STUDY OF THE INULIN COMPLEX OF DAHLIA ROOT BULBS OF KEN’S FLAME SPECIES AND ITS STANDARDIZATION

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1. Introduction

The assortment of medicines in the Ukrainian market, including inulin, is very limited. At the same time, herbal preparations are absent. Inulin complex was obtained from dahlia’s tubers of the ‘Ken’s Flame’ species (with conventional name ‘Jorzhin-Ch’). This complex is fructan, and the active ingredient is inulin. Inulin is an active pharmaceutical ingredient that is used in the complex treatment of lipid and carbohydrate metabolism disorders of the cardiovascular system and the immune system. ‘Jorzhin-Ch’ is a promising substance for creation of drugs of various forms for treatment of diseases described above.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Recently, scientific interest in one of the more interesting classes of biologically active substances — phytopolysaccharides, which are isolated from herbal material — has increased.

In medical technology, phytopolysaccharides are frequently used as auxiliary substances (stabilizers, emulsifiers, fillers, slides, etc.) when creating various medical forms. Nowadays, phytopolysaccharides act as medicinal and prophylactic agents, due to wide range of their properties (antiviral, antitumor, antimicrobial, anti-inflammatory, etc.) and low toxicity [1, 2]. Therefore, phytopolysaccharides are promising sources for the study and development of active pharmaceutical ingredients, and in the future they could be used as new drugs or biologically active additives.

Due to the fact that there is no data concerning chemical structure and biological role of a number of polysaccharides, these compounds are frequently classified according to isolation sources, despite the fact that the same polysaccharide can be obtained from completely different sources. Further classification of polysaccharides in these groups is carried out partly by isolation sources, and partly in accordance with chemical structure.

This classification makes it possible to divide natural polysaccharides into three large groups: herbal polysaccharides or phytopolysaccharides, microorganisms polysaccharides (including some bacteria and yeasts), and polysaccharides of animal organisms (zoology polysaccharides). Naturally, phytopolysaccharides perform skeletal or reserve functions. The latter include starch polysaccharides and fructans.

Thus, the actual task is to determine the structure of the inulin complex and to carry out its standardization in order to use it for treatment as a prebiotic or nutritional supplement for children.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

Fructans are polysaccharides made of O-fructose residues that act as an energy reserve for the body, and,
moreover, their presence is necessary to support the so-called "turmeric solution".

Fructans are found in some higher plants, especially in members of complex flowers and cereals. There are two types of plant fructans: the type of inulin and the type of flanee [3]. Inulin and its solutions are used as a diagnostic tool for kidneys glomerular filtration, and are parts of some drugs, which gives them an adaptogenic action [4]. Inulin is used as a prebiotic, supporting the activity of gastrointestinal tract bifidoflora [5]. Inulin improves condition of patients with lipid and carbohydrate disorders, cumulates inflammatory processes with colitis [6]. There is evidence that inulin has a positive effect on the immune system and improves the adsorption of calcium and magnesium, and has immunomodulatory properties [7].

Fructo-oligosaccharides arouse interest of scientists [8]. According to the literature, it is known that the dahlia tubers contain inulin [9]. Therefore, recent study of dahlias is an actual trend in science [10]. It is known that in the United States and Europe, rootlets use dill as a source of inulin, while in Ukraine they were not given proper attention.

The main method to determine the structure of polysaccharides is the splitting of molecules into fragments [3]. For this purpose, full acidic hydrolysis of all glycosidic ligaments or partial splitting of molecules is used, resulting in oligosaccharides or monosaccharides formation, that in turn allows determining which of the given monosaccharides is necessary polysaccharide. Partial hydrolysis is carried out in such way to split only a portion of the glycoside bonds labile to acids and accumulate fragments with more tight bonds. As a result of the splitting of the polysaccharide, partial hydrolysis becomes less random and more selective.

The most widely used methods of qualitative analysis are chromatographic methods: paper and thin-layer chromatography, electrophoresis on paper and in polyacrylic gel [11, 12]. There are modern methods for determining monosaccharides, such as gas-liquid chromatography, and high-performance liquid chromatography [13]. Also, for accurate determination of monosaccharides in raw materials, highly effective anion exchange chromatography with a pulse amperometric detector is used, as well as combined methods [14]. To determine the qualitative composition of the monosaccharide mixture, a preliminary cleavage of the polysaccharide molecule was carried out. Qualitative analysis of the mixture of monosaccharides by chromatography allows determining the number of components in the sample under test, the mobility of each component relative to the solvent front (RF) or the standard sample (Rs).

According to the literature, the main monosaccharide in the hydrolysis of inulin is fructose, which belongs to ketogexose [8, 11].

For the quantitative determination of fructose, many color reactions are used [15]. For the analysis, the most sensitive reaction of fructose with α-naphthol, called the reaction of Dische, was chosen [15]. The basis of this reaction lies in the ability of furfural in an acidic medium to form a trivalent methane chromogen with α-naphthol, which turns into a colored quinidic compound having a violet coloration.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

At present, the study of inulin, which has been isolated from herbal material, is paying more attention to the creation of medicines based on it. In Ukraine, the problem of standardization of inulin is not solved, but a monograph for inulin and an inulin injection solution is included in the Pharmacopoeia of the United States of America [16].

To standardize inulin, it is necessary to study the kinetics of acid hydrolysis of the inulin complex, establish a monomeric composition, and select the conditions for the reaction of fructose with alpha-naphthol with additives.

5. Formulation of goals (tasks) of Article

Purpose: to study composition of the inulin complex obtained from the root bulbs of the “Ken's Flame” species and its standardization.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

The object of the study was the inulin complex isolated from dahlia’s tubers of the “Ken's Flame” species [17].

The inulin complex was isolated from dahlia’s tubers of the “Ken's Flame” species. To determine the composition of inulin complex monosaccharides, paper chromatography method of was used. Quantitative determination of fructose was carried out by adsorption spectrophotometry.

Stationary phase used: paper FN 4, FN 17 and FN 1; the following mobile phases were used: acetic acid – chloroform – water (70:60:10) and n-butanol-acetic acid-water (4:1:5). To optimize the mentioned methods, cost-effective standard samples (ES): R fructose and glucose R were selected as reference solution. For optimal values RF conducted distillation chromatogram. On paper chromatography strip, cut along the fiber, the solution of fructose and glucose standard samples was applied, and then it was chromatographed in the conditions listed above.

The studies allowed: to select a paper (FN 1 or FN 17), the distance for chromatography, which provides the optimal separation of fructose and glucose, the method of applying the test solution and the reference solutions as a strips. Optimal detection conditions: air drying; detection of fructose and glucose after spraying with a solution of α-naphthol with phosphoric acid and aniline tin reagent respectively. Air drying for 15 minutes and evaluation after drying the chromatogram in an oven at 105 °C for 5–10 minutes were carry out.

To identify monosaccharides of the polysaccharide complex, acid hydrolysis was carried out within 5–30 minutes.

The quantitative determination of fructose in the inulin complex was carried out by spectrophotometry using Spectrophotometer “Specord-200” at a wavelength of 571±2 nm.

Results and discussion. On a strip of chromatography paper, the solutions of standard samples of fructose
P and glucose P were applied. Chromatography was carried out using up-flow chromatography under conditions given for standard samples. Solutions of fructose P and glucose P were applied in the amount of $1 \cdot 10^5 \, \mu g$ and $2.5 \cdot 10^5 \, \mu g$, respectively. In the study of hydrolysates of the inulin complex, according to the technique, it was found that the chromatographic profile had spots identified as fructose (Rf 0.59±0.02) and glucose (Rf 0.52±0.02) and 2 additional spots (Fig. 1).

Reaction of fructose and glucose with α-naphthol was studied. The reaction with α-naphthol is also specific for aldogexose, but its sensitivity is much lower. Absorption spectra of glucose and fructose solutions (3.3·10^6 µg/ml) with α-naphthol in acidic medium are shown in Fig. 2, 3

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As soon as urea and boric acid were added to the acidic medium (urea 3 mg/ml and boric acid 6 mg/ml), the sensitivity of the fructose reaction with α-naphthol increased approximately 6-fold, and the glucose sensitivity decreased 2-fold.

The increase in the additive (urea and boric acids) in the solution of fructose and glucose with α-naphthol did not succeed, as the optical density of fructose drops significantly, and the optical glucose solution slightly increases (Fig. 6, 7).
Fig. 6. Absorption spectrum of fructose solution with α-naphthol in acidic medium with addition of urea (6 mg/ml) and boric acid (12 mg/ml).

Fig. 7. Absorption spectrum of glucose solution with α-naphthol in acidic medium with urea additives (6 mg/ml and boric acid 12 mg/ml).

Based on the color reaction of fructose and glucose, the following concentration of additives was added: urea – 3 mg/ml, boric acid – 6 mg/ml, which increased the sensitivity of the fructose reaction by 6 times.

Studies on the dependence of the optical density of fructose on α-naphthol (urea 3 mg/ml and boric acid 6 mg/ml) have shown that it is possible to use this method for fructose standardization. During the research, it was necessary to pick up the dilution so that the absorption rate of solutions was in the range from 0.2 to 0.7, to establish the concentration, to study time stability of the optical density of solutions of fructose. The conducted studies allowed setting the heating time – 15 minutes. The linear dependence of the optical density of the solution on the concentration of fructose was studied (Fig. 8).

Stability of solutions of fructose for 2 hrs was studied. It was found that solutions are stable for 1 hour. The optical density of solutions decreases by 0.36 %, and after 2 hours by 0.89 %. Therefore, the optical density of solutions of fructose must be measured immediately after preparation. The confirmation of the specificity of the absorption spectrophotometry method is based on the need to prove that the relative systematic error introduced by the auxiliary substances is insignificant in comparison with the maximum permissible uncertainty of the analysis. The evaluation of the influence of excipients was carried out for measuring the optical density: the optical density of the solution, prepared by the method for fructose and without fructose, was measured.

The average values of 3 measurements of optical density are: Ablank =0.0041; Aasbest =0.4097. The systematic error was:

\[ \delta_{exc} = \frac{100 \cdot 0.0041}{0.4097} = 1.00\% \cdot \]

As it can be seen, inequality (1.00 %≤1.024 %) was carried out – hence background absorption is insignificant and the technique is characterized by permissible specificity.

The studies concerning dependence of the optical density of the solution of fructose on α-naphthol (with additives of urea 3 mg/ml and boric acid 6 mg/ml) in the above mentioned conditions allowed to set the heating time of 15 minutes, the stability of the obtained color is 1 year.

The linear dependence of optical density on the concentration of the fructose solution (Fig. 8).

On the basis of the conducted studies, a spectroscopy method for standardization of fructose with α-naphthol in an acidic medium with additives was developed (Table 1).

Table 1

| Taken X_i | found X | S | S_{cp} | P | t(P,v) | E, % | S_x | RSD_x, % |
|-----------|---------|---|--------|---|--------|------|-----|---------|
| 0.146;    |         |   |        |   |        |      |     |         |
| 0.153;0.157 | 0.151 | 1.48·10^{-3} | 0.0016 | 0.95 | 2.78   | 2.89 | 0.01059 | 1.059   |
| 0.149;0.153 | 0.152 |             |        |      |        |      |       |         |
In order to standardize the inulin complex derived from the root bulbs of the “Ken's Flame” species, it was necessary to study the kinetics of hydrolysis and optimal conditions for the formation of colored compounds, the spectral characteristics of colored products of the fructose reaction with α-naphthol, the kinetics of hydrolysis, and, on the basis of the conducted studies, to select the optimal conditions for these reactions. For this purpose, the study of hydrolysis kinetics of inulin complex with dilute hydrochloric acid was carried out.

The kinetics of hydrolysis of inulin was determined by the amount of fructose, which was allocated for a certain period of time (from 0–30 min.). The obtained experimental data allowed constructing the curves of the dependence of fructose amount on the time of the inulin hydrolysis (Fig. 9). According to experimental data, the maximum concentration of fructose appears after 15 minutes, so 15 minutes is enough for hydrolysis.

In development of the standardization method of inulin, the aliquot of the preparation was selected, the dilution of the tested and compensatory solutions, and the analytical wavelength were determined; also spectrophotometry conditions and the time during which the test solution optical density was stable were determined.

On the basis of the developed method, the determination of fructose in the inulin complex obtained from dahlias rootlets was performed. In Fig. 10, the spectrum of fructose with α-naphthol and additives in the inulin complex after hydrolysis for 15 minutes is shown.

Thus, the conducted studies allowed to develop standard conditions for the determination of fructose in the inulin complex from the dahlias’ tubers of the “Ken’s Flame” species. The method of standardization of inulin has been tested on a sample of inulin complex obtained from dandelion rootlets. The fructose content in IR is 41.86 %, based on fructose.

7. Conclusion
The composition of the inulin complex «Jorzhin-Ch» obtained from tubers of dahlia of «Ken’s Flame» species was studied and its standardization was carried out.

1. Conditions for the fructose and glucose identification by the method of paper chromatography were selected.

2. Chromatography standard conditions for the polysaccharide complex after hydrolysis were selected. The paper, the distance by chromatography, the method and amount of application of the tested solutions, and the method of processing chromatograms were chosen. It has been determined that the chromatographic profile of the inulin complex has two spots: fructose and glucose. Possibly there are 2 unidentified monosaccharides

3. Method for standardization of fructose with alpha-naphthol in the presence of glucose in the inulin complex obtained from dahlias’ tubers of the “Ken’s Flame” species using a color reaction with alpha-naphthol in an acidic medium with additives was developed.

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