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Research Article

Analysis of carboxylic acids of *Crambe cordifolia* Steven

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Abstract

Crambe cordifolia Steven is a perennial herb and contains many biologically active substances, including amino acids, quercetin and glycosides of kaempferol. In continuation of the investigation of these plant compounds, it is advisable to study the qualitative composition and quantitative contents of carboxylic acids. Using a HPLC method the quantitative content of the following organic acids was identified and determined: pyruvic (40.66 mg/g), isocitric (12.88 mg/g), citric (8.71 mg/g), succinic (38.03 mg/g) and malic (0.75 mg/g). Among fatty acids the saturated and unsaturated acids were determined by the GC/MS method. The content of polyunsaturated fatty acids of the total fatty acids was 56.97%, saturated – 38.53% and monounsaturated – 4.50%. Linolenic and palmitic acids dominated among the determined 7 fatty acids, their content was 9.68 mg/g (47.87%) and 4.88 mg/g (24.14%). The results of the study show that *Crambe cordifolia* Steven leaves is a source of carboxylic acids.

Keywords

Crambe cordifolia Steven, fatty acids, GC/MS, HPLC, organic acids

Introduction

The importance of medicinal plants did not reduce the annual increase in the number of synthetic medicines, which often model biologically active substances of plants and are their chemical equivalents (Slobodianiuk et al. 2019). The most interesting are plants that have a long-standing history of usage (Stoiko and Kurylo 2018; Darzuli et al. 2019). 10,000-year-old traces of cultivation give evidence that plants in the family Brassicaceae are among the oldest cultivated plants known. These plants grow under various climatic conditions and accumulate different bioactive compounds that are important for human health, food and animal feed (Jahangir et al. 2009; Björkman et al. 2011; Avato and Argentieri 2015; Vergun et al. 2018).

One of the most interesting plants of Brassicaceae are the genus *Crambe* L. species, that indicate the need for their widespread introduction and investigation due to promising properties such as food, decorative, medicinal etc. (Kalista 2017; Vergun et al. 2018).

Genus *Crambe* L. is the largest genus in the family Brassicaceae. Much work has been done upon different species of *Crambe* in order to evaluate their industrial and pharmaceutical importance (Bukhari et al. 2013). For instance, *Crambe orientalis* can inhibit seed germination and shows phytotoxic effects (Razavi and Nejad-Ebrahimi 2009). *Crambe abyssinica* contains membrane active compounds (Salakhutdinov et al. 1998). According to another study, it also shows a good ratio of phenolic and flavonoid compounds (Matthäus 2002). Another member of this genus, *Crambe cordifolia*, contains amino acids, growth inhibitors and is used as livestock feed (Dudkin et al. 1977; Bukhari et al. 2013).

Nowadays the study with crambe seeds for bio diesel production has been carried out, mainly due to the high

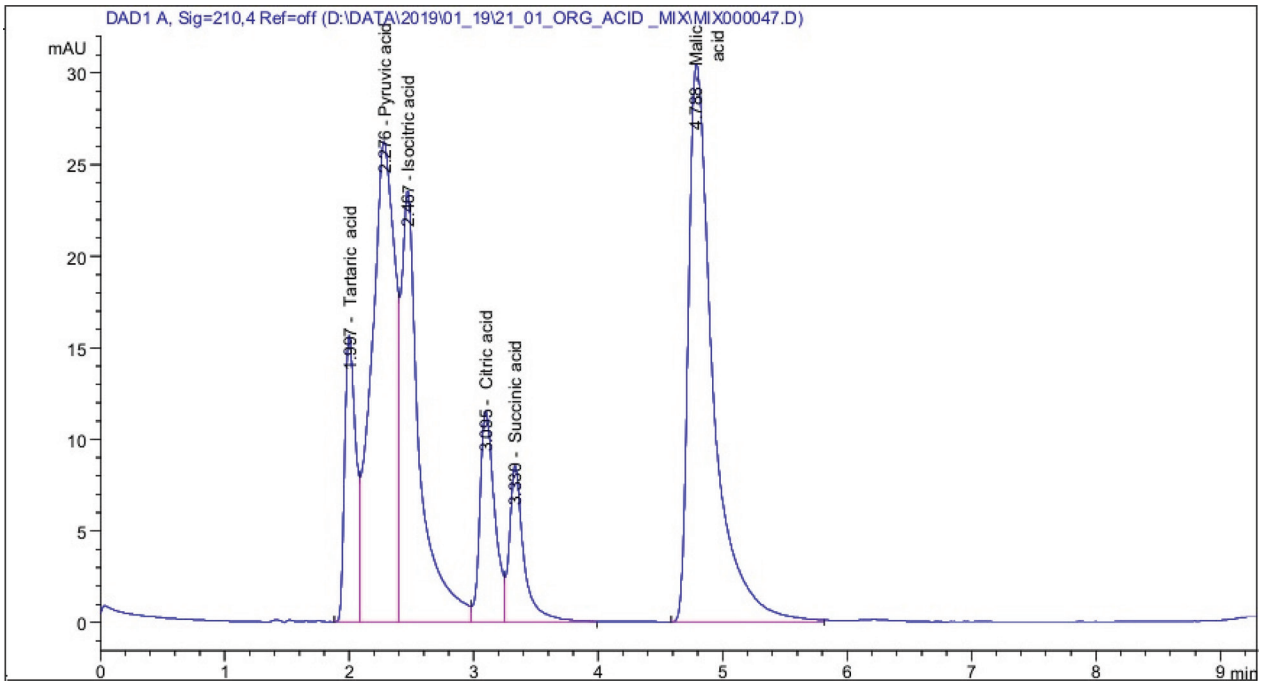

Figure 1. HPLC chromatogram of a standard mixture of organic acids.

content of oil in it (Toledo et al. 2011). As reported Wazilewski et al. (2013) the crambe biodiesel is more stable than the soybean biodiesel. Raw material of these plants has an antioxidant activity due to content of phenolic compounds and flavonoids (Bukhari et al. 2013). Plants of the genus *Crambe*, as other species of Brassicaceae, contain glucosinolates.

Crambe cordifolia Steven. is a perennial herb that may reach the height of 182 cm. It has lobed leaves with small white flowers. It contains many chemical compounds including amino acids, quercetin and glycosides of kaempferol (Dudkin et al. 1977; Aguinagalde and Del Pero Martinez 1980; Rashid et al. 2018). Typically, it contains kaempferol 3-(p-coumaroyl) glucoside-7, 4'-diglucoside and quercetin 3-feruloylglucoside-7, 4'-diglucoside. (Aguinagalde and Del Pero Martinez 1980; Bukhari et al. 2013).

The literature survey shows that there have not been many reported studies about some primary metabolites, such as organic and fatty acids in *Crambe cordifolia* Steven. This deficiency in experimental data motivated us to determine these compounds.

Materials and methods

Plant materials

Leaves of the *Crambe cordifolia* Steven. were selected as the objects of the study. The raw materials were provided by the Department of cultural flora of M. Gryshko National Botanic Garden of the National Academy of Sciences of Ukraine. The leaves were collected in summer 2018. The leaves were dried using a conventional method and stored in paper bags in a dry place (Husak et al. 2018). The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). A voucher specimen no. 253 is kept at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine.

Chemicals and standards

Standards of organic acids, including tartaric acid, pyruvic acid, isocitric acid, citric acid, succinic acid and malic acid, obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) were of analytical grade (≥95% purity) (Figs 1, 2).

Applied reagents (acetonitrile, undecanoic acid, methanol, heptane, toluene) were of the highest purity and purchased from the Sigma-Aldrich Chemical Company.

Sample preparation, HPLC method determination of organic acids

HPLC analysis of organic acids was performed using Agilent 1200 (Agilent Technologies, USA) (Basaraba 2019). Mobile phase A – acetonitrile (ACN); mobile phase B – 0.1% solution of phosphoric acid in water (1:99, v/v). Elution was performed in isocratic mode. Separation was performed on a Zorbax SB-Aq chromatographic column (4.6 mm ± 150 mm, 3.5 μm) (Agilent Technologies, USA), column flow rate 0.5 ml/min, the temperature of the thermostat column is 30 °C, volume injection 3 μl. Detection was performed using a diode-matrix detector with signal registration at 210 nm and fixation of absorption spectra in the range of 210–700 nm.

Standard solutions. Standard solutions (1000 ppm) of tartaric, pyruvic, isocitric, citric, succinic and malic

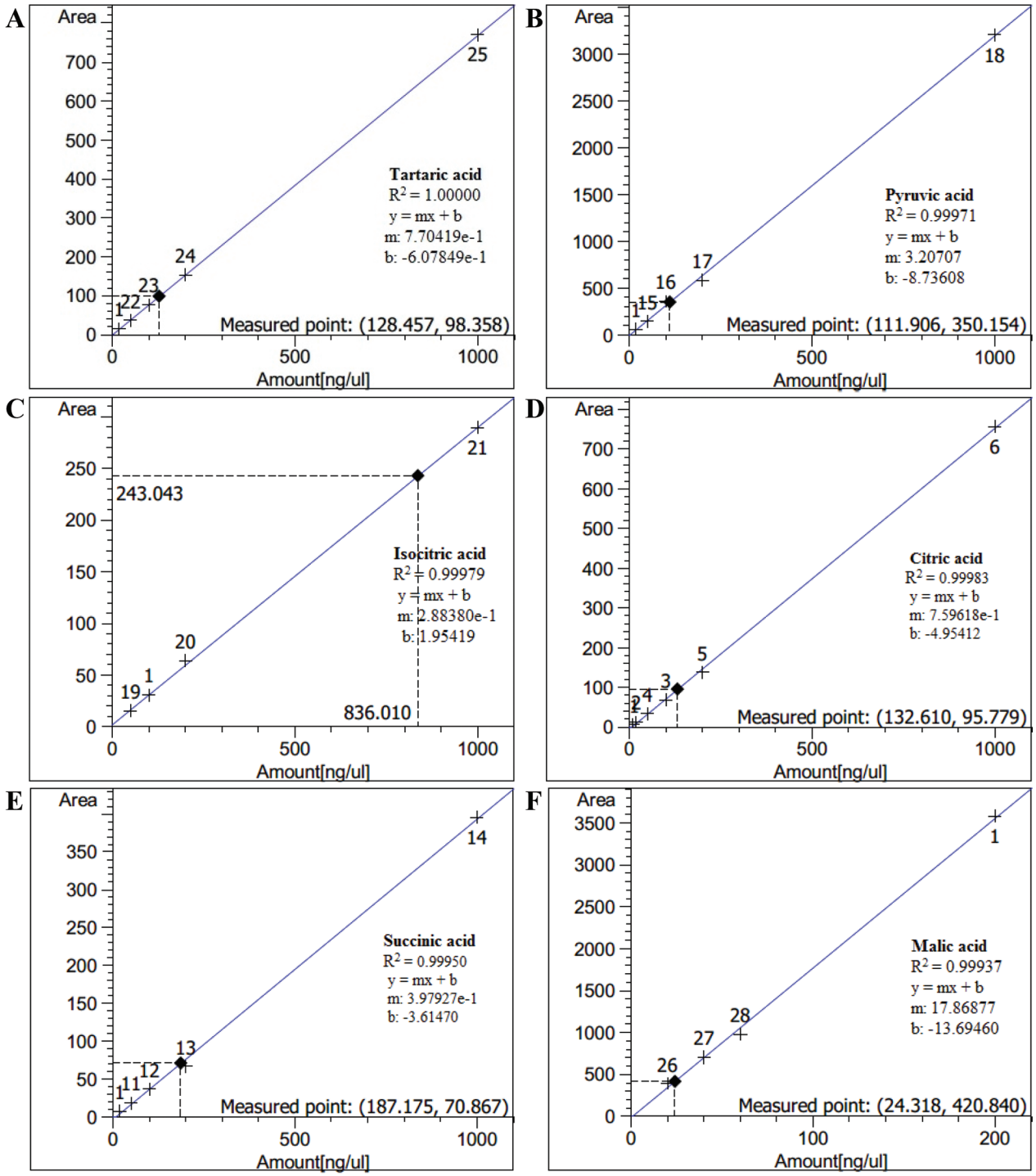

Figure 2. Analysis of organic acids by HPLC. (**A**) Calibration curves for tartaric acid; (**B**) Calibration curves for pyruvic acid; (**C**) Calibration curves for isocitric acid; (**D**) Calibration curves for citric acid. (**E**) Calibration curves for succinic acid; (**F**) Calibration curves for malic acid.

acids were prepared in the mobile phase consisting of 0.1% phosphoric acid solution. Stock solutions of every organic acid were made in the mobile phase by respective dilutions.

Extraction of organic acids from leaves of the *Crambe cordifolia* Steven. 700 mg of powdered raw material was placed in a vial and extraction in 10 ml of 0.1% solution of phosphoric acid. Extraction was performed in the ultrasonic bath at 80 °C for 4 h. 8.3 ml of the obtained extract was centrifuged at 3000 rpm/min for 30 minutes and filtered through Supelco Discovery DSC-18 filter and then concentrated to the residual volume of the extract of 20 µl.

Identification and quantification content of organic acids was performed using standard solutions of dicarboxylic compounds (tartaric, pyruvic, isocitric, citric, succinic and malic acids). The content of organic acids in µg/g was calculated by the following equation 1:

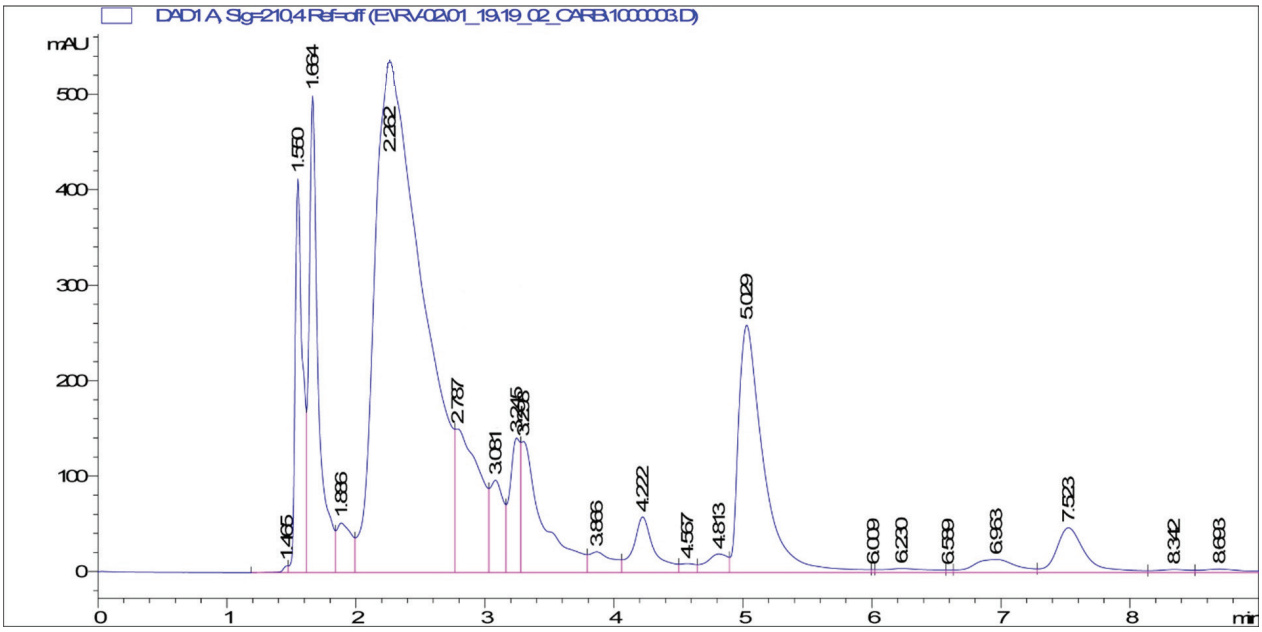

Figure 3. HPLC chromatogram of organic acids in the *Crambe cordifolia* Steven leaves.

$$X = \frac{C \times V_{\text{solv.}} \times V_{\text{inj.}}}{m \times V_{\text{actual inj.}} \times 1000},$$

where:

C – concentration, obtained from the chromatogram by calculating the reference solution and the test solution;

$V_{solv.}$ – the volume of solvent for extraction;

$V_{inj.}$ – the volume of injection standard of organic acid;

$V_{actual\ inj.}$ – the volume of injection of extract;

m – is a mass of the raw material (Ergönül and Nergiz 2010; Agius et al. 2018; Basaraba 2019).

GC/MS determination of fatty acids

GC/MS analysis of fatty acids was performed using gas chromatograph Agilent 6890N with mass detector 5973 inert (Agilent Technologies, USA) (Stoyko et al. 2015; Iosypenko et al. 2019). Samples were analyzed on a silica capillary column HP-5MS (apolar) length – 30 m, internal diameter – 0.25 mm, the diameter of sorbent grain 0.25 μm. The interface and were operated at 250 and 380 °C respectively. The initially set up oven temperature at 60 °C for 4 min, then at the rate of 4 °C/min 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 (38–400 m/z).

0.5 g (accurately mass) 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 solution). 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 rpm. Then 0.5 ml of the upper heptane phase with containing methyl esters of fatty acids was selected.

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

The amount of fatty acids in mg/g was calculated according to the following equation 2:

$$X = \frac{Sx \times M\text{inst} \times 1000}{S\text{inst} \times m},$$

where:

S*x* – is a peak area of each fatty acid,

M*inst* – is a mass of the internal standard,

S*inst* – is a peak area of the internal standard,

m – is a mass of a plant material (Garcés and Mancha 1993; Atolani et al. 2015; Stoyko et al. 2015; Iosypenko et al. 2019).

Results and discussion

In total, five organic acids were determined in the *Crambe cordifolia* Steven leaves, including pyruvic, isocitric, citric, succinic and malic acids by means of the HPLC method (Fig. 3, Table 1).

As shown in Table 1, the highest pyruvic (40.66 mg/g) and succinic (38.03 mg/g) acids content were found in *Crambe cordifolia* Steven leaves. Pyruvic acid is an important keto-carboxylic acid and has vital importance in energy metabolism and used for the productions of pharmaceuticals, such as alanine, tyrosine, etc. by existing technologies (Pal et al. 2017). The more exploitable ability of pyruvic acid encompasses exercise trim down of

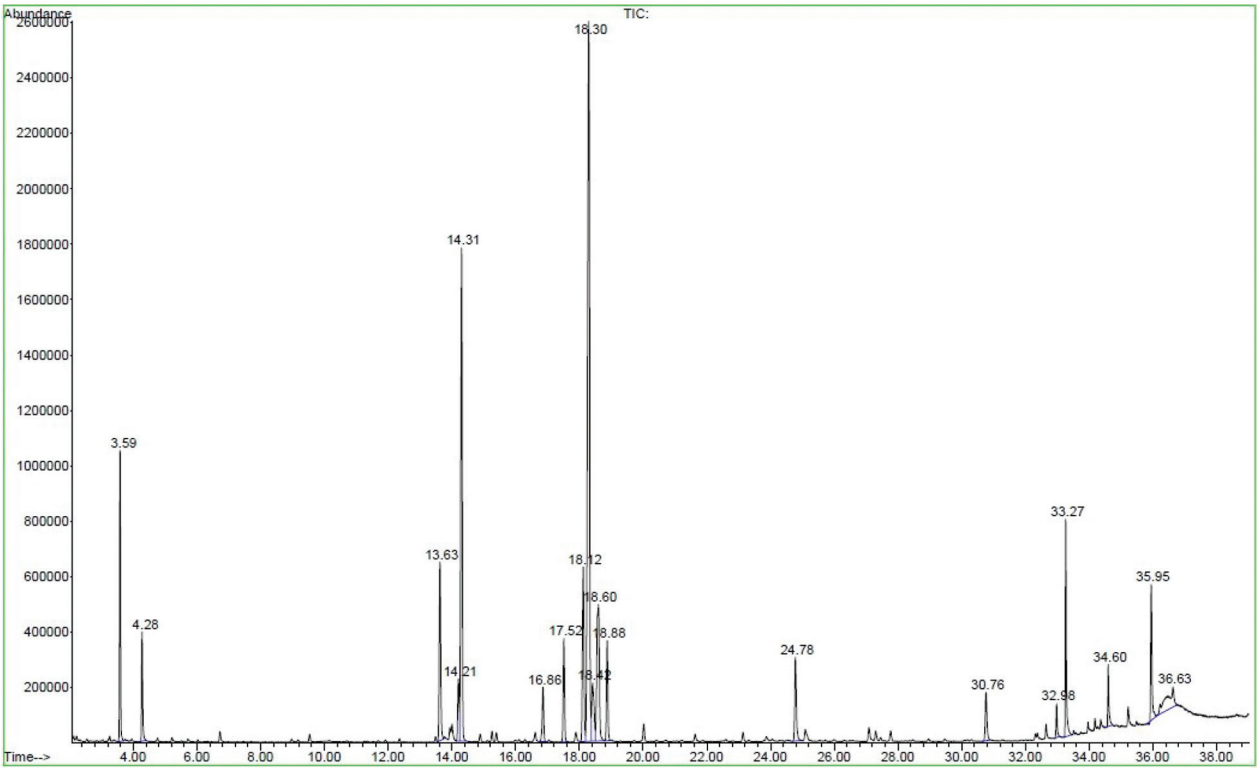

Figure 4. GC/MS chromatogram of fatty acids in the *Crambe cordifolia* Steven leaves.

Table 1. The results of the organic acids determination in *Crambe cordifolia* Steven leaves.

No.	Retention time	Common name of organic acid (IUPAC)	Molecular formula	Quantitative content (mg/g) $x^- \pm \Delta x^-$
1.	2.262	Pyruvic (2-oxopropanoic) acid	$C_3H_4O_3$	40.66 ± 0.84
2.	2.787	Isocitric (1-hydroxypropane-1,2,3-tricarboxylic) acid	$C_6H_8O_7$	12.88 ± 0.26
3.	3.081	Citric (2-hydroxypropane-1,2,3-tricarboxylic) acid	$C_6H_8O_7$	8.71 ± 0.16
4.	3.298	Succinic (butanedioic) acid	$C_4H_6O_4$	38.03 ± 0.83
5.	4.813	Malic ((2*S*)-2-hydroxybutanedioic) acid	$C_4H_6O_5$	0.75 ± 0.02

Table 2. The results of the determination of fatty acids in *Crambe cordifolia* Steven leaves.

No.	Retention time	Common name of fatty acid (IUPAC)	Chemical nomenclature	Quantitative content of methyl esters of fatty acids	
				mg/g	% of the total
Saturated acids					
1.	3.59	Undecylic (undecanoic)	C 11:0	internal standard	
2.	14.31	Palmitic (hexadecanoic)	C 16:0	4.88	24.14
3.	18.88	Stearic (octadecanoic)	C 18:0	0.92	4.55
4.	30.76	Lignoceric (tetracosanoic)	C 24:0	0.54	2.67
5.	33.27	Cerotic (hexacosanoic)	C 26:0	1.45	7.17
Monounsaturated acids (ω-9)					
6.	18.42	Oleic (octadecenoic)	C 18:1	0.91	4.50
Polyunsaturated acids (ω-3 and ω-6)					
7.	18.12	Linoleic (octadecadienic, ω-6)	C 18:2	1.84	9.10
8.	18.30	Linolenic (octadecatrienic, ω-3)	C 18:3	9.68	47.87
The amount of saturated fatty acids				**7.79**	**38.53**
The amount of unsaturated fatty acids				**12.43**	**61.47**
Total				**20.22**	**100**

cholesterol, persuasive antioxidant and generation of free radical. Owing to its applications in agriculture, chemical and food, pharmaceutical industries, pyruvic acid demand is ever increasing (Li et al. 2001; Li et al. 2001). Succinic acid is the predecessor of a wide range of bio-compounds, particularly it is important in accumulation of mitochondrial metabolite succinate and during ischemia controls reperfusion injury through mitochondrial reactive oxygen production (Jarukas et al. 2018). The followed ones isocitric and citric acids, the content of which were 12.88 mg/g and 8.71 mg/g respectively in the raw material. The citric acid is a natural component and prevalent metabolite of plants and is the most universal and widely used organic acid in pharmaceuticals and foods. Citric acid and its salts are used in many industrials as a buffering, pH adjustment, derivatization and chelating (Crolla and Kennedy 2001; Celik et al. 2014). The requirements for isocitric acid and its salts are also increasing. Monopotassium salt of threo-D-(S)-(+)-isocitric acid is used in some biochemical experiments (NADP-isocitrate dehydrogenase, NAD-isocitrate dehydrogenase, measurement of aconitate hydratase, isocitrate lyase) (Kamzolova et al. 2008). Moreover, there is apparently increasing interest in isocitric acid as a useful pharmaceutical additive (Celik et al. 2014). The malic acid content in the analyzed sample was 0.75 mg/g. Malic acid is a four-carbon dicarboxylic acid. It has many applications in the pharmaceuticals industry and also food industry as flavor and an acidulant enhancer. In the chemical industries the malic acid also used as a feedstock for chemical synthesis of polymalic acid (Chi et al. 2016).

The quantitative content of fatty acids is present in Table 2.

As shown in Fig. 4, Table 2, *Crambe cordifolia* Steven leaves contain a mixture of fatty acids saturated (7.79 mg/g; 38.53%) and unsaturated (12.43 mg/g; 61.47%).

The biological role of saturated fatty acids is that they are a source of energy for the human body. They are also involved in the construction of cell membranes, hormone synthesis, transfer and absorption of vitamins and trace elements. Unsaturated fatty acids play an important role in the body's vital functions.

The results of the study showed that the major components of raw material were linolenic acid (9.68 mg/g; 47.87%), palmitic acid (4.88 mg/g; 24.14%), and linoleic acid (1.84 mg/g; 9.10%). *Crambe cordifolia* Steven leaves is a source of essential fatty acids omega-3 (linolenic acid), omega-6 (linoleic acid) that must be supplied in the diet because the body needs them but cannot synthesize them. Obtained linolenic and linoleic acids are the starting point for the synthesis of a variety of other unsaturated fatty acids (De Lorgeril et al. 2001).

Linoleic acid performs a number of vitally important functions, such as the production of bile acids in the liver, the normalization of metabolic processes, the production of prostaglandins, the normalization of hormonal balance, and the improvement of digestive enzymes. Linoleic acid is converted in the body into γ-linolenic, which is the most active and turns into prostaglandin E1, which increases immunity. Prostaglandins also suppress inflammatory processes, regulate brain function, reduce the probability of vascular and cardiac diseases, normalize the nervous system, regulate metabolism and insulin levels. Linolenic acid provides the production of prostaglandins, normalizes blood pressure and blood cholesterol (Stoyko et al. 2015).

The organic and fatty acids profile plays an important role in the chemical properties therefore this is the useful information for the next researches.

Conclusion

The results of the research indicate that *Crambe cordifolia* Steven leaves are the source of carboxylic acids. Using the HPLC method, we determined the composition of organic acids in the raw material. The dominant organic acids in the studied *Crambe cordifolia* Steven were pyruvic and succinic acids, the content of which was 40659.83 μg/g and 38034.83 μg/g respectively. The qualitative composition and quantitative content of fatty acids were studied by GC/MS method. We determined 7 fatty acids in the *Crambe cordifolia* Steven leaves. The content of polyunsaturated fatty acids was 56.97%, monounsaturated was 4.50% and saturated was 38.53%. Linolenic and palmitic acids dominated among the fatty acids, their content was 9.68 mg/g and 4.88 mg/g that were 47.87% and 24.14% respectively of the total fatty acids. Thus, *Crambe cordifolia* Steven displayed particular composition of organic and fatty acids which could be of great interest for pharmaceutical industries, and this plant raw material can be used as a source for new medicines in the future.

References

Agius C, Von Tucher S, Poppenberger B, Rozhon W (2018) Quantification of sugars and organic acids in tomato fruits. MethodsX 5: 537–550. https://doi.org/10.1016/j.mex.2018.05.014

Aguinagalde I, Del Pero Martinez M (1980) The occurrence of acylated flavonol glycosides in the cruciferae. Phytochemistry 21(12): 2875–2878. https://doi.org/10.1016/0031-9422(80)85059-X

Atolani O, Adeniyi O, Kayode OO, Adeosun CB (2015) Direct preparation of fatty acid methyl esters and determination of in vitro antioxidant potential of lipid from fresh *Sebal causarium* Seed. Journal of Applied Pharmaceutical Science 5: 24–28. https://doi.org/10.7324/JAPS.2015.50305

Avato P, Argentieri MP (2015) Brassicaceae: a rich source of health improving phytochemicals. Phytochemistry Reviews 14: 1019–1033. https://doi.org/10.1007/s11101-015-9414-4

Basaraba RYu (2019) Pharmacognosy study of common pussytoes (Antennaria dioica) in order to develop new medicines. PhD thesis, Ternopil, Ukraine: I. Horbachevsky Ternopil State University of the Ministry of Health of Ukraine. [in Ukrainian]

Björkman M, Klingen I, Birch AN, Bones AM, Bruce TJ, Johansen TJ, Meadow R, Molmann J, Seljasen R, Smart LE, Stewart D (2011) Phytochemicals of Brassicaceae in plant protection and human health – influences of climate, environment and agronomic practice. Phytochemistry 72(7): 538–556. https://doi.org/10.1016/j.phytochem.2011.01.014

Bukhari SM, Simic N, Siddiqui HL, Ahmad VU (2013) Determination of antioxidant activity of *Crambe cordifolia*. World Applied Sciences Journal 22(11): 1561–1565.

Celik G, Ucar FB, Akpınar O, Çorbacı C (2014) Production of citric and isocitric acid by *Yarrowia lipolytica* strains grown on different carbon sources. Turkish Journal of Biochemistry 39(3): 285–290. https://doi.org/10.5505/tjb.2014.92005

Chi Z, Wang ZP, Wang GY, Khan I, Chi ZM (2016) Microbial biosynthesis and secretion of l-malic acid and its applications. Critical Reviews in Biotechnology 36(1): 99–107. https://doi.org/10.3109/07388551.2014.924474

Crolla A, Kennedy KJ (2001) Optimization of citric acid production from *Candida lipolytica* Y-1095 using *n*-paraffin. Journal of Biotechnology 89(1): 27–40. https://doi.org/10.1016/S0168-1656(01)00278-4

Darzuli N, Budniak L, Hroshovyi T (2019) Selected excipients in oral solid dosage form with dry extract of *Pyrola rotundifolia* L. International Journal of Applied Pharmaceutics 11(6): 210–216. https://doi.org/10.22159/ijap.2019v11i6.35282

De Lorgeril M, Salen P, Laporte F, De Leiris J (2001) Alpha-linolenic acid in the prevention and treatment of coronary heart disease. Europe-

an Heart Journal Supplements 3: D26–D32. https://doi.org/10.1016/S1520-765X(01)90115-4

Ergönül PG, Nergiz C (2010) Determination of organic acids in olive fruit by HPLC. Czech Journal of Food Sciences 28: 202–205. https://doi.org/10.17221/1379-CJFS

Garcés R, Mancha M (1993) One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. Analytical Biochemistry 211(1): 139–143. https://doi.org/10.1006/abio.1993.1244

Husak L, Dakhym I, Marchyshyn S, Nakonechna S (2018) Determination of sugars and fructans content in *Stachys sieboldii*. International Journal of Green Pharmacy 12: 70–74.

Iosypenko OO, Kyslychenko VS, Omelchenko ZI, Burlaka IS (2019) Fatty acid composition of vegetable marrows and zucchini leaves. Pharmacia 66(4): 201–207. https://doi.org/10.3897/pharmacia.66.e37893

Jahangir M, Kim HK, Choi YH, Verpoorte R (2009) Health-affecting compounds in Brassicaceae. Comprehensive Reviews in Food Science and Food Safety 8(2): 31–43. https://doi.org/10.1111/j.1541-4337.2008.00065.x

Jarukas L, Kamarauskaitė J, Marksa M, Trumbeckaitė S, Banienė R, Ivanauskas L (2018) Bio-based succinic acid sample preparation and derivatization procedure optimisation for Gas chromatography-mass spectrometry analysis. ScienceRise – Pharmaceutical Science 4(14): 9–13. https://doi.org/10.15587/2519-4852.2018.135132

Kalista MS (2017). Underutilized medicinal species of *Crambe* L. of the flora of Ukraine. Agrobiodiversity for improving nutrition, health and life quality 1: 216–220. https://doi.org/10.15414/agrobiodiversity.2017.2585-8246.216-220

Kamzolova SV, Finogenova TV, Morgunov IG (2008) Microbiological production of citric and isocitric acids from sunflower oil. Food Technology and Biotechnology 46(1): 51–59.

Li Y, Chen J, Lun S (2001) Biotechnological production of pyruvic acid. Applied Microbiology and Biotechnology 57: 451–459. https://doi.org/10.1007/s002530100804

Li Y, Fu W, Chen J (2001) Recovery of pyruvate from fermentation broth by using ion exchange resin chromatography. Journal of Wuxi University of Light Industry 20(4): 335–339.

Matthäus B (2002) Antioxidant activity of extracts obtained from residues of different oilseeds. Journal of Agricultural and Food Chemistry 50(12): 3444–3452. https://doi.org/10.1021/jf011440s

Pal D, Keshav A, Mazumdar B, Kumar A (2017) Production and Recovery of Pyruvic Acid: Recent Advances. Journal of the Institution of Engineers (India) – Series E 98: 165–175. https://doi.org/10.1007/s40034-017-0101-4

Rashid MA, Akhtar MN, Ashraf A, Nazir S, Ijaz A, Noradila AO, Noor S, Basit A, Tareen RB (2018) Chemical composition and antioxidant, antimicrobial and haemolytic activities of *Crambe cordifolia* roots. Farmacia 66(1): 165–171.

Razavi SM, Nejad-Ebrahimi S (2009) Chemical composition, allelopatic and cytotoxic effects of essential oils of flowering tops and leaves of *Crambe orientalis* L. from Iran. Natural product research 23(16): 1492–1498. https://doi.org/10.1080/14786410802611303

Salakhutdinov BA, Tadzhibaeva ÉT, Zamaraeva MV, Tukfatullina II, Aripov TF, Mezhlum'yan LG, Redina ÉF, Yuldashev PK (1998) Membrane-active properties of crambin. Chemistry of Natural Compounds 34: 62–67. https://doi.org/10.1007/BF02249689

Slobodianiuk L, Budniak L, Marchyshyn S, Basaraba R (2019) Determination of amino acids and sugars content in *Antennaria dioica* Gaertn. International Journal of Applied Pharmaceutics 11(5): 39–43. https://doi.org/10.22159/ijap.2019v11i5.33909

Stoiko L, Kurylo Kh (2018) Development of optimal technology of alcohol extract *Centaurium erythraea* Rafn. herb. Archives of the Balkan Medical Union 53(4): 523–528. https://doi.org/10.31688/ABMU.2018.53.4.06

Stoyko LI, Gusak LV, Marchishin SM, Demidyak OL (2015) Research of fatty acid composition of common centaury herbs and crosne herbs. Journal of Siberian Medical Sciences 6: 1–9.

Toledo MZ, Teixeira RN, Ferrari TB, Ferreira G, Cavariani C, Cataneo AC (2011) Physiological quality and enzymatic activity of crambe seeds after the accelerated aging test. Acta Scientiarum – Agronomy 33(4): 687–694. https://doi.org/10.4025/actasciagron.v33i4.8248

Vergun OM, Rakhmetov DB, Shymanska OV, Fishchenko VV (2018) The accumulation of nutrients in underground parts of plants of the genus *Crambe* L. spp. Plant Introduction 78(2): 3–11.

Wazilewski WT, Baricatti RA, Martins GI, Secco D, de Souza SNM, Rosa HA, Chaves LI (2013) Study of the methyl crambe (*Crambe abyssinica* Hochst) and soybean biodiesel oxidative stability. Industrial Crops and Products 43: 207–212. https://doi.org/10.1016/j.indcrop.2012.07.046