin is a complex carbohydrate that belongs to a class of compounds known as inulin-type fructans. Inulin-type fructans are oligo-/polymers of D-fructose joined by β (2-1) bonds with an α (1-2) linked D-glucose at the end of the molecule. Molecules with degree of polymerization (DP) between 3 and 10 are referred to as 'oligofructose', and those with a DP between 10 and 65 are known as 'inulin'. Inulin is found to share many of the properties of soluble dietary fibers, such as the ability to lower blood lipids and stabilize blood glucose level. Additionally, inulin-type fructans, galactooligosaccharides and lactulose have been shown to enhance the growth of bifidobacteria and lactobacillus, and promote the gut environment [7] already after a short feeding period. Bifidobacteria are able to break down and utilize inulin-type fructans because of their possession of the β-fructofuranosidase enzyme that provides a competitive advantage in a mixed-culture environment such as the human gut. Therefore, inulin-type fructans are able to

*** Corresponding author**
e-mail: kov.serg71@gmail.com, ewa.poleszak@umlub.pl

exhibit bifidogenic effects at various daily intakes in healthy humans.

These information drew much interest in the development of prebiotics containing the nondigestible oligosaccharides [8,9]. Oligosaccharides are sugars consisting of between ~2 and 20 saccharide units, i.e., they are short-chain polysaccharides [10]. Examples include inulin-type fructans, trans-galactooligosaccharides, isomaltooligosaccharides, xylooligosaccharides, soyoligosaccharides, glucooligosaccharides, and lactosucrose [11].

It was proved that inulin dose of 5-8 g/d should be sufficient to elicit a positive effect on the gut microbiota [12,10]. The common target microorganisms for this action are bifidobacteria, and the observed increase in their number upon the administration of inulin-type fructans was measured as 0.5-1.0 log10. This constitutes a major upheaval in the gut microbiota toward a 'healthier' composition [13-15].

While no universally accepted definition for dietary fiber exists, it is generally accepted that this term includes saccharides (+lignin) that are not hydrolyzed or absorbed in the upper part of the gastrointestinal tract. These materials reach the colon, where they may be totally fermented, partially fermented, or remain unfermented. In addition, fibers contribute to fecal bulking. Inulin and oligofructose do not digest in the upper part of the gastrointestinal tract, or they are absorbed and metabolized in the glycolytic pathway, or directly stored as glycogen – like 'sugars' or starches.

None of the molecules of fructose and glucose that form inulin and oligofructose appears in the portal blood. These materials are quantitatively fermented by the colonic microflora. Moreover, it has been demonstrated that this fermentation leads to the selective stimulation of the growth of the bifidobacteria population.

Based upon their chemical composition, origin and physiological effects, inulin and oligofructose can be considered to be dietary fibers [16]. They share the basic common characteristics of dietary fibers, such as being saccharides of plant origin, having resistance to digestion and absorption in the small intestine, and inducing fermentation in the colon to produce short-chain fatty acids, that are absorbed and metabolized in various parts of the body. In addition, this fermentation induces the bulking effect [17-19].

Therefore, the aim of the study is to determine the composition of *Erigeron annuus* L. – an insufficiently studied plant – in terms of its polysaccharide composition. For this purpose, the GC-MS analysis will be conducted and free sugars and sugars released in the hydrolysis will be analyzed.

MATERIALS AND METHODS

The object of study was herb of *Erigeron annuus* L., collected in the flowering phase in 2020 (Kharkiv region, Ukraine). Herbarium specimens No 030601820-10061820 are stored in the Pharmacognosy Department (National University of Pharmacy, Kharkiv, Ukraine).

Samples preparation

For the extraction, 500 mg of powdered flowering herb was used. It was placed in a glass vial to which methyl alcohol solution (solid to liquid ratio 1:5 w/v) (reagent grade, Merck, Darmstadt) was added and enriched in an internal standard of 500 μg. The content of the vial was then mixed thoroughly and placed in an ultrasonic bath at 80°C for 4 hours. The obtained extract was centrifuged to sediment the plant material, whereas the supernatant was used for further analysis. Half of the obtained extract was used for enzymatic hydrolysis of inulin, that included the use of inulinase, whereas the rest of the extract was used to determine the free fructose content [20].

Enzymatic hydrolysis

Half of the obtained extract was directed to the determination of inulin content. The portion of extract was divided into two halves again. The first half was cooled to 60°C, brought to the pH of 7,2 [21] and mixed with 100 μl (41.67 IU) of fructozyme enzyme (Sigma Aldrich, St. Louis, MO, USA), and was kept in an ultrasonic bath for 30 min. The total fructose content was determined in this sample. At the same time, the other half was used to determine the free fructose content – without inulin hydrolysis (without the addition of enzyme). This has come about because preliminary studies have revealed that the raw material contains sucrose disaccharide, which also releases fructose during hydrolysis. Therefore, the quantitative content of sucrose was also taken into account when calculating the inulin content.

Derivatization of carbohydrates

An aliquot of the extract (the remaining half) was evaporated to dryness on a rotary evaporator to obtain aldonitrile monosaccharide derivatives, and 0.3 ml of derivatizing reagent (32 mg/ml hydrochloric acid hydrochloride in pyridine/methanol (4:1 v/v)) was added. The dissolved extract was kept for 25 min. at 75°C. Acetylation of aldonitrile derivatives of monosaccharides was carried out for 15 min at 75°C. To the reaction mixture was added 1 ml of dichloroethane, and the excess of derivatization reagents was removed by double extraction with 1N hydrochloric acid and water. The dichloroethane layer was dried to dryness, and was dissolved in 300 μl of heptane/ethyl acetate (1:1 v/v) [21,22].

GC-analysis

The determination of fructose in the study samples was performed by gas chromatography-mass spectrometry. Chromatographic separation was performed on an Agilent 6890N/5973 inert gas chromatographic mass spectrometric system (Agilent technologies, USA). We used a capillary column HP-5ms (30m×0.25mm×0.25mkm, Agilent technologies, USA). Evaporator temperature was 250°C, interface temperature was 280°C. The separation was performed in temperature programming mode – the initial temperature of 160°C was maintained for 8 minutes, and then raised with a gradient of 5°C/min to 240°C. The final temperature was maintained for 6 minutes. The sample of 1μl was introduced in the 1:50 flow separation mode. Detection was performed in SCAN mode in the range (38-400 m/z). The flow rate of carrier gas through the column was 1.2 ml/min. The identification of the monosaccharides of the study mixture was performed by comparing the retention times of standard monosaccharides, and using the NIST 02 mass spectra

library. Quantitative analysis was performed by adding an internal standard solution to the study sample [21-23].

Calculation and expression of results

The quantitative content of inulin is calculated as the difference between the total content of fructose after fermentation and free fructose, taking into account the amount of fructose released by decomposition of sucrose according to the formula:

$$X\ (mg/ml) = \frac{A \times (F1 - F2 - F3)}{P},$$

where:
F1 – is the concentration of total fructose (mg/ml);
F2 – is the concentration of free fructose (mg/ml);
F3 – is the concentration of fructose released from sucrose (mg/ml, F3=S/B, where: S – is the sucrose concentration;
B – is the empirical factor for the conversion of fructose to sucrose (2.13);
A – is the empirical factor for the conversion of fructose to inulin (1.03);
P – is the mass of the sample of plant material (mg).

The empirical factor for the conversion of fructose to inulin and sucrose (the factor of conversion of inulin to fructose and sucrose to fructose) was determined by sequential processing of samples with different amounts of enzyme using rhamnose as an internal standard, and assessing the amount of fructose release [20].

RESULTS

GC-MS method for the detection of single sugars

For the detection of fructans, inulin was used as a representative compound of this sugar group. Inulin is one of five types of fructan, and it is a mixture of fructose-derived polysaccharides with 2-1 linkages between fructosyl residues of varying chain length, with one terminal glucose molecule [24]. The use of inulin as standard has been initiated by the food industry, where inulin-type fructans are most frequently found [25]. The GC-MS analysis of the *E. annuus* samples identified the presence of the monosaccharides arabinose, glucose and fructose. The peaks were identified according to the retention time in comparison with arabinose, glucose and fructose standards, as well as the fragmentation profile at the mass spectra of oxime-silylated derivatives. This was in addition to consultation with the NIST 02 Mass Spectral Library Database.

The results of studies of carbohydrates in the herb of *E. annuus* are presented in (Table 1,2).

The GC-MS chromatogram (Fig. 1) showed a major peak that corresponded to fructose units at retention times of 19.001 and 19.256 min, while glucose peaks were identified at retention time of 12.80. In addition, arabinose peaks were identified at retention time of 5.873 and the internal standard of sorbitol was observed at 16.381 min. The GC-MS chromatogram (Fig. 2) peaks at retention times 18.996 and 19.251 min. were assigned as fructose units, and the peak with retention time of 12.813 min. was identified as glucose. Peaks of arabinose were identified at retention time of 5.885. Peaks of disaccharide sucrose were identified

Table 1. The content of fermented carbohydrate in the herb of *E. annuus, mg/g d.w.*

No	Name of the substance	RT, min	Concentration of mg/g
1	Arabinose	5.873	13.10
2	Glucose	12.80	63.01
3	Fructose 1	19.001	7.74
4	Fructose 2	19.256	7.85
∑ carbohydrates			91.70

Table 2. The free carbohydrate content in the herb of *E. annuus mg/g of d.w.*

No	Name of the substance	RT, min	Concentration of mg/g
1	Arabinose	5.885	13.10
2	Glucose	12.813	40.65
3	Fructose 1	18.996	5.54
4	Fructose 2	19.251	5.65
5	Sucrose	32.834	4.89
∑ carbohydrates			69.83

at retention time of 32.834 min. The method of GC-MS identified and chemically characterized 5 free (69.83 mg/g) and 4 fermented (91.70 mg/g) carbohydrates from *E. annuus* herb.

As expected, the investigated plant was found to contain a variety of saccharides. According to previous studies inulin is a typical metabolite synthesized by all plants from the Asteraceae family [26]. The measured content of fructose, that appears in the decomposition process of inulin, in the studied herb was equal to 7.74 and 7.85 mg/g d.w. for the fermented herb and 5.54 and 5.65 mg/g d.w. for fructose in the free carbohydrates fraction. Similar results concerning the free carbohydrates content were described for other

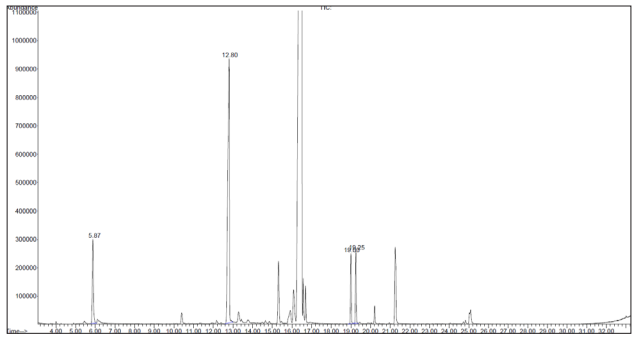

Figure 1. GC-MS chromatography of fermented carbohydrates of *E. annuus* herb.

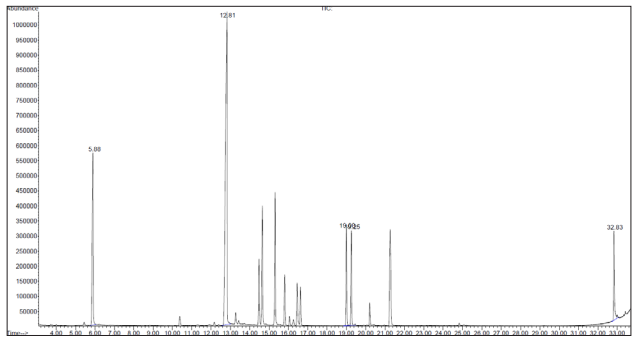

Figure 2. GC-MS chromatography of free carbohydrates of E. annuus herb.

plant species whish were found to be distinct sources of carbohydrates. In the studies of Almeida and co-investigators *Trimezia juncifolia*, *Trimezia cathartica*, *Sisyrinchium vaginatum*, *Cipura xanthomelas*, and *Cipura paludosa* were found to contain 3.8, 1.5, 0.9, 0.7, and 7.2 mg/g d.w. of fructose in the polysaccharide fraction, respectively [27].

In view of the finding presented above, inulin-type fructans were identified in the *E. annuus* herb. This information explains the potential use of the plant material as a "prebiotic" as it is expected to selectively stimulate growth and/or activity of a number of health-stimulating intestinal bacteria. The obtained results let us propose the herb of *E. annuus* to be used in combination with "probiotics" or live bacteria that are added to the host's diet to promote health. The combinations of pre- and probiotics have synergistic effect, referred to as 'synbiotic', because in addition to the action of prebiotics that promote the growth of existing strains of beneficial bacteria in the colon, inulin and oligofructose also act to improve the survival, implantation and growth of newly added probiotic strains [28,29].

CONCLUSIONS

We isolated and chemically characterized several FOS molecules from *E. annuus* herb in the present study. This came about by applying the GC-MS method. In doing so, we noted the presence monosaccharides of arabinose, glucose, fructose and sucrose disaccharide after free enzymatic hydrolysis. The examined raw material can be considered a promising source for obtaining FOS in high yield. The present findings emphasize the importance of investigating alternative and sustainable sources to obtaining molecules with prebiotic potential that can serve as food nutrients that promote health. We hope to continue this work despite the current situation in free Ukraine induced by our Russian neighbour.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID iDs

Sergey Kovalev https://orcid.org/0000-0002-3529-0146
Vladimir Kovalev https://orcid.org/0000-0001-7852-7783
Ewa Poleszak https://orcid.org/0000-0003-4359-3953
Elshan Akhmedov http://orcid.org/0000-0001-6727-8259
Olga Bobrytska https//orcid.org/0000-0002-5368-8094

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