

DETERMINATION OF CARBOHYDRATES CONTENT IN *GENTIANA CRUCIATA* L. BY GC/MS METHOD

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ABSTRACT

Objective: Thus, the aim of our research was to determine the qualitative composition and quantitative content of carbohydrates in the studied plant material with the prospect of its application as a medicinal plant raw material.

Methods: The carbohydrates of the herb of *Gentiana cruciata* L. determined by GC/MS method. Identification of monosaccharides was based on comparing their retention times with retention times of standards of the mass spectral library NIST 02. Quantification was done by using sorbitol added to the sample.

Results: The quantitative content of 4 free carbohydrates such as D-saccharose (38.39 mg/g), D-Pinitol (12.01 mg/g), D-glucose (10.05 mg/g) and D-fructose (1.69 mg/g) was established in the herb of *Gentiana cruciata* L. Also, this method established the qualitative composition and quantitative content of eight carbohydrates (monosaccharides and their derivatives after hydrolysis): D-glucose (29.66 mg/g), D-Pinitol (22.24 mg/g), L-arabinose (4.26 mg/g), D-galactose (3.55 mg/g), D-xylose (1.80 mg/g), L-rhamnose (1.49 mg/g), D-Dulcitol (0.76 mg/g) and D-mannose (0.44 mg/g).

Conclusion: The results of the study showed that carbohydrates from the *Gentiana cruciata* L. can be used as important resources of new ingredients for the pharmaceutical industry.

Keywords: *Gentiana cruciata* L., Carbohydrates, GC/MS, Herb

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INTRODUCTION

The *Gentianaceae* family has 99 genera and about 1736 species (24 species grow in Ukraine), which are distributed mainly in subtropical and moderately warm regions of both hemispheres of Earth, and are also found in mountainous areas of the tropics [1, 2]. Many *Gentians* species are widely distributed in the Himalayas, Pyrenees and alpine mountains across Eurasia, with the largest species variety occurring on the Qinghai-Tibet Plateau [3-6]. In temperate latitudes and in the mountains, *Gentianaceae* is dominated by perennial, rarely annual plants; in subtropics and tropics the family is represented by shrubs, vines, small trees up to 5 m tall [1]. Plants of this family have a long history of usage, minor side effects, and high tolerability, regardless of the age of patients, and are the objects of interest in our society [7, 8]. The following genera belong to the *Gentianaceae* family: *Gentiana* L., *Menyanthes* L., *Centaureum* Hill., *Swertia* L., etc. The genus *Gentiana* L. is the largest genus of the family *Gentianaceae*, which includes more than 400 species [5]. The plants of the family *Gentianaceae* perform a major role in human lives because of their pharmacological (production of rich, specific secondary metabolites) and horticultural values (beauty of the flowers; transformation in shape and size of the leaves) [9, 10]. They accumulate flavonoids, xanthenes, iridoids, secoiridoids and other metabolites, typical for exceptional species used in medicine [2, 11]. The major important substances responsible for the bioactivities of the *Gentiana* species are swertiamarin, gentiopicroside and loganic acid [12, 13]. The therapeutic values of *Gentiana* are extensive, including analgesic, antirheumatic, diuretic, anti-inflammatory, hypoglycemic and antipyretic properties [12, 14-16]. Many species of *Gentiana* are highly used in the world for their pharmaceutical purposes, *Gentiana lutea* L. in Europe, *Gentiana kurroo* Royle in Pakistan and India, *Gentiana tibetica* King in China [17-19]. Some *Gentiana* species are rare or endangered plants because of their usage by humans. But

modern biotechnological methods in combination with *in vitro* cultures, offer alternative approaches to traditional cultivation methods, which leads to rapid micropropagation of *Gentianaceae* species, such as *Gentiana cruciata* L. [20, 21]. *Gentiana cruciata* L. owing to its blue flowers and easy cultivation, is popular in gardening [21]. Its aboveground and underground parts are the source of secoiridoid glycosides such as swertiamarin, sweroside and gentiopicroside [22-26]. There is established the pharmacological usage of *Gentiana cruciata* L., such as antimicrobial, anticholinesterase, hepatoprotective, antigenotoxic and antioxidant [27]. Its root has been used for its sedative and stomachic effects in folk medicine. Besides, it stimulates the production of white blood cells [28, 29]. Fatma Senol and other Turkish scientists have established the inhibitory effect of ethyl acetate extract of *Gentiana cruciata* L. root on butyrylcholinesterase, which is contained in structures located mostly outside the central nervous system [30]. Its choleric activity was also experimentally established [31]. Based on this review, it is apparent that *Gentiana cruciata* L. should get more attention as the source of bioactive compounds and phytochemicals. Considering the lack of study of carbohydrates, their definition in *Gentiana cruciata* L. is relevant. Accordingly, the aim of our research was to determine the qualitative composition and quantitative content of carbohydrates in the studied plant material with the prospect of its application as medicinal plant raw material.

MATERIALS AND METHODS

Plant materials

The herb of *Gentiana cruciata* L. was collected in Western Ukraine, at the territory of Volove, Ternopil region (N 49°21'13.0" E 26°05'24.1") during the flowering period in 2017. The herb was authenticated by professor Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). A voucher specimen no. 135 is kept at the Department of

Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine. The study plant material was dried using conventional method and stored in paper bags in a dry place [32].

Chemicals and standards

All standards of polysaccharides were of analytical grade 99% purity). Standards of polysaccharides including D-fructose (Fru), D-xylose (Xyl), D-glucose (Glc), D-mannose (Man), D-arabinose (Ara), L-rhamnose (Rha), D-galactose (Gal), D-ribose (Rib), D-saccharose (Sac), D-sorbitol were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Sample preparation, gas chromatography coupled with mass spectrometry (GC/MS) determination of carbohydrates

The monosaccharides composition of *Gentiana cruciata* L. herb was determined by GC/MS method on gas chromatograph Agilent 6890N with 5973inert mass detector (Agilent Technologies, USA). Samples were analyzed on a capillary column HP-5MS of 30 m in length and an internal diameter of 0.25 mm, a thickness of the stationary phase is 0.25 μ m. Firstly, we set up an oven temperature at 160 °C and held for 8 min, then raised to 240 °C at the rate of 5 °C/min and kept at this point for 6 min. At a constant flow rate of 1.2 sm³/min Helium was used as the carrier gas. Detection was performed in the SCAN mode at the width range of 38–400 m/z.

Extraction of free monosaccharides. 0.5 mg of methanol solution with internal standard (sorbitol) was added to 500 mg of powdered raw materials. The extraction was performed at the ultrasonic water bath at 80 °C for 4 h.

Extraction and hydrolysis of bonded monosaccharides. For the extraction of bonded monosaccharides or monosaccharides after hydrolysis 500 mg of the powdered herb of the *Gentiana cruciata* L. was placed into the flask and added 5 ml of 2 M trifluoroacetic acid. Hydrolysis was performed under 100 °C for 6 h. 2 ml of obtained hydrolysate was evaporated and was added 2 ml of an internal standard.

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 incubated 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 dichloromethane was added and the excess of the derivatization reagents was removed by the double extraction with water and 1 M hydrochloric acid. The dichloroethane layer was dried and dissolved in 300 μ l of the mixture of ethyl acetate/heptane (1:1, v/v).

Identification of monosaccharides was based on comparing their retention times with retention times of standards of the mass spectral library NIST 02. Quantification was done by using sorbitol added to the sample [33-36].

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

$$X = \frac{S_x \times \text{Minst} \times 1000}{S_{\text{inst}} \times m}$$

Where: S_x —is a peak area of each monosaccharide or disaccharide;

Minst —is a mass of the internal standard;

S_{inst} —is a peak area of the internal standard;

m —is a mass of plant material [37].

Statistical analysis

The assays were carried out three times. The obtained results were expressed as mean values and standard deviation. Values were determined using the Statistica v 10.0 (StatSoft I nc.) program. The level of significance was set at * $p < 0.05$ for all statistical analyses.

RESULTS AND DISCUSSION

The GC/MS method determined the qualitative composition and quantitative content of carbohydrates. Free carbohydrates in the herb of the *Gentiana cruciata* L. included D-saccharose (D-Sac), D-fructose (D-Fru), D-glucose (D-Glc) and D-Pinitol (fig. 1).

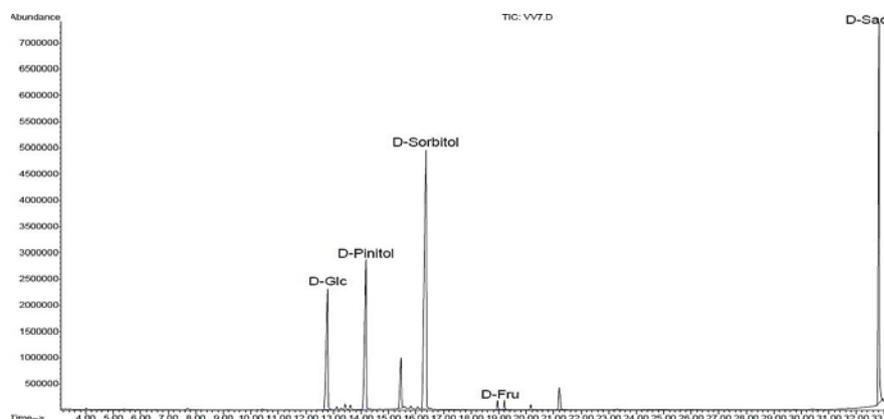


Fig. 1: GC/MS chromatogram of free carbohydrates of *Gentiana cruciata* L. herb

Table 1: The content of monosaccharides, their derivatives after hydrolysis and free carbohydrates of *Gentiana cruciata* L

The name of the carbohydrate	The content of the carbohydrate, mg/g $\bar{x} \pm \Delta \bar{x}$, n=3, P<0.05	
	Free carbohydrates	Monosaccharides and their derivatives after hydrolysis
L-rhamnose	—	1.49±0.03
L-arabinose	—	4.26±0.08
D-mannose	—	0.44±0.01
D-glucose	10.05±0.13	29.66±0.36
D-galactose	—	3.55±0.04
D-xylose	—	1.80±0.03
D-fructose	1.69±0.04	—
D-Pinitol	12.01±0.21	22.24±0.29
D-Dulcitol	—	0.76±0.01
D-Sorbitol	internal standard	—
D-saccharose	38.39±0.35	—

Note: — not found.

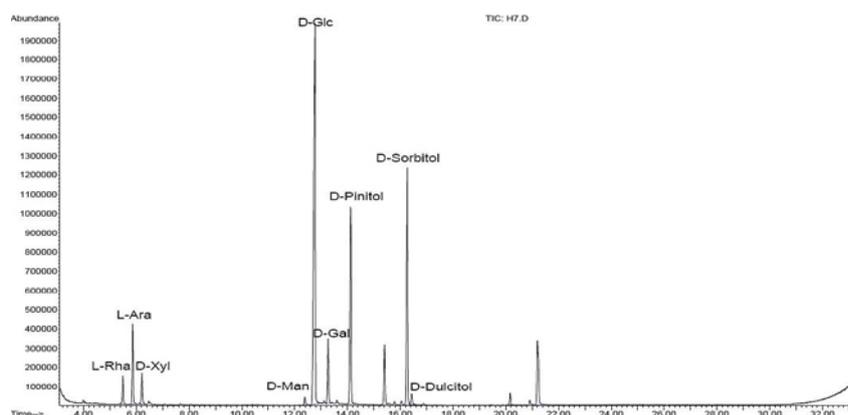


Fig. 2: GC/MS chromatogram of monosaccharides and their derivatives after hydrolysis of *Gentiana cruciata* L. herb

In this analyzed material, after acidic hydrolysis and derivatization with acetylated aldonitriles, L-rhamnose (L-Rha), L-arabinose (L-Ara), D-mannose (D-Man), D-glucose (D-Glc), D-galactose (D-Gal), D-xylose (D-Xyl), D-Pinitol, D-Dulcitol were identified too (fig. 2).

The quantitative content of carbohydrates is presented in table 1.

The GC/MS method identified 4 free carbohydrates of the herb of *Gentiana cruciata* L. (fig. 1). Free carbohydrate D-saccharose was presented in the studied raw material in the greatest amount 38.39 mg/g. Saccharose is a disaccharide that is produced only by plants and is a substrate for fructan synthesis [38]. It is an easily assimilated macronutrient that provides a quick source of energy to the body [39]. Also, the GC/MS method identified 8 carbohydrates (monosaccharides and their derivatives after hydrolysis) of *Gentiana cruciata* L. herb (fig. 2).

A simple monosaccharide D-glucose was present in the herb of *Gentiana cruciata* L. in the greatest amount, 29.66 mg/g. Glucose is a source of energy for many organisms, from bacteria to humans. The brain is very demanding in terms of energy metabolism [40]. Its functions, such as cognitive learning, memory and thinking, are interlinked to the efficient utilization of glucose [41, 42]. Red blood cells and neurons have a big energy demand too [43].

Pinitol, chemically known as 3-O-methyl-D-chiro-inositol, was the second for the amount among monosaccharides and their derivatives after hydrolysis and free carbohydrates [44]. D-Pinitol's content in Free State was 12.01 mg/g and in the bonded state 22.24 mg/g. It protects the plant from adverse environmental conditions, among them water lack and a great level of salinity [45]. Moreover, D-pinitol helps to lower plasma glucose levels, promote the lipid profile, has antidiabetic and antioxidant effects [46, 47]. Also, the protective effect against the dangerous aftermath of oxidative stress suffered by the renal and hepatic tissues in mamma cancer was established [48]. D-pinitol's anti-tumor effect against 7,12-dimethylbenzanthracene (DMBA)-initiated carcinogenesis has been investigated *in vivo* and found lower tumor growth by modulating hormones and interleukins and stimulation apoptosis in cancer cells due to inhibition of necrosis factor- α [34].

In the herb of *Gentiana cruciata* L. was defined as the lower content of L-arabinose 4.26 mg/g, D-galactose 3.55 mg/g, D-xylose 1.80 mg/g. Arabinose is a prevalent component of plant cell walls and is broadly distributed in the plant world. This pentose has the potential to be used as a nutrition additive to support good health and correct obesity [49]. Galactose is a physiological nutrient that is chemically a reducing carbohydrate. It can be combining with glucose and form the disaccharide lactose [50, 51]. Xylose or wood sugar has antifungal and antibacterial properties. This aldopentose particularly affects *Candida species* and gram-negative organisms. Xylose is contained in the embryos of most eatable plants. In contradistinction to saccharose, xylose supports the rise of 'friendly flora' in the bowel, thus rising absorption of all foods and strengthening the immune system to help fight diseases. In clinical medical practice, it is used as a diagnostic remedy to assess

intestinal absorption [52]. The content of other carbohydrates is little.

CONCLUSION

In conclusion, the current results of GC/MS indicated the presence of certain carbohydrates in *Gentiana cruciata* L., that have important therapeutic activity, which corresponded to the information about the medicinal activity of this plant. The data revealed that four free monosaccharide, such as D-saccharose, D-glucose, D-Pinitol, D-fructose, were present in the raw material. We also determined 8 carbohydrates after acidic hydrolysis and derivatization with acetylated aldonitriles. The main compounds identified in *Gentiana cruciata* L. herb were D-glucose (29.66 mg/g) and D-Pinitol (22.24 mg/g). Our findings suggest that polysaccharides from the *Gentiana cruciata* L. can be used as important resources of new ingredients of the pharmaceutical industry and could be used for the development of nutraceuticals and functional foods.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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