Research Article

www.ijrap.net

POLYSACCHARIDES IN *CENTAURIUM ERYTHRAEA* RAFN.

Stoiko Liliya [1], Dakhym Iryna [2]*, Pokotylo Olena[3], Marchyshyn Svitlana [4]

[1]Assistant Professor, Department of Pharmacy Management, Economics and Technology, I. Horbachevsky Ternopil State Medical University, Maidan Voli 1, Ternopil, Ukraine

[2]Associate Professor, Department of Pharmacognosy and Medical Botany, I. Horbachevsky Ternopil State Medical University, Maidan Voli 1, Ternopil, Ukraine

[3]Associate Professor, Department of Pharmacy Management, Economics and Technology, I. Horbachevsky Ternopil State Medical University, Maidan Voli 1, Ternopil, Ukraine

[4]Professor, Department of Pharmacognosy and Medical Botany, I. Horbachevsky Ternopil State Medical University, Maidan Voli 1, Ternopil, Ukraine

Received on: 18/02/17 Accepted on: 11/04/17

***Corresponding author**
E-mail: iradakhym@gmail.com

DOI: 10.7897/2277-4343.082122

ABSTRACT

We used gas chromatography–mass spectrometry (GC–MS) to determine polysaccharide and monosaccharide content of Centaury herb. The monosaccharides present in the investigated raw material were derivatized with hydroxylamine hydrochloride in the mixture of pyridine/methanol (4:1 v/v), identified by their retention time in the gas chromatograms and compared to those of pure reference compounds. The monosaccharides were quantified by comparing their response signal to that of sorbitol as an internal standard. Six components among free carbohydrates and eight components among monosaccharides after hydrolysis were identified and quantified in the Centaury herb. Among the monosaccharides after hydrolysis glucose and pinitol had the highest content at 26.86 mg/g and 15.26 mg/g respectively. Glucose (13.07 mg/g), saccharose (7.16 mg/g) and pinitol (3.71 mg/g) had the highest content among the free carbohydrates. We used gravimetric analysis to study qualitative content of polysaccharides in dried Centaury herb. We determined that 11.80±0.01 % of total mass was water-soluble polysaccharides and 2.33±0.05 % pectin substances.

Keywords: *Centaurium erythraea*, gas chromatography, gravimetric method, monosaccharides, polysaccharides

INTRODUCTION

Centaury (*Centaurium erythraea* Rafn.) is a flowering plant in the family Gentianaceae. This is a biennial plant with basal leaf rosette, growing to the height of 15–24 cm. Its stems are branched, bearing clusters of pink or red flowers[1]. Centaury grows in dry and sandy places in Europe, Western Asia, North Africa and North America. It has also naturalized throughout eastern Australia, where it is an introduced species[2]. The whole plant is utilized in herbal medicine.

Centaury is traditionally given as a bitter tonic to increase gastric secretions, to relieve dyspeptic discomfort and loss of appetite. The herb is claimed to have antidiabetic, antitumor, sedative, anticholinesterase, anti-inflammatory and antipyretic properties[3] and possess wound healing[4] activity. Centaury is included in some cosmetic and toiletry preparations for its allergy soothing and astringent properties.

Previous studies found that centaury herb contains flavonoids, hydroxycinnamic acids, tannic substances, coumarines, fatty and organic acids, aminoacids, carotenoids and chlorophylls[5,6,7]. However, there is no published information about some primary metabolites, such as polysaccharides. Polysaccharides are energy-rich molecules providing energy for life processes as well as building parts of the cell walls of plants and animals. In animals, they play crucial role in the pathogenesis, immune system, and blood clotting responses. Polysaccharides have expectorant, laxative, demulcent, sorbent, anti-inflammatory, antiulcer, anti-viral and detoxifying actions. Many polysaccharides are used as stabilizers, fillers, emulsifiers and film coating agents in pharmaceutical industry[8]. Thus, the aim of our study was to determine the content and monomeric composition of polysaccharides in aerial parts of *C. erythraea* Rafn. collected from natural habitat.

MATERIALS AND METHODS

Plant Material

Aerial parts of *C. erythraea* were collected on the outskirts of Zboriv (Ternopil region, Ukraine) during the flowering period in July 2013. The raw material was authenticated by TSMU professor Dr. Svitlana Marchyshyn. A voucher specimen no. 133 is kept at the Department of Pharmacognosy and Medical Botany, TSMU, Ternopil, Ukraine. The plant material was dried using conventional methods and then stored in paper bags in dry place.

Chemicals and Methods

Two conventional methods for quantification of polysaccharides and determining their monomeric content were used: gas chromatography with mass spectrometry (GC-MS) and gravimetry. All reagents were of analytical grade (> 95 % purity). The chemicals were obtained from Sigma-Aldrich, USA and were: D-mannose (Man), L-rhamnose (Rha), D-ribose (Rib), D-galactose (Gal), D-xylose (Xyl), D-arabinose (Ara), D-fucose (Fuc), D-glucose (Glc), D-fructose (Fru), D-saccharose (Sac), D-sorbitol; methanol, trifluoroacetic acid, hydroxylamine hydrochloride, pyridine, dichloroethane, hydrochloric acid,

heptanes, ethyl acetate, picric acid, sodium hydrogen carbonate and sodium hydroxide.

Sample Preparation and Chromatographic Analysis

Centaury herb was powdered in glass mortar. 500 mg of raw material was placed into the flask and 5 mL of 2 M trifluoroacetic acid was added for the extraction of bonded monosaccharides or monosaccharides after hydrolysis. Hydrolysis was performed under 100 °C for 6 hours. 2 mL of hydrolysate was evaporated and 2 mL of internal standard was added.

For the extraction of free monosaccharides 500 micrograms of methanol solution with internal standard (sorbitol) was added to 500 mg of powdered centaury herb. The extraction took place in the ultrasonic water bath at 80 °C for 4 hours.

To obtain acetylated aldonitriles 2 mL of extract 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. The extract was incubated 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)[9,10].

GC-MS analysis of monosaccharides was performed using gas chromatograph Agilent 6890N with 5973inert mass detector (Agilent Technologies, USA). Samples were analyzed on 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.

The polysaccharides were hydrolyzed to produce constituent monosaccharides, which were subsequently converted into acetylated aldononitriles.

Identification of monosaccharides was based on their retention times compared to Sac standard and mass library NIST 02. Quantification was done by using internal standard of sorbitol added to the sample.

The quantities of water-soluble polysaccharides and pectin substances were determined by gravimetric method reported by Marchyshyn et al[11]. All samples were analyzed in triplicates and the results were averaged.

RESULTS

Gas chromatography coupled to mass spectrometry (GC/MS) was used to identify and measure the monosaccharides content in *C. erythraea* herb.

In the analyzed material Rha, Ara, Man, Glc, Gal, Pinitol, Inositol and Mannitol were identified after acidic hydrolysis and derivatization with acetylated aldononitriles (Figure 1). The following free carbohydrates were identified from the water-methanol solution of the samples: Glc, Pinitol, Inositol, L-Iditol, Fru and Sac (Figure 2).

The quantitative content of carbohydrates in mg/g calculated in reference to the internal standard (sorbitol) is shown in the Table 1.

Among bonded monosaccharides glucose and pinitol had the highest content − 26.86 mg/g and 15.26 mg/g respectively. Glucose (13.07 mg/g) was the major compound of free carbohydrates followed by saccharose (7.16 mg/g) and pinitol (3.71 mg/g).

The content of water-soluble polysaccharides and pectin substances in centaury was determined at 11.80±0.1 % and 2.33±0.05 % of total dry weight respectively (Figure 3).

DISCUSSION

The monosaccharides identified in this study have various roles and can cause a variety of pharmacological effects when administered to mammals.

We found that in *C. erythraea* herb the predominant monosaccharide compound was glucose: 26.86 mg/g and 13.07 mg/g among monosaccharides after hydrolysis and free monosaccharides respectively. The main function of this aldohexose is to supply energy for physiological processes. Of all mammalian organs and tissues, the brain, neurons and developing red blood cells have the highest energy demand. If concentration of glucose is low, processes requiring mental effort are deteriorated[12].

Table 1: Content of monosaccharides after hydrolysis and free carbohydrates in *C. erythraea* Rafn.

No.	Carbohydrate	Content in the plant material [mg/g]	
		Monosaccharides after hydrolysis	Free carbohydrates
1	Rhamnose (Rha)	1.46	
2	Arabinose (Ara)	0.98	
3	Manose (Man)	1.08	
4	Glucose (Glc)	**26.86**	**13.07**
5	Galactose (Gal)	3.50	
6	Pinitol	**15.26**	**3.71**
7	Inositol	1.02	0.28
8	L-Iditol		0.45
9	Mannitol	0.81	
10	Sorbitol	internal standard	
11	Fructose (Fru)		0.45
12	Saccharose (Sac)		**7.16**

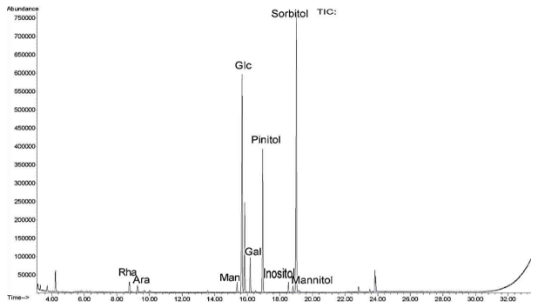

Figure 1: GC-MS chromatogram of *C. erythraea* monosaccharides after hydrolysis

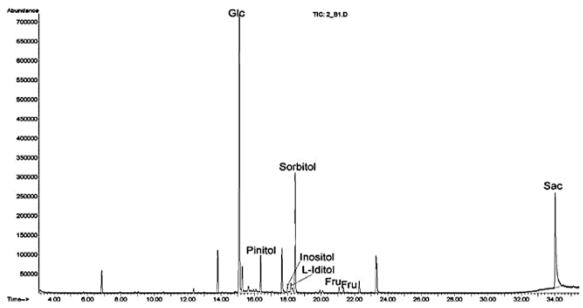

Figure 2: GC-MS chromatogram of *C. erythraea* free carbohydrates

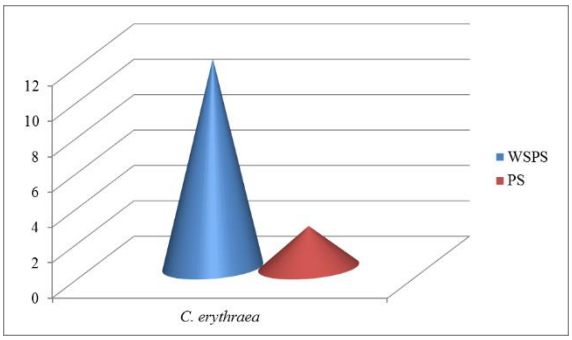

Figure 3: Water-soluble polysaccharides (WSPS) and pectin substances (PS) in *C. erythraea* Rafn.

Glucose is also a precursor for the synthesis of biologically active molecules such as vitamin C (ascorbic acid) and is involved in the synthesis of polysaccharides.

The second major identified sugar was pinitol (3-O-methyl-D-chiro-inositol). Its content in the bonded state was 15.26 mg/g and in free state 3.71 mg/g. The anti-diabetic action of pinitol was shown in normal, obese-diabetic (ob/ob) and streptozotocin (STZ)-diabetic mice when administered orally (p.o.) and intraperitoneally (i.p.)[13].

The anti-tumor effect of D-pinitol against 7,12-dimethylbenzanthracene (DMBA)-initiated rat mammary carcinogenesis have been investigated *in vivo* and its mechanism of action was determined. D-pinitol mitigates tumor growth by modulating interleukins and hormones and induces apoptosis in rat breast cancer cells through inhibition of necrosis factor-α[14].

Saccharose in free carbohydrates was present in the analyzed samples in the amount of 7.16 mg/g. It is an easily assimilated macronutrient that provides a quick source of energy.

CONCLUSION

In this study, we determined composition and quantified polysaccharides, monosaccharides and monosaccharide derivatives in dry herb of *Centaurium erythraea* Rafn., a plant used in traditional medicine preparations. We used gas chromatography coupled to mass spectrometry as a relatively simple and efficient method to determine these compounds, and these methods can be adapted to determine poly- and monosaccharide content in other herbal material. The main compounds identified in Centaurea herb were glucose, pinitol and saccharose as well as water-soluble polysaccharides and pectin substances, which, based on published animal studies, can be involved in therapeutic properties of the herb.

These results point to the need for further studies of pharmacological effects of the plant and of poly-and monosaccharides.

REFERENCES

1. Vaughan JG, Judd PA. The Oxford Book of Health Foods. Oxford: Oxford University Press; 2006.
2. Kumarasamy Y, Nahar L, Cox P J, Jaspars M, Sarker SD. Bioactivity of secoiridoid glycosides from *Centaurium erythraea*. Phytomedicine 2003; 10: 344-347.
3. Berkan T, Ustunes L, Lermioglu F, Ozer A. Anti-inflammatory, analgesic, and antipyretic effects of an aqueous extract of *Erythraea centaurium*. Planta Med 1991; 57:34-37.
4. Kupeli EA, Suntar I, Keles H, Aktay G. Evaluation of the wound healing activity of *Buxus sempervirens* L. and *Centaurium erythraea* (L.) Rafn. by using in vivo and in vitro Methods. Planta Med 2013; 13:1155.
5. Marchyshyn SM, Stoiko LI. Definition of Phenolic compounds in herbs of *Centaurium erythraea* Rafn. by HPLC. Pharmaceutical review 2014; 1:15-17.
6. Marchyshyn SM, Stoiko LI. The composition of lipophilic fraction from the herbs of *Centaurium erythraea* Rafn. Ukr Biopharm J 2015; 1:65-68.
7. Marchyshyn SM, Stoiko LI. Volatile compounds of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. Phytotherapy. Review 2016; 3:45-48.
8. Kyslychenko VS, Zhuravel IO, Marchyshyn SM, Minarchenko VM, Khvorost OP Pharmacognosy. 1st ed. Kharkiv: Golden Pages; 2015.
9. Chen Y, Xie MY, Wang YX, Nie SP, Li C. Analysis of the monosaccharide composition of purified polysaccharides in *Ganoderma atrum* by capillary gas chromatography. J Phytochem Anal 2009; 20:503.
10. Kozachok S, Marchyshyn S, Ostapchuk A, Zavyalova L. Monosaccharide composition of *Herniaria glabra* L. and

Herniaria polygama J.Gay. Curr Issues Pharm Med Sci 2016; 29: 142-145.
11. Marchyshyn SM, Demydyak OL, Dakhym IS, Berdei TS, Kozyr HR. Investigation of Polysaccharide complexes of Asteraceae Family Plants. Sci J ScienceRise 2015; 10(4, Suppl 15):31-36.
12. Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. Trends Neurosci. 2013; 36:587-597.
13. Bates SH, Jones RB, Bailey CJ. Insulin-like effect of pinitol. Br. J. Pharmacol. 2000; 130:1944-1948.
14. Rengarajan T, Nandakumar N. D-pinitol mitigates tumor growth by modulating interleukins and hormones and induces apoptosis in rat breast carcinogenesis through inhibition of NF-κB. J Physiol Biochem 2015; 71:191-204. Rengarajan T, Nandakumar N, Rajendran P, Ganesh MK, Balasubramanian MP, Nishigaki I.

Cite this article as:

Stoiko Liliya *et al*. Polysaccharides in *Centaurium erythraea* Rafn. Int. J. Res. Ayurveda Pharm. 2017;8(Suppl 2):252-255 http://dx.doi.org/10.7897/2277-4343.082122

Source of support: Nil, Conflict of interest: None Declared