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Research Article

Quantitative analysis of fatty acids and monosaccharides composition in *Chamerion angustifolium* L. by GC/MS method

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Abstract

Chamaenerion angustifolium L. widely known as fireweed, has significant natural resources in Ukraine. It has long been used in folk medicine as a sedative, analgesic, astringent, antimicrobial, anti-inflammatory, antisclerotic, wound healing, and diaphoretic agent. These properties are the result of the availability of many groups of biologically active compounds such as flavonoids, hydroxycinnamic acids, tannins, ascorbic acid, polyphenols, macro- and microelements. The aim of the study was to determine the content of fatty acids and monosaccharides of the herb. The qualitative composition and quantitative content of these biologically active compounds were determined using a GC/MS method. The results of the analysis showed that the herb of fireweed has monosaccharides, such as D-ribose, D-arabinose, D-mannose, D-glucose, D-galactose, and Myo-inositol. D-glucose presented in raw materials in the highest amount, it is content in the herb was 11.23 mg/kg. Among fatty acids, the mono-, polyunsaturated and saturated acids were determined. The content of polyunsaturated fatty acids of the total fatty acids was 53.19%, monounsaturated – 4.92%, and saturated – 41.89%. Linolenic, palmitic, and linoleic acids dominated among the determined twelve fatty acids, their content was 1.82 mg/kg, 1.74 mg/kg, and 1.10 mg/g.

Keywords

Chamerion angustifolium L., fireweed, fatty acids, monosaccharides, GC/MS

Introduction

Medicinal plants, especially those that mankind has long used in folk medicine, are a source of many biologically active substances (flavonoids, hydroxycinnamic acids, tannins, etc.). The complex of substances contained in plants determines the polyvalence of their action, affecting various systems and organs of the human body (Garnyk et al. 2002; Voloshyn et al. 2003; Ruban et al. 2012; Voloshyn et al. 2013; Budniak and Vasenda 2021e; Savych and Polonets 2021i). The purpose of using plants is the control of metabolic disorders (Marchyshyn et al. 2021c). Newly, there is a tendency to appoint herbal drugs in modern medicine, despite substantive advances in the development of synthetic drugs for the treatment of various diseases (Darzuli et al. 2019; Budniak et al. 2021c, f; Savych et al. 2021a, d; Slobodianiuk et al. 2021b; Vasenda et al. 2021). Conditioned the ever-growing needs of the industry in herbal raw materials for the manufacture of drugs, a prominent task of modern pharmaceutical science is to elongate existing and search for new plant sources (Budniak et al. 2021a; Marchyshyn et al. 2021b).

In recent decades, scientists have been interested in the phytochemical composition and pharmacological properties of plant materials belonging to the willowherb family (*Onagraceae*). Initially, this was due only to chemotaxonomy, as different species of this family contain large amounts of flavonoids, which are often considered important chemotaxonomic markers (Hevesi et al. 2006; Stechyshyn et al. 2021). However, it was later found that plants of this genus due to the diversity of representatives and versatility of biologically active substances are promising raw materials for studying their composition and properties for further reasonable use in medical practice (Granica et al. 2014; Marchyshyn et al. 2021d).

Among many promising medicinal plants, *Chamaenerion angustifolium*, widely known as fireweed (Onagraceae), which has significant natural resources in Ukraine. *C. angustifolium* has long been used in folk medicine as a sedative, antimicrobial, adstringent, and analgesic, wound healing, anti-inflammatory, diaphoretic, emollient and antisclerotic agent (Safonov 2008; Feshchenko et al. 2021a, Slobodianiuk et al. 2021a). Also, it is used in the traditional medicine of various countries around the world (Schepetkin et al. 2016; Feshchenko et al. 2021b).

C. angustifolium, which grows in the Krasnodar territory, contains a large number of polysaccharides, cellulose and lignins. Also, the plant contains carotene (3.16 mg%), rutin (15.68 mg%) and anthocyanins (30.11%) (Polezhaeva 2007a). Polyphenols are the main active compounds of aboveground parts of *C. angustifolium*, which are dominated by flavonoids, phenolic acids and tannins (enotein B) (Granica et al. 2014; Prasad et al. 2018).

The leaves of *C. angustifolium* contain organic acids, tannins, anthocyanins, carotenoids, flavonoids (quercetin, kaempferol, myricetin), ascorbic acid, pectins, polysaccharides, lignins, coumarins, tannins, sterols, triterpenes, carotenes, plain phenols and polyphenolic compounds (Hunecr et al. 1967; Polezhaeva 2007b; Monschein et al. 2015; Tsarev et al. 2016; Oleinyts et al. 2018). The flowers of *C. angustifolium* contain essential oil, the main components of which are: 3-hexen-1-ol, α-pinene, camphene, benzaldehyde, delta 3-carene, limonene, camphor, benzoacetaldehyde, terpineol, linalool, linalyl propionate, eugenol (Monschein et al. 2015).

Studies by Lithuanian scientists have also shown the presence of phenolic compounds in samples of freshly harvested and dry raw materials of *C. angustifolium*. Enotein B, rutin and quercetin predominated in all samples; caffeic and chlorogenic acids were also identified. Volatile compounds were studied and it was found that all investigated samples contain α- and β-caryophyllenes (Kaškonienė et al. 2015).

Previous studies revealed various groups of biologically active substances in *C. angustifolium*, but monosaccharides and fatty acids were not studied. Based on the literature data and analysis of the chemical composition of biologically active substances of *Chamerion angustifolium*, it is important to study the monosaccharides and fatty acids composition of this plant.

Materials and methods

Plant materials

The aboveground part of *C angustifolium* was collected during flowering period in the Ternopil region (Ukraine) in 2017 (Feshchenko 2021b). The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). The voucher specimen no. 217 is kept at the Department of Pharmacognosy and Medical Botany, TNMU (Savych and Mazur 2021f; Slobodianiuk et al. 2021c). The plant material was air-dried at room temperature (25 °C) and stored in paper bags in a dry place (Kurylo et al. 2020; Slobodianiuk et al. 2021d).

Standards and chemicals

All applied reagents were of analytical grade (≥ 95% purity). Standard reagents, including D-mannose, L-rhamnose, D-ribose, D-galactose, D-xylose, D-arabinose, D-glucose, D-fructose, D-saccharose, D-fucose, and D-sorbitol, were purchased from Sigma-Aldrich Chemical Co. (USA), as well as nonadecanoic acid, methanol, hydroxylamine hydrochloride, pyridine, hydrochloric acid, dichloroethane, ethyl acetate, and heptane (Savych et al. 2021e).

Fatty acids were identified by the reference standard mixture FAME (Supelco, Belle Fonte, PA, USA).

GC/MS determination of monosaccharides

GC/MS analysis of monosaccharides composition of the herb was performed using gas chromatograph Agilent 6890N with 5973inert 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 (Savych et al. 2021b,c). The mass spectrometer recorded the entire spectrum (SCAN mode) in range from 38 to 400 *m/z*, using electronic ionization energy at 70 eV (Budniak et al. 2021b). Scan rate (electronic) was 10 000 amu/s. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min (Husak et al. 2018; Huzio et al. 2020; Savych et al. 2021j).

Sample Preparation. For the extraction of free monosaccharides, 10 mL of methanol and 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 h. In this regard, 2 mL of the extract was evaporated to dryness 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 kept at 75 °C for 25 min. Then, for acetylation of aldonitrile derivatives, 1 mL of acetic anhydride was subsequently added to the samples and incubated at 75 °C for 15 min. Two mL of dichloroethane was added and the excess of the

derivatization reagents was removed by 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) (Budniak et al. 2021d).

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

GC/MS determination of fatty acids

GC/MS analysis of fatty acids was performed using gas chromatograph Agilent 6890N with mass detector 5973 inert (Marchyshyn et al. 2020; Savych et al. 2020; Savych and Nakonechna 2021h). First, the oven temperature was set at 60 °C for 4 min, then at a rate of 4°C/min was raised to 250 °C and kept at this point for 6 min and maintained at a final temperature for 7 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The sample with a volume of 1 µl was injected in a splitless mode using 7683 series Agilent Technologies injector. Detection was performed in SCAN mode in the range from 38 to 400 *m/z*, using electronic ionization energy at 70 eV (Marchyshyn et al. 2021a).

Accurate mass (0.5 g) of the raw material was refluxed with a 3.3 mL mixture containing (methanol: toluene: sulfuric acid (44:20:2 v/v)) and 1.7 mL of internal standard solution (undecanoic acid in heptane). The sample was maintained in the ultrasonic water bath at 80 °C for 2 h. The resulting mixture was allowed to cool and centrifuged for 10 min at 5000 × g. Then 0.5 mL of the upper heptane phase containing methyl esters of fatty acids were separated (Atolani et al. 2015).

The compositions of the product obtained were identified by comparison of their mass-spectrums with data obtained from the NIST 2008 database. The quantitative content of fatty acids was done using the internal standard of undecanoic acid in heptane solution added to the sample.

Method validation

The analytical method was validated in terms of linearity, detection limit, precision, stability, repeatability, and recovery. A total of 10 standard monosaccharides (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 a 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.

The performance of the proposed method of determining fatty acids was tested using a number of qualitative and quantitative parameters. The identification criteria for working range determination (calibration curve range) were LOQ, linearity, and calibration model fits (correlation) (Savych and Milian 2021g). Table 2 shows the values obtained for the linearity ranges, the determination coefficients (R^2), and the limits of detection (LOD) and quantification (LOQ). The working range was set as the range of concentrations from the LOQ to the maximum of the calibration curve, maintaining the correlation coefficient (R^2) above 0.995. The calibration curves were constructed over the range of 0.4–0.004 mg/mL by replicate injections (n = 3) of standard mixtures. The calibration curves, determined by the least-squares regression method were linear over the range, with R^2 above 0.995 (Table 2). It was found that the linear fit was an appropriate calibration model for all fatty acids in the analyzed samples.

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

Monosaccharide	Regression equations	Correlation coefficient R^2	Limit of detection LOD, µmol/L	Limit of quantification LOQ, µmol/L
ribose	$y = 0.0308x + 0.6322$	0.9998	0.19	0.63
rhamnose	$y = 0.0728x + 0.7383$	0.9999	0.83	2.76
arabinose	$y = 0.0211x + 0.1918$	0.9999	0.22	0.73
fucose	$y = 0.0535x + 0.1376$	0.9998	0.54	1.80
xylose	$y = 0.0254x + 0.4005$	0.9995	1.15	3.83
mannose	$y = 0.0318x + 0.1231$	0.9999	0.37	1.23
glucose	$y = 0.0235x + 0.1588$	0.9999	0.29	0.97
galactose	$y = 0.0271x + 0.7549$	0.9998	0.74	2.47
fructose	$y = 0.0177x + 0.5006$	0.9996	0.57	1.90
saccharose	$y = 0.0163x + 0.5701$	0.9991	0.78	2.60

Table 2. Performance parameters of the fatty acid determination method.

Fatty acid	Regression equations	Correlation coefficient R^2	Limit of detection LOD, µmol/mL	Limit of quantification LOQ, µmol/mL
undecanoic acid	$y = 0.0015x + 0.0236$	0.999	0.0086	0.0143
palmitic acid	$y = 0.0013x + 0.0237$	0.997	0.0435	0.0522
palmitoleic acid	$y = 0.0069x + 0.0273$	0.995	0.0176	0.0293
heptadecanoic acid	$y = 0.0102x + 0.0292$	0.995	0.0204	0.034
linoleic acid	$y = 0.0164x + 0.0487$	0.998	0.0128	0.0256
linolenic acid	$y = 0.0106x + 0.0402$	0.999	0.015	0.0432
stearic acid	$y = 0.0117x + 0.0295$	0.999	0.0265	0.0318
11-eicosenoic acid	$y = 0.0475x + 0.0402$	0.996	0.015	0.0249
arachidic acid	$y = 0.0027x + 0.0125$	0.997	0.0157	0.0262
behenic acid	$y = 0.0032x + 0.0144$	0.995	0.0206	0.0343
hexacosanoic acids	$y = 0.0132x + 0.0552$	0.996	0.0192	0.032

Results and discussion

The monosaccharides extracted from *C. angustifolium* was determined based on GC/MS analysis of the alditol acetates, formed after acid hydrolysis. The number of monosaccharides components of the herb is represented in Table 3. D-ribose, D-arabinose, D-mannose, D-glucose, D-galactose, and Myo-inositol were detected in the aerial parts after acidic hydrolysis and subsequently derivatized in acetylated aldononitriles (Fig. 1).

The major monosaccharide detected was D-glucose (11.23 mg/g). Glucose is one of the three dietary monosaccharides, along with galactose and fructose, which are absorbed directly into the bloodstream during digestion (Caballero et al. 2016). Brain is the main place of daily glucose intake and is 75% (Khowala et al. 2008). The contents of D-galactose and Myo-inositol were also the highest in the herb 1.36 mg/kg and 1.37 mg/kg, respectively (Table 3). Galactose is a reducing sugar resulting from the hydrolysis of lactose (milk sugar) by intestinal lactase to produce glucose and galactose. Two enzymes normally metabolize D-galactose in the intestinal tract: D-galactokinase and galactose-1-phosphate uridyltransferase (Lai et al. 2009). Myo-inositol is the harbinger of inositol triphosphate, a second messenger regulating a variety of hormones such as TSH, FSH, and insulin (Bizzarri and Carlomagno 2014).

The content of fatty acids is presented in Fig. 2, Table 4.

Table 3. Content of free monosaccharides of *C. angustifolium* herb.

No.	Retention time	Name	Content of the free monosaccharides, mg/kg
1.	10.96	D-ribose	0.31±0.02
2.	11.41	D-arabinose	0.35±0.01
3.	17.08	D-mannose	0.5±0.02
4.	17.36	D-glucose	11.23±0.06
5.	17.85	D-galactose	1.36±0.03
6.	20.07	Myo-inositol	1.37±0.03

The content of mono- and polyunsaturated fatty acids in the herb was greater than saturated fatty acids. The unsaturated coefficient was 1.39.

C. angustifolium herb contained palmitic acid as the main saturated acid. Its content was 1.74 mg/kg (31.69% of the fatty acids total content). The amount of other saturated acids such as behenic, stearic, arachidic, margaric, and cerotic was much lower. Polyunsaturated fatty acids of the sample were represented by linolenic and linoleic acids, their content was 1.82 mg/kg, and 1.10 mg/kg. Linoleic and linolenic acids are essential for human nutrition and are called vitamin F (Zinchenko and Kyslychenko 2011). Linolenic acid is a ω-3 fatty acid and transforms to prostaglandin E1 has blood cholesterol-reducing properties and increases immunity (Karpe et al. 2011; Kaur et al. 2014; Blondeau et al. 2015; Sears and Perry 2015; Leontiiev et al. 2019). Linoleic acid is a ω-6 fatty acids. This polyunsaturated acid ensures the production of bile acids in the liver, affects the hormonal balance, and influences the production of prostaglandins (Brown et al. 2019). A mixture of linoleic and linolenic is often used as a dietary supplement (Jalc et al. 2007).

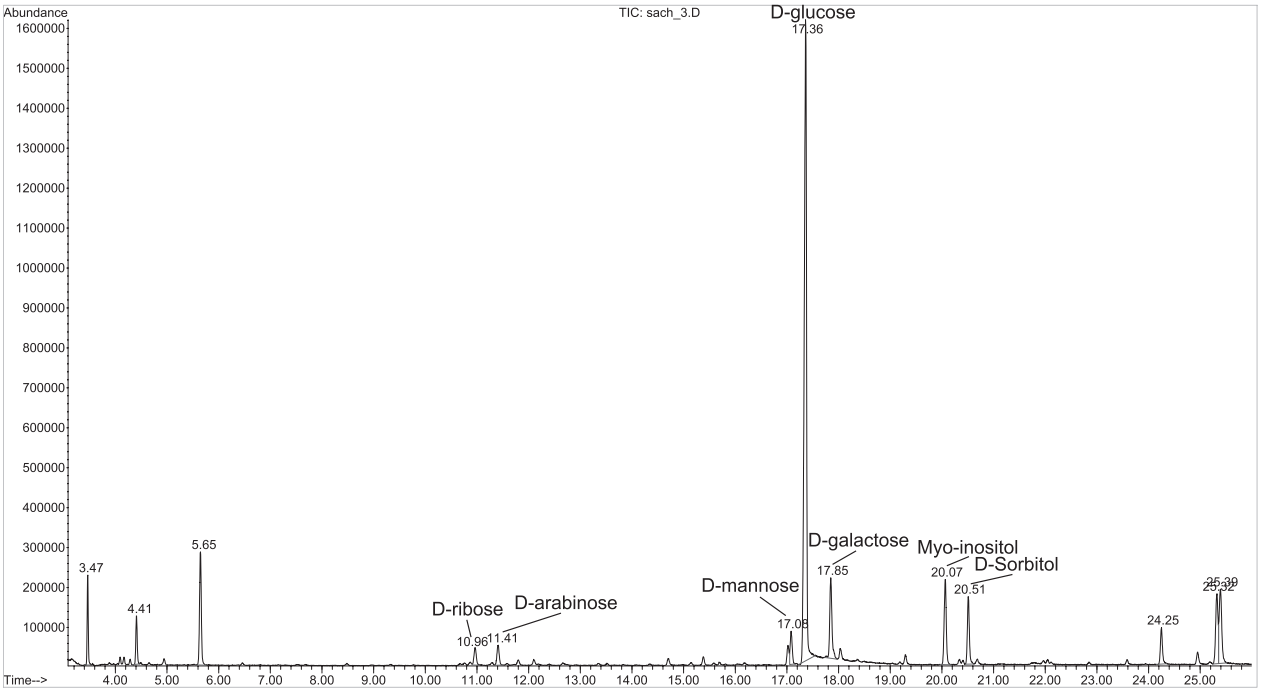

Figure 1. GC/MS chromatogram of monosaccharides in *C. angustifolium* herb

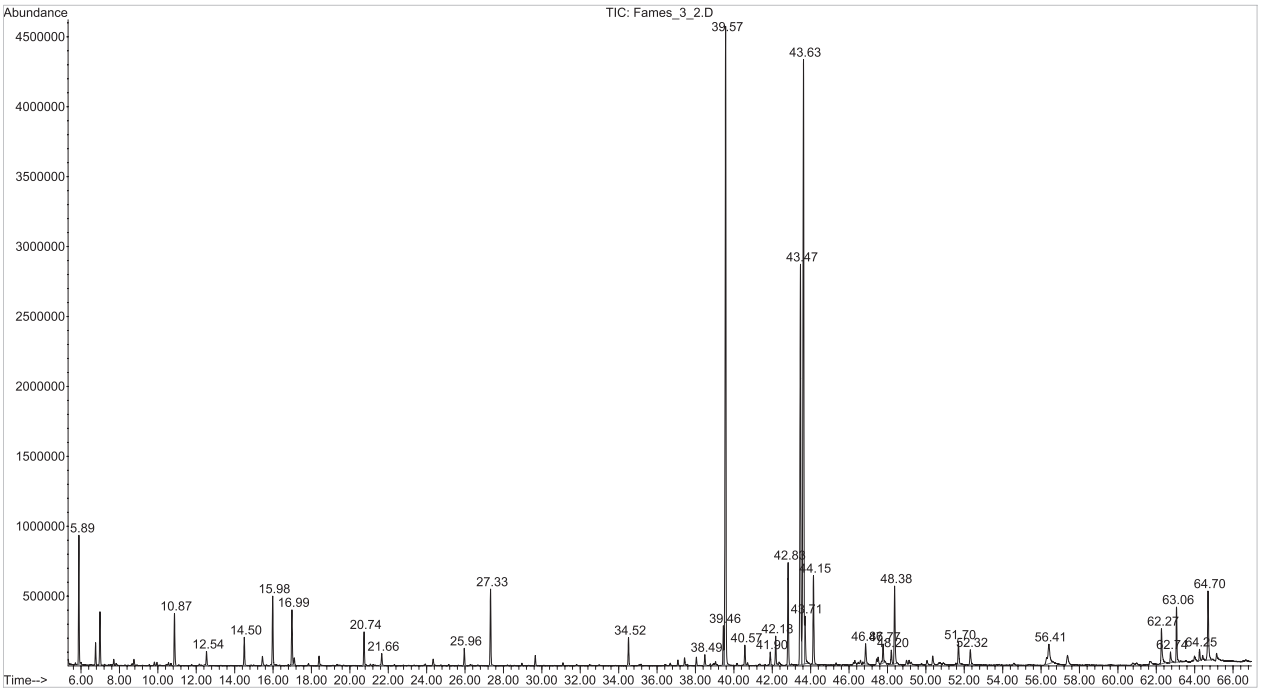

Figure 2. GC/MS chromatogram of fatty acids in the sample

Table 4. The fatty acid composition content of the herb.

No.	Retention time	Common name of fatty acid (IUPAC)	Chemical nomenclature	Quantitative content of methyl esters of fatty acids	
				mg/kg	% of the total
Saturated acids					
1.	39.57	Palmitic (hexadecanoic)	C 16:0	1.74	31.69
2.	41.90	Margaric (heptadecanoic)	C17:0	0.03	0.55
3.	44.15	Stearic (octadecanoic)	C 18:0	0.23	4.19
4.	48.38	Arachidic (eicosanoic)	C 20:0	0.22	4.00
5.	52.32	Behenic (docosanoic)	C 22:0	0.05	0.91
6.	62.74	Cerotic (hexacosanoic)	C 26:0	0.03	0.55
Monounsaturated acids (ω-7 and ω-9)					
7.	39.46	Palmitoleic (9-hexadecenoic, ω-7)	C 16:1	0.10	1.82
8.	43.71	Oleic (octadecenoic, ω-9)	C 18:1	0.13	2.37
9.	48.20	Gondoic (11-eicosenoic, ω-9)	C20:1	0.04	0.73
Polyunsaturated acids (ω-3 and ω-6)					
10.	43.47	Linoleic (octadecadienic, ω-6)	C 18:2	1.10	20.04
11.	43.63	Linolenic (octadecatrienic, ω-3)	C 18:3	1.82	33.15
Amount of saturated fatty acids				**2.3**	**41.89**
Amount of unsaturated fatty acids				**3.19**	**58.11**
Total				**5.49**	**100**

Three monounsaturated acids: palmitoleic, oleic, and gondoic acids were also determined among the unsaturated fatty acids in the herb. Palmitoleic acid is one of the most common ω-7 fatty acids. This acid is a lipokine that has potential nutraceutical use to treat nonalcoholic fatty liver disease. Also, palmitoleic acid has anti-inflammatory and antidiabetic activities (Souza et al. 2014). Oleic acid is a ω-9 fatty acid and it is used as a solubilizing or emulsifying agent in aerosol products and an excipient in pharmaceuticals. It may hinder the progression of adrenoleukodystrophy, a fatal disease that affects the adrenal glands and brain, and it may help boost memory (Choulis 2014).

Conclusion

The fatty acids and monosaccharides present in the herb of fireweed have been studied by GC/MS analysis. The results of monosaccharides analysis showed that the herb of fireweed accumulated D-glucose (11.23 mg/kg), Myo-inositol (1.37 mg/kg), D-galactose (1.36 mg/kg), D-mannose (0.5 mg/kg), D-arabinose (0.35 mg/kg) and D-ribose (0.31 mg/kg). The content of polyunsaturated fatty acids of the total fatty acids was 53.19%, monounsaturated – 4.92%, and saturated – 41.89%. Linolenic, palmitic, and linoleic fatty acids were dominant, these content was 1.82 mg/kg (33.15%), 1.74 mg/kg (31.69%), and 1.10 mg/kg (20.04%).

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