Analysis of inulin and fructans in *Taraxacum officinale* L. roots as the main inulin-containing component of antidiabetic herbal mixture

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Abstract

Herbs and their combinations due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of development of diabetes mellitus and its complications. One of such combinations is an antidiabetic herbal mixture with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in previous pharmacological study in vivo that including an inulin-containing component – *Taraxacum officinale* L. roots. Thus, the aim of this study was to determine the quantitative content of inulin and fructans in *Taraxacum officinale* L. roots. Quantity content of inulin was determined by the difference between fructose as a product of enzymatic hydrolysis and D-fructose, a constituent of sucrose and free D-fructose, taking into account the empirical factor for the conversion of D-fructose from inulin. Carbohydrates used in the calculation of inulin were separated by gas chromatography-mass spectrometry after conversion into volatile derivatives as aldononitrile acetate. According to the results, *Taraxacum officinale* L. roots contain 436.29 mg/g of inulin. Total content of fructans was determined by spectrophotometric analysis as a product of acid hydrolysis of 5-(hydroxymethyl)furfural. The results show that *Taraxacum officinale* L. roots contain 39.49% of fructans. The obtained results are evidence that this plant component should be included in the herbal antidiabetic mixture, because due to the presence of fructans and inulin causes hypoglycemic, hypolipidemic and detoxification activity.

Keywords

Diabetes mellitus, fructans, herbal mixture, inulin, GC-MS, spectrophotometric assay, *Taraxacum officinale* L. roots

Introduction

Diabetes mellitus is one of World Health Organization priorities matters, which requires immediate solutions, as the epidemiological situation is alarming – the number of patients is growing rapidly each year, leading to increased disability and mortality due to the development of macro- and microangiopathies (Harding et al. 2019; American Diabetes Association 2021). According to the official information of International Diabetes Federation (2019) the number of diabetics will increase to 700 million by 2045. Therefore, the implementation of pharmacotherapy optimization, the search and study of new drugs for the prevention and treatment of this disease and its dangerous complications is a topical issue of pharmacy and medicine.
Modern pharmacotherapy is increasingly taking into account the centuries-old experience of folk medicine using phytomedicines as monotherapy and in combination with synthetic drugs. It became quite justified, after all as phytherapy has a number of advantages over traditional therapy with using synthetic agents, namely, it is low-toxic, has a mild pharmacological effect and possibility to be used for long periods of time without significant side effects and is well combined with synthetic drugs (Gothai et al. 2016; Governa et al. 2018). In addition, herbal preparations usually have a wide range of pharmacological properties, which is realized through various groups of phytochemicals (Oh and Jun 2014; Kooti et al. 2016). Particular attention deserve the combinations of different medicinal plants, because such herbal mixtures will have more biologically active substances that will influence on the all links of the pathogenetic mechanism of development of diabetes mellitus and its complications (Savych et al. 2020a, b, c, d, e, f; Savych et al. 2021a, b, c, d, e, f, g).

One of such combinations is an antidiabetic herbal mixture (Urtica dioica L. leaf, Taraxacum officinale L. roots, Vaccinium myrtillus L. leaf, Rosa majalis L. fruits, Mentha piperita L. herb) with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreaticotrophic activity in pharmacological study in vivo (Savych et al. 2020b, c, d, e, f) and the defined phytochemical composition that determines such pharmacodynamics (Savych et al. 2020a; Savych et al. 2021a, b, c, d, e, f, g).

Biologically active substances of plant origin have a wide range of pharmacological action and a variety of mechanisms of influencing on the development of diabetes and its angiopathies (Oh and Jun 2014; Kooti et al. 2016; Skyler et al. 2017). One of the most influential phytochemical components is fructans, in particular inulin because it has the ability to regulate the lipid metabolism, a disorder of which occurs in diabetes and leads to the development of cardiovascular diseases and microcirculatory complications – diabetic nephropathy, neuropathy and retinopathy, the formation of diabetic foot. Important influence of inulin on lipid metabolism is manifested by a decrease in triglycerides and cholesterol (Hiel et al. 2018; Mistry et al. 2018).

Inulin as a representative of fructans has hypoglycemic activity due to its ability to increase glucagon-like peptide-1 (GLP-1), which increases the secretion of insulin, inhibits the secretion of glucagon and somatostatin, causes the proliferation and neogenesis of β-cells and increases the response of β-cells to glucose (Kietsirirote et al. 2018; Paternoster and Falasca 2018).

Fructans, including inulin, have a powerful detoxifying effect due to the formation of a healthy intra-intestinal environment that protects against pathogens, toxins and free radicals resulting from lipid peroxidation. This is achieved by stimulating the growth of beneficial bacteria in the colon, including Bifidobacteria and Lactobacilli (Shang et al. 2018; Hoffman et al. 2019).

The main plant component in the studied mixture containing fructans and inulin, according to the literature, is Taraxacum officinale L. roots (Wirnigo et al. 2016). As for other components of the herbal mixture, such as Urtica dioica L. leaf, Vaccinium myrtillus L. leaf, Rosa majalis L. fruits and Mentha piperita L. herb, no literature data were found on the presence of inulin in them, so these components were not considered by us as objects of this study.

**Aim of the research**

The aim of this study was to determine the quantitative content of inulin and fructans in Taraxacum officinale L. roots, as the main inulin-containing component of antidiabetic herbal mixture.

**Materials and methods**

**Plant materials**

It was used the herbal raw materials of Taraxacum officinale L. harvested from September to October 2020 in Ternopil region (Ukraine) during the study. The raw materials were then dried, crushed and stored according to the general GACP requirements (WHO 2003). The plant was identified at the Department of Pharmacognosy with Medical Botany, Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. Sample of herbal raw materials has been deposited in Departmental Herbarium for future record.

**Chemicals and standards**

All applied reagents were of analytical grade (≥ 99% purity). Chemical reference substances (CRS) of carbohydrates including D-arabinose, D-glucose, D-fructose and saccharose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Water used in the studies was produced by MilliQ Gradient water deionization system (Millipore, Bedford, MA, USA). Inulinase, acetate buffer, methanol, hydroxylamine hydrochloride, pyridine, dichloroethane, hydrochloric acid, heptanes, ethyl acetate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

**Chromatographic condition**

Quantity content of inulin in Taraxacum officinale L. roots was studied by GC-MS method with small modifications (Marchyshyn et al. 2020). Chromatographic separation was performed on a gas chromatomo-mass spectrometric system model 6890N/5973inert (Agilent Technologies, USA) using a capillary column HP-5ms (30 m × 0.25 mm × 0.25 mm, Agilent Technologies, USA). Evaporator temperature was 250 °C, the interface temperature – 280 °C. Separation was performed in the mode of temperature programming – the oven temperature was initially at 160 °C, held for 8 min, then ramped at the rate of 5 °C/min to 240 °C and finally held at this temperature for 6 min. Samples 1 μL were administered in a 1:50 flow divider.
mode. Detection was held in the SCAN mode in the range of (38–400 m/z). Carrier gas flow rate through a column was 1.2 mL/min.

**Sample preparation for GC-MS**

Sample of herbal raw materials was grinded into a powder by laboratory mill, then about 50–80 mg (accurately weighed mass) was placed in a glass vial and 4 mL of 0.1 M acetate buffer (pH 4.5) was added. Extraction of inulin was performed in ultrasonic bath at 80 °C for 3 hours. Resulting extract was centrifuged at 3000 rpm for 10 min and the supernatant was evaporated to dryness on a rotary evaporator. One part of an extract was used for enzymatic hydrolysis of inulin with 100 μL of inulinase at 60 °C for 30 min (Vendrell-Pascuas et al. 2000). Rest of an extract of *Taraxacum officinale* roots was used for the determination of free D-fructose (Guan et al. 2010).

**Pre-column derivatization**

Aliquots of 0.6 mL from extracts were taken and 0.3 mL of a derivatizing reagent (32 mg/mL of hydroxylamine hydrochloride in the mixture of pyridine/ methanol (4:1, v/v)) was added to obtain an aldonitrile monosaccharide derivatives. Sample was incubated in a preheated water bath shaker at 75 °C for 25 min. After incubation 1.0 mL of acetic anhydride was subsequently added to the sample and incubated at 75 °C for 15 min. 2 mL of dichloromethane was added to the mixture, the excess of the derivatization reagents was removed by the double extraction with 1 M hydrochloric acid solution and water. Dichloromethane layer was dried and dissolved into 300 μL of the mixture of heptane/ethyl acetate (1:1, v/v) (Chen et al. 2009).

**Identification and calculation for GC-MS**

Identification of enzymatic hydrolysis products, free monosaccharides and disaccharide – sucrose was performed by comparing of retention time (tR) of the mixture of CRS nosaccharides and disaccharide – sucrose was performed by laboratory mill, then about 50–80 mg (accurately weighed mass) was placed in a glass vial and 4 mL of 0.1 M acetate buffer (pH 4.5) was added. Extraction of inulin was performed in ultrasonic bath at 80 °C for 3 hours. Resulting extract was centrifuged at 3000 rpm for 10 min and the supernatant was evaporated to dryness on a rotary evaporator. One part of an extract was used for enzymatic hydrolysis of inulin with 100 μL of inulinase at 60 °C for 30 min (Vendrell-Pascuas et al. 2000). Rest of an extract of *Taraxacum officinale* roots was used for the determination of free D-fructose (Guan et al. 2010).

**Spectrophotometric condition**

Quantitative content of fructans in *Taraxacum officinale* roots L. was determined by spectrophotometric methods, using spectrophotometer Shimadzu 1800-UV (Japan).

Extract: 3.00 g (accurately mass) of powdered herbal raw materials were extracted by 100 mL of water at the water bath under reflux at 80 °C for 1 hour.

Stock solution: an extract was cooled, filtered and the volume was completed to 100 mL by water.

Test solution: 2 mL of stock solution was placed into 100 mL volumetric flask and 50 mL of 5% hydrochloric acid solution was added. Obtained solution was hydrolyzed at the water bath under reflux for 2 hours. Then 2.0 mL of cooled hydrolyzate was placed into 50 mL volumetric flask and solution of 5% hydrochloric acid was added to the mark.

Reference solution: 2 mL of stock solution was placed into 50 mL volumetric flask and solution of 5% hydrochloric acid was added to the mark.

The absorbance of the test solution was measured at wavelength (Amax) of 284 nm relative to the blank.

The total content of fructans in *Taraxacum officinale* L. roots was calculated, as a product of acid hydrolysis of 5-(hydroxymethyl)furfural (5-HMF) (Marchyshyn et al. 2020).
**Results and discussion**

During GC-MS analysis it was detected an inulin by the products of its enzymatic hydrolysis after conversion into volatile derivatives as aldononitrile acetate in *Taraxacum officinale* L. roots, which was the main inulin-containing component of antidiabetic herbal mixture (Fig. 1). However, chromatographic determination of free carbohydrates, in particular *D*-fructose and sucrose, was performed to exclude their content from the total amount of enzymatic hydrolysis products by inulinase (Fig. 2).

Result of quantitative determination of carbohydrates formed due to enzymatic hydrolysis, free *D*-fructose, *D*-glucose and sucrose in *Taraxacum officinale* L. roots has showed in Table 1. It was established that content of inulin was 436.29 mg/g in *Taraxacum officinale* L. roots by a number of calculations.

After acid hydrolysis of an aqueous extract of *Taraxacum officinale* L. roots by a solution of 5% hydrochloric acid was formed 5-HMF, which was confirmed by the absorption spectrum at λmax 284 nm (Fig. 3). During spectrophotometric analysis it was found that the *Taraxacum officinale* L. roots contain 39.49% of fructans.

The obtained results are an important stage of phytochemical study of antidiabetic herbal mixture and its plant components, because it allows already establish a correlation between biologically active substances and its pharmacodynamics. Fructans and their representative – inulin have a leading position in the prevention and treatment of diabetes, as confirmed by numerous preclinical and clinical studies (Kietsiriroje et al. 2018; Mistry et al. 2018; Hoffman et al. 2019). Inulin-type fructans help control glycemia in type 2 diabetes and prediabetes by a variety of mechanisms, including by reducing insulin resistance, increasing glucagon-like peptide (GLP-1), and glucagon-like peptide 2 (GLP-2) (Kietsiriroje et al. 2018; Paternoster and Falasca 2018). In addition, the hypoglycemic effect of inulin is realized by delaying the rate of gastric

![Figure 1. GC-MS chromatogram of carbohydrates formed as a result of enzymatic hydrolysis in *Taraxacum officinale* roots (1 – *D*-arabinose, internal standard; 2 – *D*-glucose; 3, 4 – *D*-fructose).](image)

**Table 1.** Result of the GC-MS analysis of carbohydrates, which are taken into account when calculating the inulin content in *Taraxacum officinale* L. roots.

| No. of peak | tR, min (SD±0.008) | Identified substance | Derivatization products | Content, mg/g |
|------------|--------------------|---------------------|------------------------|--------------|
| **CARBOHYDRATES AFTER HYDROLYSIS** | | | | |
| 1. | 9.02 | *D*-arabinose | 2,3,4,5-tetra-O-acetyl-*D*-arabinonitrile | Internal standard |
| 2. | 15.42 | *D*-glucose | 2,3,4,5,6-penta-O-acetyl-*D*-gluconitrile | 10.86±0.56 |
| 3. | 18.68 | *D*-fructose | naphthalene-1-carboxylic acid, 4-butyramino-6,7-dimethoxy-2-methyl-ethyl ester | 394.66±1.87 |
| 4. | 18.94 | *D*-fructose | 1-nitro-4-phenoxyninthraquinone | 65.74±0.83 |
| **FREE CARBOHYDRATES** | | | | |
| 1. | 9.02 | *D*-arabinose | 2,3,4,5-tetra-O-acetyl-*D*-arabinonitrile | Internal standard |
| 2. | 15.42 | *D*-glucose | 2,3,4,5,6-penta-O-acetyl-*D*-gluconitrile | 3.58±0.25 |
| 3. | 18.68 | *D*-fructose | naphthalene-1-carboxylic acid, 4-butyramino-6,7-dimethoxy-2-methyl-ethyl ester | 7.10±0.29 |
| 4. | 18.97 | *D*-fructose | 1-nitro-4-phenoxyninthraquinone | 2.39±0.19 |
| 5. | 34.05 | sucrose | sucrose octaacetate | 58.19±0.64 |

**Note:** Values are expressed as mean ± SD (n = 5).
emptying, thereby slowing the flow of glucose into the blood and reducing the degree of alimentary hyperglycemia that occurs after meals (Hiel et al. 2018). Fructans as probiotics have many health benefits, such as improving immune function, lowering blood pressure, improving blood lipids and reducing inflammation (Mistry et al. 2018).

That is why the phytochemical study of the content of inulin and total content of fructans confirms the feasibility of introducing *Taraxacum officinale* L. roots into the antidiabetic herbal mixture with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreaticoprotective activity in pharmacological study in vivo (Savych et al. 2020b, c, d, e, f).

**Conclusion**

We established the quantity content of inulin in *Taraxacum officinale* L. roots as the main inulin-containing component of antidiabetic herbal mixture with hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreaticoprotective activity and defined phytochemical composition by GC-MS method. The inulin content was 436.29 mg/g. During spectrophotometric analysis it was detected that the *Taraxacum officinale* L. roots contain 39.49% of fructans, as a product of acid hydrolysis of 5-(hydroxymethyl)furfural. The obtained results are evidence that this plant component should be included in the herbal antidiabetic mixture, because due to the presence of fructans and inulin causes hypoglycemic, hypolipidemic and detoxification activity.

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