RESEARCH ARTICLE

Determination of sugars and fructans content in *Stachys sieboldii*

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Abstract

Aim: This study aims to determine carbohydrate content of *Stachys sieboldii* herb and tubers, in particular, its sugars and fructans composition. The resulting data will be used with the further purpose to produce new hypoglycemic drugs of natural origin. **Materials and Methods**: Tubers and herb of Chinese artichoke (*S. sieboldii* Miq.) were analyzed for the content of sugars by gas chromatography coupled with mass spectrometry (GC/MS). Content of fructans was measured using spectrophotometry. **Results and Discussion**: The results of GC/MS analysis showed that in *S. sieboldii* herb glucose had the highest content (16.08 mg/g), followed by fructose (22.49 mg/g) and saccharose (24.34 mg/g). In the tubers, saccharose was the main compound (392.94 mg/g). In *S. sieboldii,* tubers were determined to have higher fructans content (39.92 ± 0.15%) compared to herb (20.48 ± 0.14%). **Conclusion:** The results show that *S. sieboldii* is a rich source of these important biologically active substances. These data on sugars and fructans content can be used in further studies on the spectrum of hypoglycemic action.

Key words: Fructans, gas chromatography coupled with mass spectrometry, *Stachys sieboldii*, sugars, ultraviolet spectrophotometric method

INTRODUCTION

The genus *Stachys* (Lamiaceae) includes around 300 species in the world. Many *Stachys* species are used in decoctions or infusions for the treatment of skin, stomach, rheumatic disorders, asthma, and vaginal cancer. Some members of genus have been reported to be used as anti-inflammatory and antibacterial agents. Furthermore, their antioxidant and antinephritic properties have been also reported.[1,2]

Stachys sieboldii Miq., Chinese artichoke, is native to Northern China and widely distributed in North America, Asia, and Europe.[3] The entire *S. sieboldii* plant is used as medicine, and its tuber is used as food in China, Russia, and Japan. Recent studies demonstrated that *S. sieboldii* has antianoxia action, while its inhibitory action on hyaluronidase activity is utilized in Chinese folk medicine for the treatment of ischemic stroke, senile dementia, and various gastrointestinal problems.[4] Previous phytochemical and pharmacological studies of this plant reported the isolation of terpenes, flavonoids, and phenolic compounds, and their antimicrobial, antioxidant, and antitumor actions were evaluated.[5-7]

Recently, fructans, such as stachyose, have been identified as potential development prospects found in *S. sieboldii*.[8] Fructans can affect animals and humans in various ways: They can improve intestinal health, modify lipid metabolism, modulate the immune response, and decrease the risk of intestinal and systemic diseases. Studies have shown that oligosaccharides, particularly stachyose, have good hypoglycemic effect.[9,10]

This study aimed to determine carbohydrate content of *S. sieboldii* herb and tubers, in particular, its sugars and fructans composition. The resulting data will be used with the further purpose to produce new hypoglycemic drugs of natural origin.

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MATERIALS AND METHODS

Plant Materials

Tubers and herb of Chinese artichoke (*S. sieboldii* Miq.) were collected on research grounds of Educational and Scientific Centre "Institute of Biology and Medicine," Taras Shevchenko National University of Kyiv in November 2016. The tubers and herb were dried using conventional methods and then stored in paper bags in dry place. A voucher specimen was deposited in the laboratory herbarium of the Department of Pharmacognosy and Medical Botany (TSMU, Ternopil, Ukraine). For spectrophotometric analysis of fructans, fresh tubers and herb were used. They were stored in the dark place at 5°C.

Chemicals and Standards

All reagents used were of analytical grade (≥95% purity). Standard reagents including D-mannose, L-rhamnose, D-ribose, D-galactose, D-xylose, D-arabinose, D-fucose, D-glucose, D-fructose, D-saccharose, and D-sorbitol were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 5-(hydroxymethyl) furfural (≥99% purity) was from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Sample Preparation and Gas Chromatography Coupled with Mass Spectrometry (GC-MS) Analysis

GC–MS analysis of sugars was performed using gas chromatograph Agilent 6890N with 5973 inert mass detector (Agilent Technologies, USA). Samples were analyzed on a capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm) with a (5% phenyl) methylpolysiloxane stationary phase. 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.

Identification was based on their retention times compared to the NIST 02 mass library and standards. Quantification was done using internal standard of sorbitol (1 mg/ml) added to the sample.

For the extraction of sugars, 10 ml of methanol solution with inner standard (sorbitol) (500 μg per sample) was added to 500 mg of powdered raw materials. The extraction took place in the ultrasonic water bath at 80°C for 4 h. Extracts were evaporated to dryness.

To obtain acetylated aldonitriles, 2 mL of extracts was evaporated to dry and 0.3 mL of derivatization reagent (32 mg/mL of hydroxylamine hydrochloride in pyridine/methanol [4:1 v/v]) was added. Extracts were kept at 75°C for 25 min. For acetylation of aldonitrile derivatives, 1 mL of acetic anhydride was subsequently added to the samples 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. Dichloroethane layer was dried and dissolved in 300 μL of the mixture of heptane/ethyl acetate (1:1 *v*/*v*).[11]

The amount of carbohydrates in mg/g was calculated according to the following equation 1:

$$X = \frac{Sx \times Mint \times 1000}{Sinst \times m}$$

Where: Sx - is a peak area of each monosaccharide or disaccharide,
Minst - is a mass of the internal standard,
Sinst - is a peak area of the internal standard,
m - is a mass of a plant material.

Identification of Fructans

For qualitative identification of sugar residues (fructans) in *S. sieboldii* tubers, we conducted a histochemical reaction. A small amount of Molisch's reagent (15% alcohol solution of α-naphthol) and concentrated sulfuric acid were applied to inner surface of the tubers.

General Method to Determine Total Fructans Content

The quantitative analysis for fructans employed spectrophotometric method using Lambda 25 ultraviolet (UV) spectrophotometer (PerkinElmer, USA).

The raw material of *S. sieboldii* (1.00 g) was extracted under reflux conditions (80°C) with 100.0 mL of water during 60 min. The extract was cooled to room temperature and filtered, and the volume was restored to 100 mL with water (resulting in the stock solution).

An aliquot (2.0 mL) of the stock solution was transferred to a 100.0 mL volumetric flask and 50 mL of 5% hydrochloric acid was added. Solution was hydrolyzed for 2 h under reflux conditions on water bath. A second aliquot (2.0 mL) of cooled hydrolysate was transferred to another 50.0 mL volumetric flask and made to volume with 5% hydrochloric acid, producing test solution.

The absorbance of the test solution was measured at 285 nm against blank solution. As the blank solution, we used 2.0 mL of stock solution before hydrolysis made to volume 50.0 mL with 5% hydrochloric acid.

The total content of fructans in analyzed objects was calculated, as 5-(hydroxymethyl)-2-furfural (HMF),

using equation 2, and it represents the average of five determinations. The results were expressed as the amount of fructans (%) in raw material:

$$X = \frac{D \times 100 \times 50 \times 50}{E \times 2 \times 2 \times m}$$

Where: X – the content of fructans, %;
D – optical density of analyzed object;
100 – volume of the flask, used for extract collection, mL;
50 and 50 – volumes of the flasks, used for dilution, mL;
E – specific absorption of HMF at the wavelength 285 nm;
2 and 2 – aliquots of extract for dilution and analysis, mL;
m – sample weight, g.[12]

Figure 1: Histochemical reaction to identify fructans in tubers of *Stachys sieboldii*

RESULTS AND DISCUSSION

Molisch's Test

The presence of fructans in plant tissue (positive reaction) was indicated by the appearance of purple color [Figure 1].

GC Chromatography

Gas chromatography coupled with mass spectrometry (GC/MS) was used to identify and measure the sugars content in *S. sieboldii* herb and tubers.

Identification of monosaccharides was based on their retention times compared to the NIST 02 library and standards [Figure 2].

The components of sugars from *S. sieboldii* herb were identified as glucose, galactose, undecanal, myoinositol, fructose, saccharose, and mannofuranoside [Figure 3]. The predominant ones were glucose (16.08 mg/g), fructose (22.49 mg/g), and saccharose (24.34 mg/g) [Table 1]. In tubers, we identified six sugars with the predominant one being saccharose (392.94 mg/g) [Figure 4, Table 2].

Table 1: Sugars and saccharose in ***S. sieboldii*** herb

RT, min	Peak identification number	Content of monosaccharides, (mg/g)	Substance
13.65	1	16.08	2,3,4,5,6-Penta-O-acetyl-D-gluconitrile (D-Glucose)
14.19	2	5.01	2,3,4,5,6-Penta-O-acetyl-D-galactonitrile (D-Galactose)
16.46	3	2.90	Undecanal
16.66	4	11.34	Myoinisitol, hexaacetate
19.77	5	22.49	2,3,4,5,6-Penta-O-acetyl- D-fructonitrile (D-Fructose)
33.21	6	24.34	Sucrose octaacetate (D-Saccharose)
35.77	7	6.00	β-D-mannofuranoside

S. sieboldii: Stachys sieboldii

Table 2: Sugars and saccharose of *S. sieboldii* tubers

RT, min	Peak identification number	Content of monosaccharides, (mg/g)	Substance
13.34	1	2.13	2,3,4,5,6-Penta-O-acetyl-D-manonitrile (D-Manose)
13.58	2	1.47	2,3,4,5,6-Penta-O-acetyl-D-gluconitrile (D-Glucose)
14.13	3	3.82	2,3,4,5,6-Penta-O-acetyl-D-galactonitrile (D-Galactose)
19.72	4	1.30	2,3,4,5,6-Penta-O-acetyl- D-fructonitrile (D-Fructose)
33.29	5	392.94	Sucrose octaacetate (D-Saccharose)

S. sieboldii: Stachys sieboldii

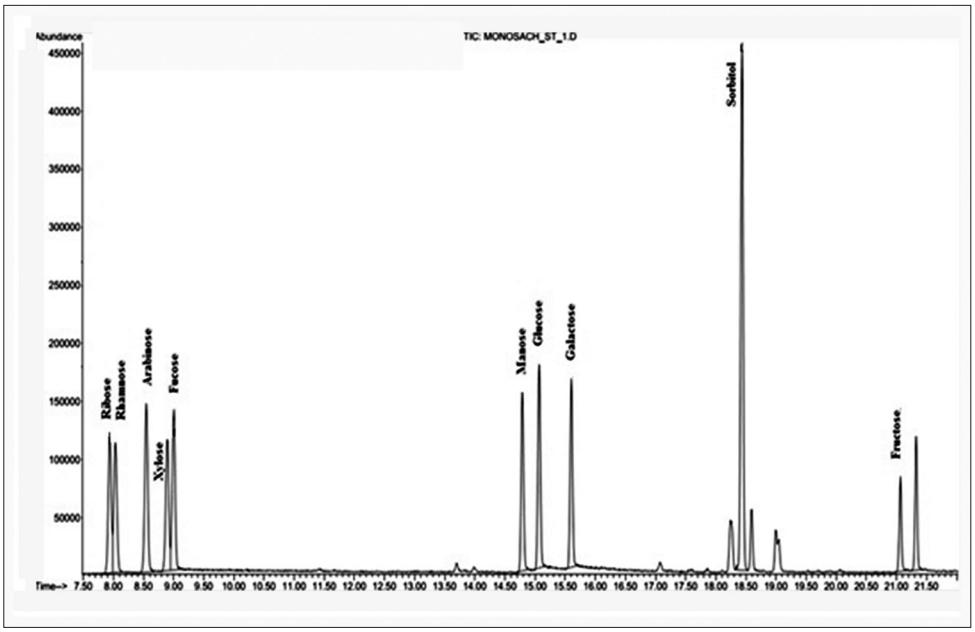

Figure 2: Gas chromatography coupled with mass spectrometry chromatogram of sugars standards

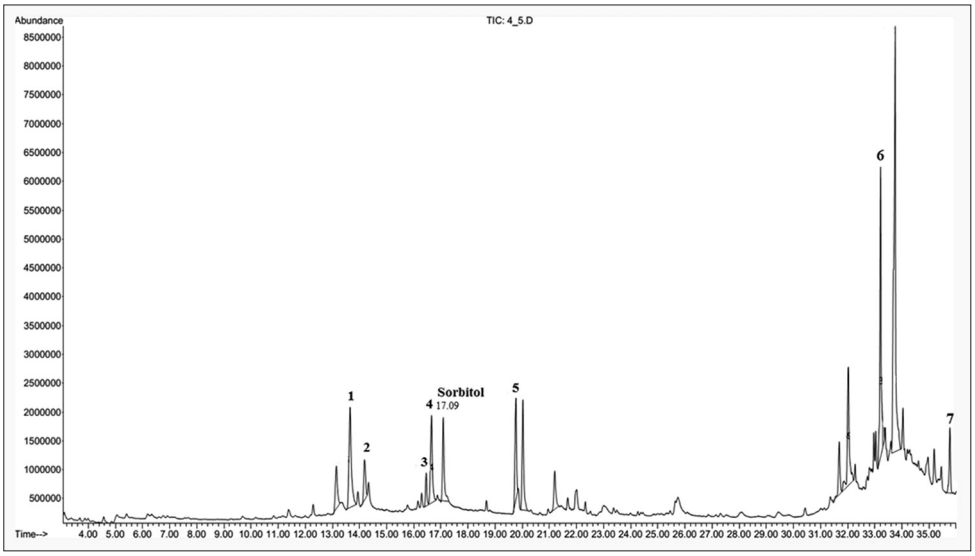

Figure 3: Gas chromatography coupled with mass spectrometry chromatogram of sugars and saccharose of *Stachys sieboldii* herb: 1 – D-glucose; 2 – D-galactose; 3 – undecanal; 4 – myoinisitol; 5 – D-fructose; 6 – D-saccharose; 7 – β-D-mannofuranoside

Spectrophotometric Analysis of Fructans

This technique is based on the formation of furfural derivatives, namely, HMF, during acid hydrolysis of sugars (fructose and saccharose) while heating with concentrated acids. Furfural derivatives have absorption at the wavelengths of 200–380 nm.

The peak amount of HMF formed after 2 h of hydrolysis start and the maximum absorption was observed at 285 nm [Figure 5].

Results of quantification of fructans in different parts of *S. sieboldii* plant indicate that tubers have higher content of fructans (39.92 ± 0.15%) in comparison to the herb (20.48 ± 0.14%).

CONCLUSION

GC/MS method was used to determine the composition of sugars. UV spectrophotometric analysis was utilized to quantify fructans. The results show that *S. sieboldii* is a rich source of these important biologically active substances. These data on sugars and fructans content can be used in further studies on the spectrum of hypoglycemic action.

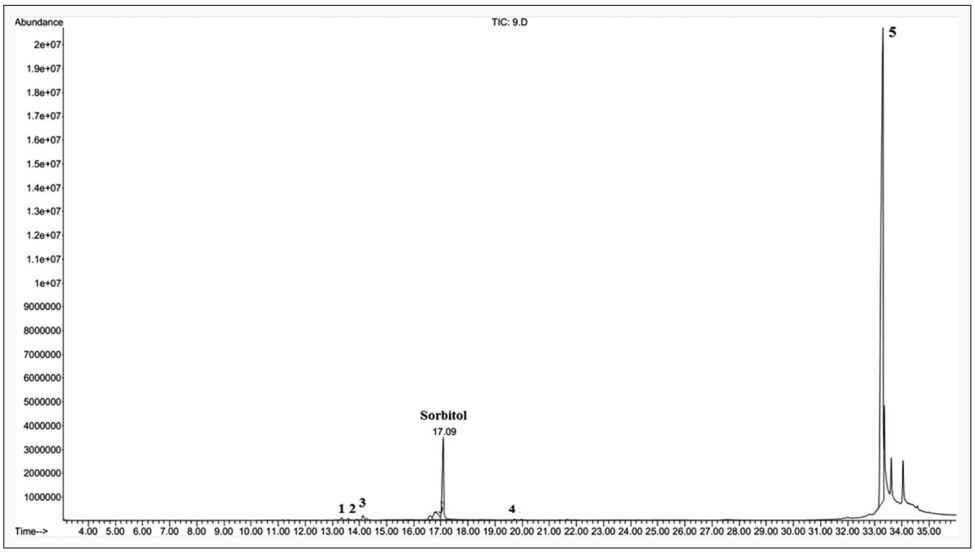

Figure 4: Gas chromatography coupled with mass spectrometry chromatogram of sugars and saccharose of *Stachys sieboldii* tubers: 1 – D-manose; 2 – D-glucose; 3 – D-galactose; 4 – D-fructose; 5 – D-saccharose

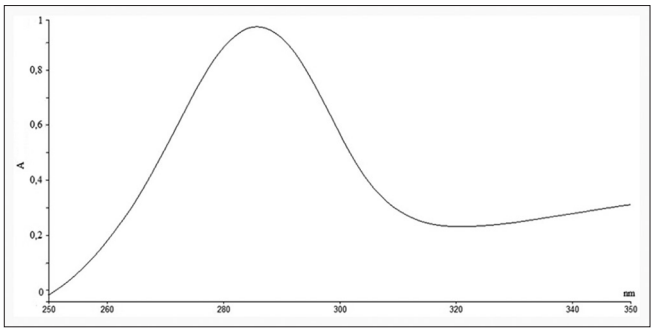

Figure 5: Ultraviolet spectrum for pure 5-(hydroxymethyl)-2-furfural

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