

DETERMINATION OF CARBOHYDRATES IN BURNET SAXIFRAGE (*PIMPINELLA SAXIFRAGA* L.)

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Abstract

Pimpinella saxifraga L. known as burnet saxifrage, belongs to the family *Apiaceae* genus *Pimpinella*. Burnet saxifrage has many properties, including expectorant activity. This property is maybe a result of the presence of carbohydrates in plant. There is a lack of data about carbohydrates content of burnet saxifrage. Thus the aim of study was to determine the content of carbohydrates *Pimpinella saxifraga* L. herb, rhizomes and roots. The carbohydrates, present in raw materials of burnet saxifrage, have been studied by gravimetric and GC/MS analysis. The fractions of pectin substances and water-soluble polysaccharides were distinguished in the herb, rhizomes and roots of burnet saxifrage, the quantitative content of which was the following: pectin substances – rhizomes and roots – 4.41%, herb 11.89%, water-soluble polysaccharides – rhizomes and roots – 11.25%, herb – 6.95%. Five carbohydrates were found in the rhizomes and roots of burnet saxifrage, two were identified, such as D-glucose (0.28 mg/kg) and D-saccharose (33.96 mg/kg). Thirteen carbohydrates were found in the herb, three were identified, namely, D-glucose (1.44 mg/kg), D-fructose (0.68 mg/kg) and D-saccharose (6.55 mg/kg). Thus, we consider that the *Pimpinella saxifraga* L. is a promising plant for medicinal purposes because of its significant role in a large number of biological functions.

Keywords: *Pimpinella saxifraga* L., burnet saxifrage, carbohydrates, herb, rhizomes and roots, GC/MS

Introduction

In recent years, drugs based on medicinal plant raw materials are becoming increasingly popular in the pharmacotherapy of many diseases. According to World Health Organization statistics, up to 80% of the world's population prefer drugs of natural origin. Today, in developed countries, herbal medicines occupy a significant part of the overall pharmaceutical market. In particular, the share of herbal medicines in the United States is about 26% of the domestic market of medicines. In Germany, the share of registered herbal medicines is about 13% of the total number of medicines [1-3]. Combinations of various medicinal plants need special attention as such herbal mixtures have a variety of biologically active substances [4-7]. The interest in the use of medicinal plants and drugs derived from them is due to the fact that when properly dosed, they are almost non-toxic, relatively affordable, and effective and in some cases, due to the complex action, have no competitors. The plant medicines are well tolerable, often used in the fight against many diseases, and have minor side effects [8-11]. The presence in plants of a wide range of action of native biologically active substances, namely: essential oil, saponins, polysaccharides and other active ingredients is of particular interest [12]. The increase in demand for herbal medicines is also due to the high efficiency, successful centuries-old experience of using many of them in folk medicine. Significant resources, availability of raw materials, the possibility of cultivation make plant raw materials a promising object of study in order to develop new herbal medicines [2, 13, 14].

A member of the family *Apiaceae* genus *Pimpinella* – *Pimpinella saxifrage* L., which contains a complex of biologically active substances (terpenoids, coumarins, furocoumarins, sugars, gums, pectins, flavonoids, tannins, saponins, essential oil, fatty oils, amino acids, phenolic carboxylic acids, acetic and benzoic acids, vitamins, bitters) with a wide range of pharmacological activity (antispasmodic, expectorant, antitussive, diuretic, antibacterial, fungicidal use), sufficient raw material base, and extensive experience in the use of folk medicine in many countries, has an important scientific and practical value [15-18]. The expectorant activity of *Pimpinella saxifrage* L. shows due to the presence of

such biologically active substances as polysaccharides, sugars [16, 19]. Thus, the purpose of our study was to establish the qualitative composition and determine the quantitative content of carbohydrates.

Methods

Plant materials

The objects of the study were the herb and rhizomes and roots of *Pimpinella saxifrage* L., which was collected in the Husiatyn district, Ternopil region (Western Ukraine), in 2017. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine) [20]. A voucher specimen of *Pimpinella saxifrage* L. is kept at the Department of Pharmacognosy and Medical Botany (TNMU) [21]. The rhizomes and roots were dried using conventional methods and then stored in paper bags in dry place [22, 23].

Standards and reagents

Standard of polysaccharides, including L-rhamnose, D-xylose, D-fucose, D-galactose, D-arabinose, D-sorbitol, D-saccharose, D-mannose, D-fructose, D-glucose, D-ribose, derived from Sigma-Aldrich were of analytical grade (> 95% purity) [24, 25]. All other reagents were of analytical grade. Reagents were purchased from the Ltd. Sfera Sim (Lviv, Ukraine) [26, 27].

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. To detect pectin substances, 0.25 ml of 0.5% carbazole solution R and 5 ml of sulfuric acid R were added to 1 ml of extract, stirred and boiled in a water bath for 10 min [19].

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. Polysaccharides were found in the aqueous extract of the studied herb according to State Pharmacopeia of Ukraine, monograph "Plantago major leaves" [28].

Determination of carbohydrates by GC/MS method

Analysis of monosaccharides composition of *Pimpinella saxifraga* L. rhizomes and roots and herb was performed using gas chromatograph Agilent 6890N with mass detector 5973 inert and capillary column HP-5MS (length 30 m, internal diameter 0.25 mm) [29-32]. The detection was executed at the width range of 38–400 m/z in the SCAN mode [33, 34]. Helium was used as the carrier gas at a constant flow rate of 1.2 ml/min [35].

Sample preparation

For the extraction of free monosaccharides, 0.500 g of powdered rhizomes and roots or herb of *Pimpinella saxifraga* L. to 10 ml of methanol solution with sorbitol was added. The extraction took place for 4 hours at the temperature of 80 °C. To obtain acetylated aldonitriles 2 ml of the extract was evaporated to dryness and was added 0.3 ml of 32 mg/ml of hydroxylamine hydrochloride in pyridine/methanol (4:1 v/v) (derivatization reagent) [36-38].

The extract was kept for 25 min at a temperature of 75 °C. To the samples was subsequently added 1 ml of acetic anhydride and incubated for 15 min at a temperature of 75 °C. 2 ml of dichloroethane was added and the excess of the derivatization reagents was removed by the double extraction with 1 M hydrochloric acid and water. The dichloroethane layer was dried and dissolved in 300 µl of the mixture of heptane/ethyl acetate (1:1 v/v).

Identification of monosaccharides was based on their retention times compared to standards and mass library NIST 02. Quantification was done by using an internal standard of sorbitol added to the sample [39-42].

Statistical analysis

All the tests were carried out five times. Obtained results were presented as mean ± SEM [43]. Results were determined using Statistica v 10.0 (StatSoft I

nc.) program. Statistical significance of differences between mean values was assessed by the Student's t-test [44, 45]. The level of significance was mounted at * $p < 0.05$ [46-48].

Results and Discussion

Polysaccharides were detected by precipitation. The appearance of flaky clots when 96% ethanol was added to the extracts indicated the presence of polysaccharides in the studied raw material of bumet saxifrage.

Detection of pectin substances was performed by reaction with carbazole. A red-violet color was observed, which indicated the presence of galacturonic acid. When free sugars were detected with the help of copper-tartrate reagent, precipitation of brick-red precipitate was observed. Flakes during sedimentation precipitated. Water-soluble polysaccharides and pectin substances were isolated from the raw materials of bumet saxifrage.

Water-soluble polysaccharides are an amorphous powder of light brown color, which is easily soluble in water, soluble in aqueous solutions of acids and alkalis and insoluble in organic solvents.

Pectin substances are an amorphous cream-colored powder, which dissolves rather slowly in water and forms a gel-like colloidal solution when heated.

It was found that the herb of bumet saxifrage contained (6.95±0.25)% water-soluble polysaccharides. The content of pectin substances in the studied object was (11.89±0.11)%, which is 1.6 times more than water-soluble polysaccharides. The rhizomes and roots of *Pimpinella saxifraga* L. contained water-soluble polysaccharides (11.25±0.15)%, pectin substances – (4.41±0.31)%.

Determination of sugars in the studied raw materials was performed by GC/MS method. The results are presented in Table 1, 2 and in Figures 1, 2. Five sugars were found in the rhizomes and roots of bumet saxifrage, two were identified; thirteen sugars were found in the herb, three were identified. D-glucose and D-saccharose were identified in both samples of plant raw materials. D-fructose was also found in the herb, the content of which was 0.68 mg/kg. It was found that the largest amount of sucrose was contained in the

underground organs of bumet saxifrage – 33.96 mg/kg.

Glucose is an aldohexose that is polyhydroxy alcohol having an aldehyde group and is one of the most important carbohydrates in biology. It is synthesized during photosynthesis and serves as the “fuel”, accrued as starch in plants and as polymer glycogen in animals [49]. The cell uses glucose as a metabolic intermediate and a source of power [50].

Saccharose is the most common disaccharide. It is formed by the glycosidic bond between the β -fructose and α -glucose molecule [49, 51]. Saccharose is most famous for its role in people's food [50]. It is a pharmaceutical necessity for syrups, it is also a demulcent and a nutrient in enough concentration in an aqueous solution sugar is preservative and bacteriostatic. Saccharose is an easily digested macronutrient that provides a fast source of power to the organism [52]. It masks disagreeable tastes in tablets and troches and slows down oxidation in some medicines [19].

Fructose is a ketone sugar found in many foods and one of the three very important blood sugars along with galactose and glucose [50]. Fructose is used as a meal for diabetic people and may be of special benefit in diabetic acidosis. Infant alimentation formulas often contain this monosaccharide. When given parenterally it produces less urinary secretion than glucose [19].

Conclusions

There is a growing interest in carbohydrates in the recent years. The carbohydrates, present in rhizomes and roots, herb of *Pimpinella saxifraga* L., have been studied by gravimetric and GC/MS analysis.

The fractions of pectin substances and water-soluble polysaccharides were distinguished in the herb, rhizomes and roots of *Pimpinella saxifraga* L., the quantitative content of which was the following: pectin substances – rhizomes and roots – 4.41%, herb 11.89%, water-soluble polysaccharides – rhizomes and roots – 11.25%, herb – 6.95%. The composition of polysaccharide complexes of rhizomes and roots revealed five carbohydrates, two of which were identified; in the herb – thirteen carbohydrates, identified – three. D-glucose and D-

sucrose were identified in both samples of the raw materials.

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Table 1. The content of carbohydrates of *Pimpinella saxifraga* L. rhizomes and roots

Retention time	The name of the compounds	The content of the carbohydrates, mg/kg
15.08	D-glucose	0.28
18.44	D-sorbitol	internal standard
34.05	D-saccharose	33.96

Table 2. The content of carbohydrates of *Pimpinella saxifraga* L. herb

Retention time	The name of the compounds	The content of the carbohydrates, mg/kg
15.08	D-glucose	1.44
18.44	D-sorbitol	internal standard
21.07	D-fructose	0.68
34.03	D-saccharose	6.55

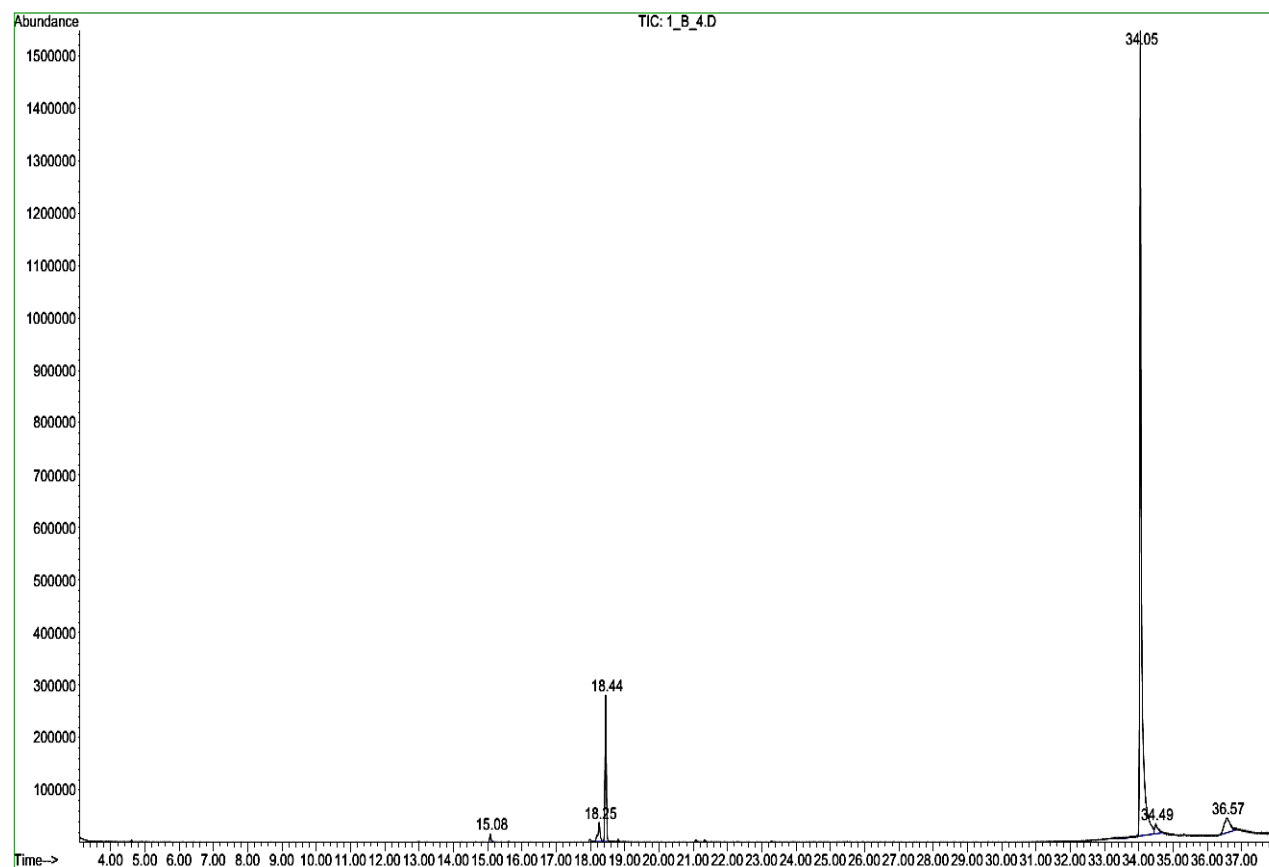
Figure 1: GC/MS chromatogram of carbohydrates of *Pimpinella saxifraga* L. rhizomes and roots

Figure 2: GC/MS chromatogram of carbohydrates of *Pimpinella saxifraga* L. herb