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Research Article

Analysis of carbohydrates in *Saponaria officinalis* L. using GC/MS method

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Abstract

Saponaria officinalis L. (common soapwort), usually named fuller's herb, is encountered in most of Europe, in Spain, France, Italy, for example, and also in Syria and North Africa. *Saponaria officinalis* L. is known in most of the world as an introduced species, often a weed, and sometimes as cultivated decorative plant. *Saponaria officinalis* contains a large amount of saponins, which foam during extraction with water. In addition to saponins, common soapwort also contains flavonoids, quillaic acid, fatty acids and different phenolic compounds. There is a lack of information about carbohydrates content of common soapwort. Thus the aim of this study was to determine the content of carbohydrates *Saponaria officinalis* L. herb and roots. The qualitative composition and quantitative content of carbohydrates in herb and roots of *Saponaria officinalis* L. were determined by using GC/MS method. The studies have shown that *Saponaria officinalis* L. herb is mainly composed of free carbohydrates such as D-glucose (3.65 mg/g), D-galactose (0.29 mg/g), D-fructose (0.20 mg/g) and D-saccharose (3.72 mg/g). In common soapwort herb, after acidic hydrolysis and derivatization with acetylated aldononitriles, D-arabinose, D-fucose, D-mannose, D-glucose, D-galactose, D-fructose and Myo-inositol were identified. Free carbohydrates in the roots of *Saponaria officinalis* L., including D-glucose, D-galactose and D-saccharose, were determined with GC/MS method too. D-saccharose was common among free carbohydrates of *Saponaria officinalis* L. in the largest amounts. Its content in herb and roots of the common soapwort was 3.72 mg/g and 25.39 mg/g respectively.

Keywords

Saponaria officinalis L., common soapwort, carbohydrates, GC/MS

Introduction

Plant metabolites are close to the metabolites of the human body, and the main effect of the herbal remedies usage is the regulation of metabolic disorders (Darzuli et al. 2019; Slobodianiuk et al. 2021). The most interesting are medicinal plants that have a long history of use for the treatment and prevention of various diseases (Stoiko and Kurylo 2018; Budniak et al. 2020; Marchyshyn et al. 2021b). Medicinal plants have great tolerability and small side effects (Kurylo et al. 2020; Darzuli et al. 2021). The typical plants for diseases' treatment are the families of *Asteraceae*, *Lamiaceae*, *Caryophyllaceae*, *Rosaceae*, *Fabaceae*, *Apiaceae*, *Poaceae*, and *Boraginaceae* (Slobodianiuk et al. 2020).

Saponaria species (family *Caryophyllaceae*) are perennial, flowering plants, characteristic to Europe and Asia, and usually known as soapworts (Petrović et al. 2018; Budniak et al. 2021b). The most familiar species within the genus is *Saponaria officinalis* L. (common soapwort), usually named fuller's herb, is encountered in most of Europe, in Spain,

France, Italy, for example, and also in Syria and North Africa. *Saponaria officinalis* L. is known in most of the world as an introduced species, often a weed, and sometimes as cultivated decorative plant (Henry 1989; Petrović et al. 2018).

The various parts of *Saponaria officinalis* have been used in traditional medicine, roots and leaves for skin diseases; roots as diuretic, diaphoretic, blood purifier; sap for scabies, to increase bile flow and hepatic eruptions. In addition, the roots are used as an anti-crystalline cholagogue that cleanses the body for medicinal purposes (Khare 2007; Talluri et al. 2018). A decoction of the herb of *Saponaria officinalis* is applied externally to treat itchy skin (Baytop 1984; Sengula et al. 2011).

Saponaria officinalis contains a large amount of saponins, which foam during extraction with water (Moniuszko-Szajwaj et al. 2013). The purified saponin fraction of *Saponaria officinalis* showed hypocholesterolemic effects in vitro, which is believed to be due to the ability of saponin to form an insoluble complex with cholesterol. Saponins also show spermicidal activity, which may be the result of their hemolytic properties (Jia et al. 2002; Bötger and Melzig 2011; Moniuszko-Szajwaj et al. 2013).

In addition to saponins, common soapwort also contains flavonoids, quillaic acid, fatty acids and different phenolic compounds (Cisowski et al. 1995; Lu et al. 2015).

The roots of *Saponaria officinalis* L. contain three oligosaccharides. Two of them were isolated namely gentiobiose and the pentasaccharide saponarose (Bukharov and Shcherbak 1969).

Petrović et al. (2018) confirmed that *Saponaria officinalis* shoots contained essential oil, rich with phytol, tricosane-6,8-dione, patchouli alcohol and tricosane, whereas patchouli alcohol, heneicosane and tricosane were dominant in the flower essential oil.

Czaban et al. (2013) reported antifungal activities of common soapwort's saponin fraction against *Gaeumannomyces graminis* var. *tritici* and *Fusarium culmorum*, which are pathogens of cereals.

Sengula M. et al. (2011) suggest that the methanol extracts of *Saponaria officinalis* contain compounds with antimicrobial properties. These exhibited properties suppose that such extracts can possibly be used as natural preservatives in the pharmaceutical industries and food.

Extracts from the roots of common soapwort are used as a substitute for existing acaricides, that can enable to achieve a significant reduction in the risks associated with the use of synthetic pesticides (Pavela 2017). The roots of *Saponaria officinalis* have been used for the production of traditional halva and other sweets in the food industry (Korkmaz and Ozcelik 2011).

However, there have been no scientific reports on the content of *Saponaria officinalis* L. carbohydrates. In this regard, this work is carried out to determine the chemical composition of these compounds in the study of raw materials.

Material and method

Plant materials

Herb and roots of *Saponaria officinalis* L. (common soapwort) are collected are collected in Western Ukraine, Chernivtsi region (48°15'33.1"N, 25°12'01.9"E). The aboveground part was collected during a mass flowering period and roots were collected in autumn after the death of the aboveground parts in 2019. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine).

Standards and chemicals

Standard of polysaccharides, including D-mannose, L-rhamnose, D-ribose, D-galactose, D-arabinose, D-fructose, D-xylose, D-glucose, D-sorbitol, D-saccharose, D-fucose, derived from Sigma-Aldrich (St. Louis, MO, USA) were of analytical grade (> 95% purity) (Figure 1). All other reagents were of analytical grade (≥ 99% purity).

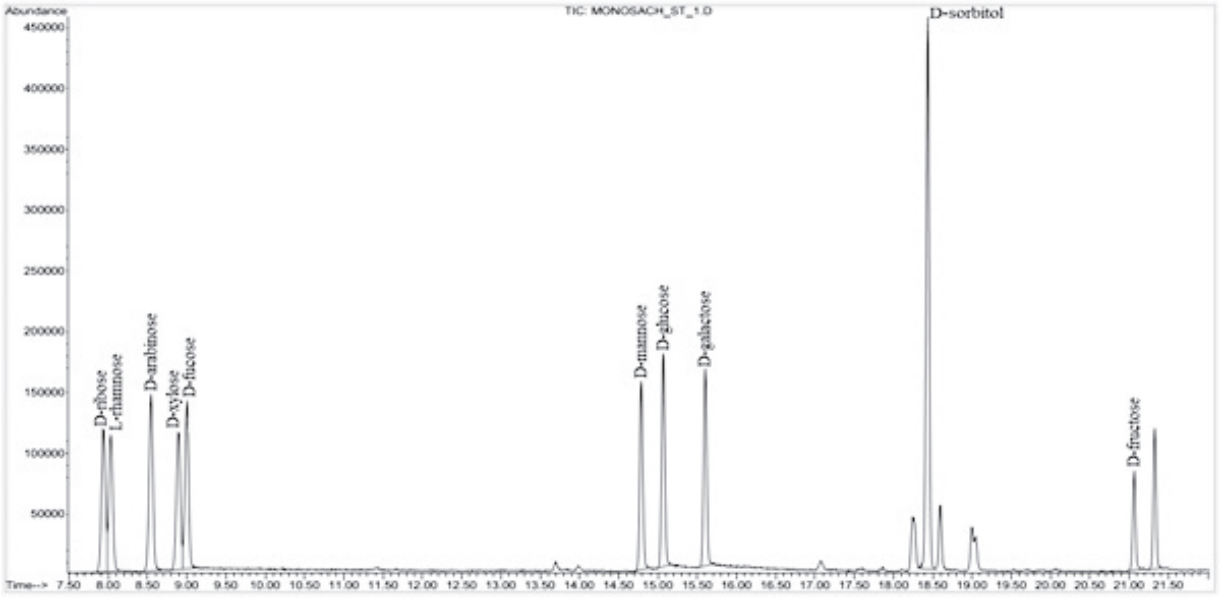

Figure 1. GC/MS chromatogram of monosaccharides standards.

GC/MS determination of carbohydrates

GC/MS analysis of monosaccharides composition of *Saponaria officinalis* L. herb and roots was performed using gas chromatograph Agilent 6890N with 5973 inert mass detector (Agilent Technologies, USA) and a capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm). The oven temperature was initially set at 160 °C, held for 8 min, then raised to 240 °C at the rate of 5 °C/min and finally kept at this point for 6 min. Injections were made in the split mode 1:50. The detection was performed in the SCAN mode at the width range of 38–400 m/z. Helium was used as the carrier gas at a constant flow rate of 1.2 ml/min.

Sample Preparation

For the extraction of bonded monosaccharides (monosaccharides after hydrolysis) 500 mg of powdered roots or herb of the common soapwort was placed into the flask and added 5 ml of 2 M trifluoroacetic acid. Hydrolysis was performed in the ultrasonic bath under 100 °C for 6 hours. Then, 2 ml of obtained hydrolysate were evaporated to dryness and 2 ml of an internal standard (sorbitol) was added (Husak et al. 2018; Marchyshyn et al. 2021a).

For the extraction of free monosaccharides, 10 ml of methanol solution with internal standard (sorbitol) (0.5 mg per sample) was added to 500 mg of powdered raw material. The extraction took place at 80 °C for 4 hours. To obtain acetylated aldonitriles 2 ml of the extract was evaporated to dryness and was added 0.3 ml of derivatization reagent (32 mg/ml of hydroxylamine hydrochloride in pyridine/methanol (4:1 v/v)). The extract was kept at 75 °C for 25 min. To the samples was subsequently added 1 ml of acetic anhydride and incubated at 75 °C for 15 min. 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 internal standard of sorbitol added to the sample (Slobodianiuk et al. 2019; Huzio et al. 2020; Budniak et al. 2021a).

Statistica v 10.0 (StatSoft I nc.) program was used for descriptive statistical analysis. The level of significance was set at $^*p < 0.05$ for all statistical analyses.

Method Validation

The analytical method was validated in terms of linearity, detection limit, precision, stability, repeatability and recovery. A total of 10 standard sugars (D-mannose, L-rhamnose, D-ribose, D-galactose, D-arabinose, D-fructose, D-xylose, D-glucose, D-saccharose, D-fucose) were used for these tests.

All calibration curves were established by plotting the chromatographic peak area of monosaccharide derivatives versus the concentration of the corresponding monosaccharide solution shown in Table 1. As a consequence, the correlation coefficients (R^2>0.9991) indicate that all calibration curves had excellent linearities within the test ranges. Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) of each analyte were determined as the concentration of standard solution with S/N = 3 (signal-to-noise ratio) and S/N = 10. The results showed that the LOD values of the 10 monosaccharides were in the range from 0.192 to 1.153 μmol/L (Table 1), indicating the sensitivity of the method.

Table 1. Calibration curves, linear ranges, limits of detection (LOD), and limit of quantification (LOQ) for individual carbohydrates after GC/MS analysis.

Carbohydrates	R^2	LOD (μmol/L)	LOQ (μmol/L)
Ribose	0.9998	0.19	0.63
Rhamnose	0.9999	0.83	2.76
Arabinose	0.9999	0.22	0.73
Fucose	0.9998	0.54	1.80
Xylose	0.9995	1.15	3.83
Mannose	0.9999	0.37	1.23
Glucose	0.9999	0.29	0.97
Galactose	0.9998	0.74	2.47
Fructose	0.9996	0.57	1.90
Saccharose	0.9991	0.78	2.60

Results and discussion

GC/MS represents an effective, comprehensive and quantitative technique for analysis of carbohydrates. Thus, the qualitative composition and quantitative content of sugars in *Saponaria officinalis* L. was determined by this method. Table 1 presents the carbohydrates composition of the studied common soapwort herb and roots.

The studies have shown that *Saponaria officinalis* L. herb is mainly composed of free carbohydrates such as D-glucose (3.65 mg/g), D-galactose (0.29 mg/g), D-fructose (0.20 mg/g) and D-saccharose (3.72 mg/g) (Figure 2).

In common soapwort herb, after acidic hydrolysis and derivatization with acetylated aldononitriles, D-arabinose, D-fucose, D-mannose, D-glucose, D-galactose, D-fructose and Myo-inositol were identified too (Figure 3).

Free carbohydrates in the roots of *Saponaria officinalis* L., including D-glucose, D-galactose and D-saccharose, were determined with GC/MS method too (Figure 4).

Various monosaccharides such as D-arabinose, D-fucose, D-xylose, D-mannose, D-glucose, D-galactose and D-fructose were observed at varying degrees in the investigated roots of common soapwort after acidic hydrolysis and derivatization with acetylated aldononitriles (Figure 5).

The quantitative content of carbohydrates is presented in Table 2.

D-saccharose was common among free carbohydrates of *Saponaria officinalis* L. in the largest amounts. Its content in herb and roots of the common soapwort was 3.72 mg/g and 25.39 mg/g respectively. Saccharose (table sugar), the most common disaccharide, is formed by glycosidic bond between the α-glucose and β-fructose molecule (Pigman 2012; Niaz et al. 2020). It is most known for its role in human nutrition (Genova et al. 2007). Saccharose is a lightly assimilated macronutrient that provides

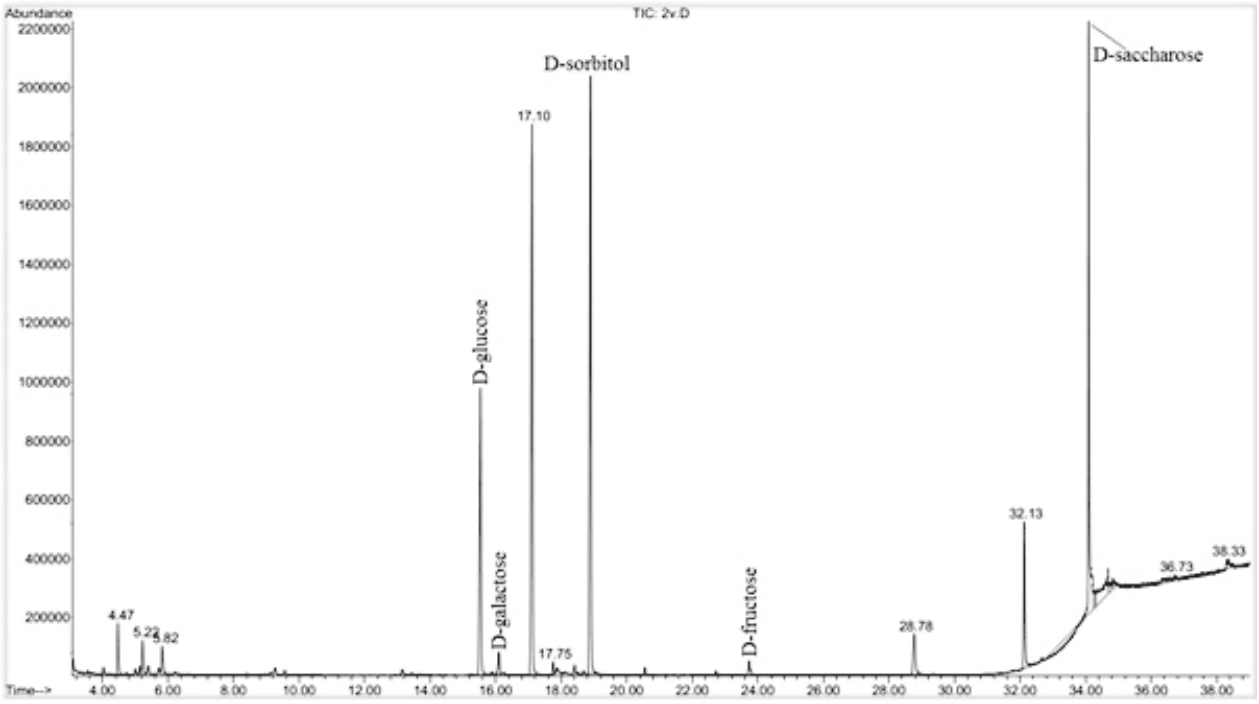

Figure 2. GC/MS chromatogram of free monosaccharides of *Saponaria officinalis* L. herb.

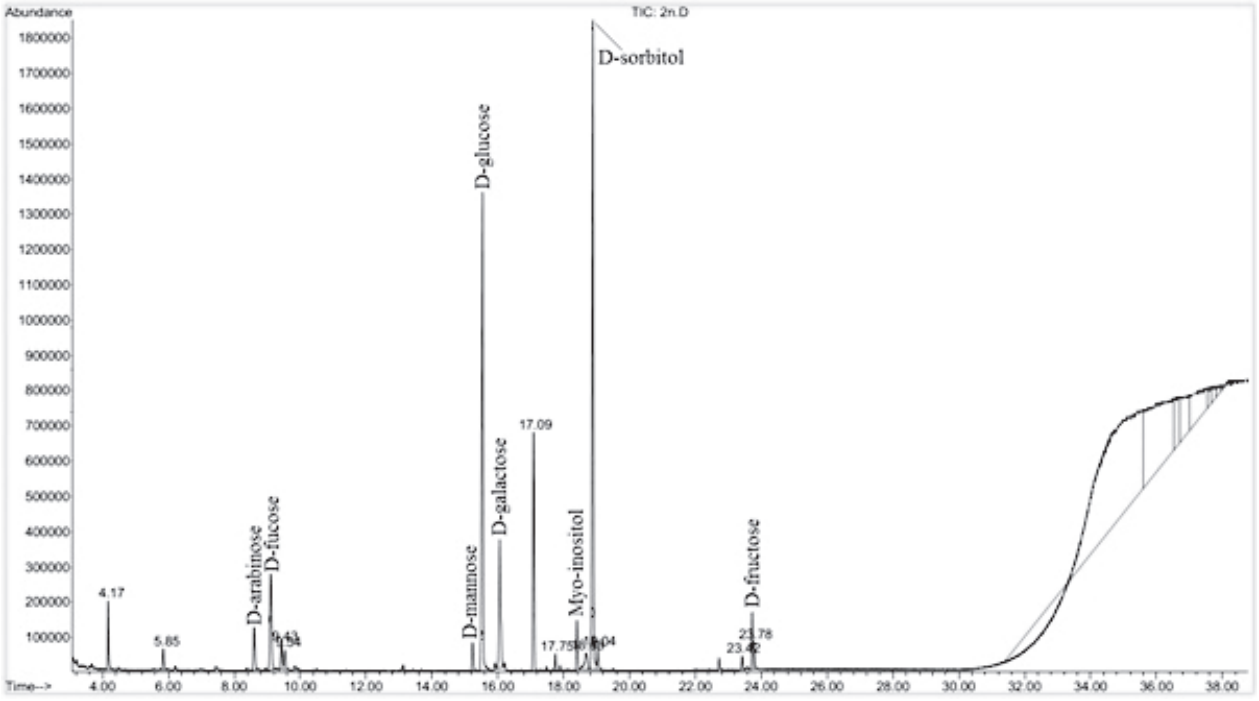

Figure 3. GC/MS chromatogram of monosaccharides and their derivatives after hydrolysis of *Saponaria officinalis* L. herb.

a fast source of energy to the organism (Khowala et al. 2008). Also, among the free carbohydrates in the herb of the common soapwort was determined a highest amount of D-glucose (3.65 mg/g). Glucose is a monosaccharide which is one of the most important carbohydrates in biology. The cell uses it as a source of energy and a metabolic intermediate (Genova et al. 2007). Glucose is synthesized during photosynthesis and serves as the "fuel" and source of energy, accrued as a polymer glycogen in animals and as starch in plants (Niaz et al. 2020). *Saponaria officinalis* L. roots were dominated by D-galactose among free carbohydrates, the content of which was 2.18 mg/g. Galactose is a nutrient, that is a reducing carbohydrate. D-galactose can be decomposed with glucose to form a lactose disaccharide (Chogtu et al. 2018).

Also, the GC/MS method identified monosaccharides and their derivatives after hydrolysis in the herb and roots of *Saponaria officinalis* L. (Figures 3, 5). D-arabino-

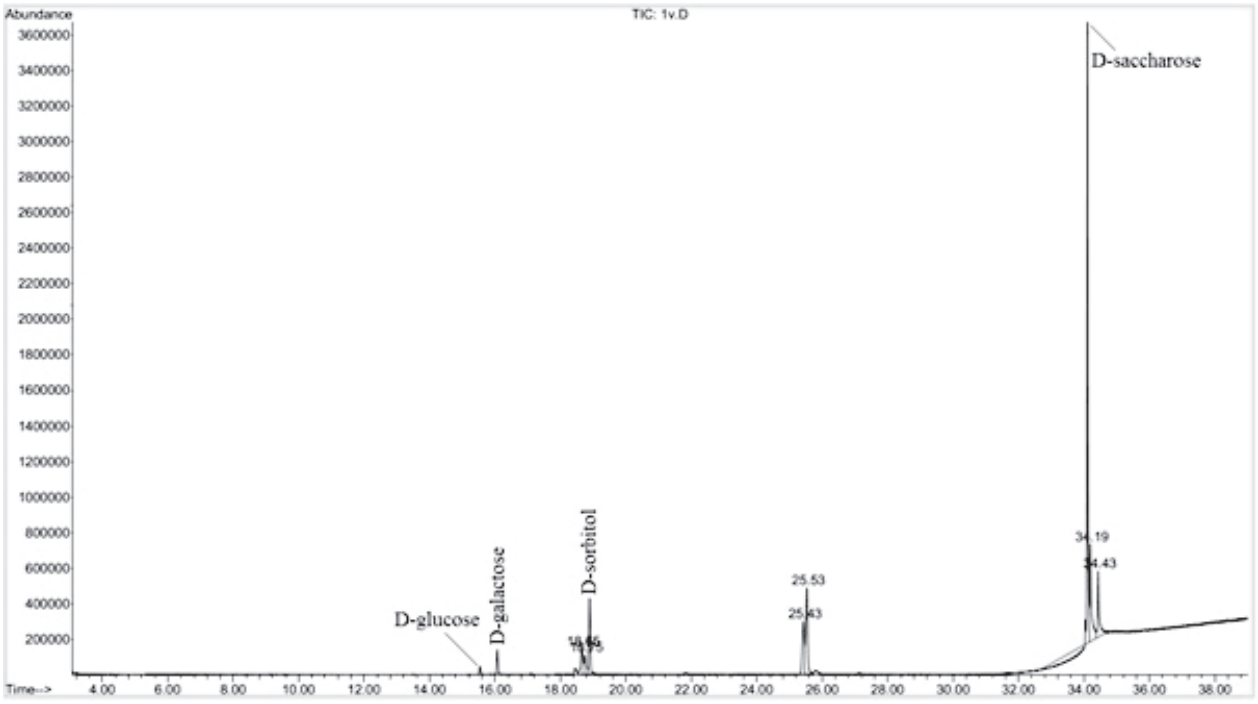

Figure 4. GC/MS chromatogram of free monosaccharides of *Saponaria officinalis* L. roots.

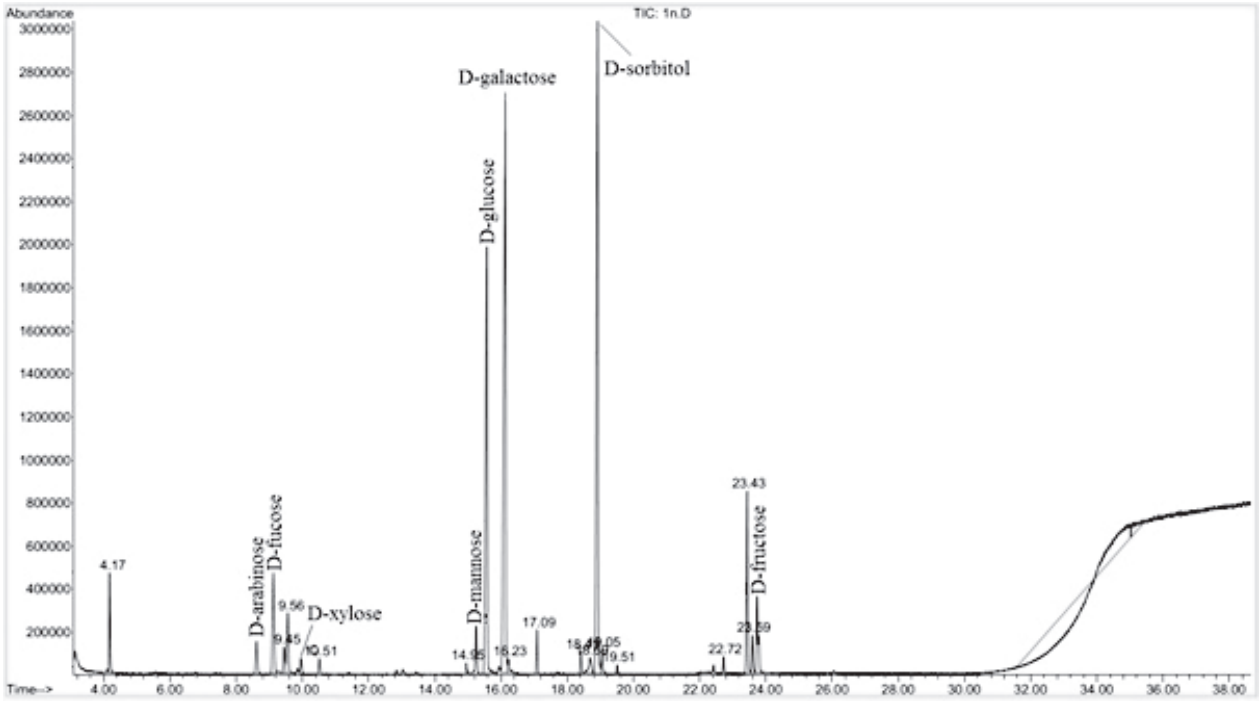

Figure 5. GC/MS chromatogram of monosaccharides and their derivatives after hydrolysis of *Saponaria officinalis* L. roots.

se, D-fucose, D-xylose and D-mannose were found in the investigated objects only after hydrolysis (Table 1). The amount of D-glucose, D-galactose and D-fructose increased significantly after hydrolysis, compared to its amount as a free sugar.

In the herb of *Saponaria officinalis* L. predominant ones were D-glucose 30.25 mg/g, D-galactose 9.17 mg/g and D-fucose 7.18 mg/g. Fucose is a deoxyhexose, which is present in a wide variety of organisms. In mammals, fucose-containing glycans play serious role in blood transfusion reactions and selectin-mediated leukocyte-endothelial adhesion. Alterations in the expression of fucosylated oligosaccharides have also been observed in several pathological processes, including atherosclerosis and cancer (Becker and Low 2003). In *Saponaria officinalis* L. herb, a great quantity of monosaccharides derivative Myo-inositol was revealed, a content of which was 2.62 mg/g. Inositol is a physiological compound appropriate to the sugar family.

Table 2. The content of monosaccharides, their derivatives after hydrolysis and free monosaccharides of *Saponaria officinalis* L.

Retention time	The name of the compounds	The content of the carbohydrates, mg/g $x^{-}\pm\Delta x^{-}$, n=3, P<0.05			
		Free carbohydrates		Monosaccharides and their derivatives after hydrolysis	
		Herb	Roots	Herb	Roots
8.61	D-arabinose	–	–	2.93±0.06	1.66±0.01
9.12	D-fucose	–	–	7.18±0.08	5.28±0.04
9.95	D-xylose	–	–	–	0.73±0.01
15.25	D- mannose	–	–	1.65±0.03	2.25±0.02
15.57	D-glucose	3.65±0.05	0.73±0.02	30.25±0.14	23.08±0.11
16.06	D-galactose	0.29±0.01	2.18±0.04	9.17±0.07	33.91±0.16
18.40	Myo-inositol	–	–	2.62±0.02	–
18.92	D-Sorbitol	internal standard			
23.73	D-fructose	0.20±0.01	–	3.68±0.05	10.79±0.09
34.11	D-saccharose	3.72±0.06	25.39±0.15	–	–

Note: – not found.

The two main stereoisomers of inositol are Myo-inositol and D-chiroinositol, which are present in our body. Myo-inositol is the harbinger of inositol triphosphate, a second messenger regulating variety hormones such as FSH, TSH and insulin (Bizzarri and Carlomagno 2014).

In the roots of common soapwort was defined the higher content of D-galactose 33.91 mg/g, D-glucose 23.08 mg/g and D-fructose 10.79 mg/g among monosaccharides after hydrolysis. Fructose is a simple monosaccharide found in many foods and one of the three very important blood sugars along with galactose and glucose (Genova et al. 2007). Fructose plays a serious role in mammalian metabolism. It is commonly regarded as being 1.73 times sweeter than sucrose (Azmat et al. 2012). D-xylose was found only after hydrolysis in the roots of *Saponaria officinalis* L. D-Xylose is a pentose sugar that is absorbed from the upper small intestinal tract, similar to the sodium-dependent active transport of glucose and amino acids (Doerfler et al. 2000). This aldopentose affects specifically gram-negative organisms. In medical practice it is used as a diagnostic remedy to assess intestinal absorption (Khowala et al. 2008).

Conclusion

To conclude, there is a growing interest in carbohydrates and their derivatives in the recent years. The carbohydrates, present in *Saponaria officinalis* L. herb and roots, have been studied by GC/MS analysis. The data revealed that four free carbohydrates, such as D-glucose, D-galactose, D-fructose and D-saccharose, were present in the herb of common soapwort. We also determined 3 free carbohydrates in *Saponaria officinalis* L. roots. The main compounds were D-glucose and D-galactose. Among the monosaccharides after hydrolysis in the herb of *Saponaria officinalis* L. prevail D-glucose (30.25 mg/g), D-galactose (9.17 mg/g) and D-fucose (7.18 mg/g). The main compounds identified in the roots were D-galactose (33.91 mg/g), D-glucose (23.08 mg/g) and D-fructose (10.79 mg/g). That allowed these carbohydrates to be considered distinguishing markers of *Saponaria officinalis* L. herb and roots. To sum up, we suggest that the common soapwort is a promising plant for medicinal purposes because of its remarkable role in a variety of biological functions.

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