

ORIGINAL ARTICLE

Gas chromatography – mass spectrometry studies of the component composition of carboxylic acids of the rhizomes of *Iris medwedewii* and *Iris carthaliniae* (Iridaceae)

Studie složení karboxylových kyselin rhizomů *Iris medwedewii* a *Iris carthaliniae* (Iridaceae) pomocí plynové chromatografie ve spojení s a hmotnostní spektrometrií

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Summary

The composition of carboxylic acids of the rhizomes of *Iris carthaliniae* and *Iris medwedewii* by the gas chromatography – mass spectrometry method has been studied for the first time. The total content of carboxylic acids for *I. carthaliniae* was 1.34%, including (%) – 0.65 fatty; 0.36 mono-, di- and tri-carboxylic; 0.33 phenol carboxylic acids. The yield of carboxylic acids for *I. medwedewii* was 1.58%, including (%) – 0.73 fatty; 0.38 mono-, di- and tri-carboxylic; 0.47 phenol carboxylic acids. The dominant fatty acids in the rhizomes of *I. carthaliniae* were myristic acid (25%), palmitic acid (14.41%), stearic acid (10.51%) and linoleic acid (6.05%). Besides, levulinic acid (15.84%) and oxalic acid (4.42%) prevailed among organic acids, while ferulic acid (2.43%), citric acid (1.88%) and malic acid (3.62%) prevailed among the hydroxy acids. The fatty acids palmitic acid (6.51%), linoleic acid (8.38%), oleic acid (3.87%) and capric acid (3.21%) were found in the

rhizomes of *I. medwedewii*, and also, levulinic (29.39%), malonic (1.45%), succinic (1.72%) acids prevailed among the organic acids and citric (25.37%), malic (3.30%) and ferulic (2.42%) acids in the rhizomes of *I. medwedewii* prevailed among the hydroxy acids.

Key words: *Iris carthaliniae* • *Iris medwedewii* • rhizomes • carboxylic acids • GC-MS analysis

Souhrn

Složení karboxylových kyselin v rhizomech *Iris carthaliniae* a *Iris medwedewii* pomocí plynové chromatografie ve spojení s hmotnostní spektrometrií dosud nebylo studováno. Celkový obsah karboxylových kyselin u *I. carthaliniae* byl 1,34 %, včetně (%) – 0,65 mastných; 0,36 mono-, di- a tri-karboxylových; 0,33 fenolických karboxylových kyselin. Výtěžek karboxylových kyselin u *I. medwedewii* byl 1,58 %, včetně (%) – 0,73 mastných; 0,38 mono-, di- and tri-karboxylových; 0,47 fenolických karboxylových kyselin. Hlavní postavení v rhizomech *I. carthaliniae* měly kyselina myristová (25 %), kyselina palmitová (14,41 %), kyselina stearová (10,51 %) a kyselina linolová (6,05 %). Kromě toho kyselina levulová (15,84 %) a kyselina šťávelová (4,42 %) převládaly mezi organickými kyselinami, zatímco kyselina ferulová (2,43 %), kyselina citronová (1,88 %) a kyselina jablečná (3,62 %) převládaly mezi hydroxykyselinami. V rhizomech *I. medwedewii* byly zjištěny mastné kyseliny – kyselina palmitová (6,51 %), linolová (8,38 %), olejová (3,87 %) a kaprinová (3,21 %). Dále bylo zjištěno, že v rhizomech *I. medwedewii* mezi organickými kyselinami převládaly kyselina levulová (29,39 %), malonová (1,45 %) a jantarová (1,72 %); kyselina citronová (25,37 %), jablečná (3,30 %) a ferulová (2,42 %) pak převládaly mezi hydroxykyselinami.

Klíčová slova: *Iris carthaliniae* • *Iris medwedewii* • rhizomy • karboxylové kyseliny • plynová chromatografie

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Introduction

The carboxylic acids are synthesized in a significant amount in the plant cell^{1, 2)}, they are products of anabolism (assimilation) and necessary for cell and organism activities. They are the products of transformation of the main nutrients: fats, proteins, and carbohydrates^{3, 4)}. Organic acids have antioxidant, anti-inflammatory and immunomodulatory properties, they are involved in metabolism, have a positive affect on digestion, and create favorable conditions for the life of beneficial intestinal microorganisms^{5, 6)}. Fatty acids are involved in the synthesis of prostaglandins and in the stabilization of cellular membranes^{1, 3, 7)}. Therefore, the search of the new plant sources rich in organic acids is a very important direction of research.

There are 40 species of iris in the flora of Azerbaijan but the most common species of iris are *I. medwedewii* Fomin and *I. carthaliniae* Fomin^{8, 9)}. They are perennial plants with thick horizontal creeping rhizomes^{8, 10)}. According to the classification of Rodionenko (1961)¹⁰⁾, *I. carthaliniae* belongs to the subgenus *Xyridion* (Tausch) Spach em Rodion. (sect. *Xyridion*, ser. *Spuria* (Diels) Lawrence em Rodion.) and *I. medwedewii* belongs to the subgenus *Iris* (sect. *Hexapogon* (Bunge) Baker em. Rodion., subsect. *Oncocyclus* (Siemss) Benth.). The *Iris* species have been cultivated as decorative plants since the ancient times. These cultivated *Irises* have a wide variety of colours in their showy and usually perfumed blossoms^{11–13)}. The plants of *Iris* species are rich in xanthones¹⁴⁾, flavonoids^{15–16)}, iridoidoids, essential oils^{17–18)}, saponins, tannins, etc. Various kinds of *Iris* species in different regions of the world are used as diuretic, hemostatic, astringent, antipyretic, tonic, anti-inflammatory, cardiac, immunomodulatory, antioxidant, antimicrobial¹⁹⁾, antiviral and tonic agents^{17–18)}. The rhizomes of this plant have been extensively used against fever, kidney infections and as ingredients of tooth powders etc. The chemical composition of secondary metabolites of these *Irises* has not been studied^{18, 20)}.

The previous papers reported the qualitative and

quantitative composition of fatty acids of the rhizomes of *I. hungarica* Waldst. et Kit²¹⁾ and *I. pseudacorus* L.²²⁾. According to the classification of Rodionenko (1961)¹⁰⁾ *I. hungarica* belongs to the subgenus *Iris*, section *Iris*, series *Elatae* Lawrence; *I. pseudacorus* – subgenus *Limniris* (Tausch) Spach em. Rodion, section *Limniris*, series *Laevigatae* (Diels) Lawrence). In the rhizomes of *I. hungarica*, 19 fatty acids have been identified (total amount – 0.5%). Myristic (C14:0; 41.0%), linoleic (C18:2ω6; 12.1%), palmitic (C16:0; 11.6%) fatty acids were prevalent. And in the rhizomes of *I. pseudacorus*, 12 fatty acids have been identified (total amount – 1.07%), the dominant components being palmitic (C16:0) 46.60%, linoleic (C18:2ω6) 28.91%, heptadecanoic (C17:0) 4.20% and linolenic (C18:3ω3) 4.73% fatty acids.

The aim of this study is to determine the qualitative and quantitative composition of carboxylic acids of the rhizomes of two Azerbaijan *Iris* species, namely *I. medwedewii* and *I. carthaliniae* by the gas chromatography-mass spectrometry (GC/MS) method.

Experimental part

Plant materials

The objects of the study were the rhizomes of *I. medwedewii* Fomin and *I. carthaliniae* Fomin, prepared in Gosmalyan and Pirasora villages of the Lerik region of Azerbaijan, in the blooming phase at the height of more than 2000 m above the sea level in May 2014. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department and Botany Department, National University of Pharmacy, Kharkiv, Ukraine, and also at the Department of Pharmacognosy and Botany, Azerbaijan Medical University, Baku, Azerbaijan.

Preparation of the extracts

The analysis of the methyl ethers of carboxylic acids was carried out by the method of chromatography-mass-spectrometry^{25–27)} on a 5973N/6890N MSD/DS



I. carthaliniae Fomin



I. medwedewii Fomin

Fig. 1. General view of the living plants and herbariums of the studied species of Irises

Agilent Technologies (USA). The internal standard (solution of tridecane (50.0 µg) in hexane) and 1.0 ml of the methylating agent (14% BCl_3 in methanol, Supelco 3-3033) were added to a weighed sample of the product (50.0 mg) in a vial of 2.0 ml. The mixture was held at 65 °C in a sealed vial for 8 hours. In this time organic acids were completely extracted from the plant material and hydrolysis and methylation of fatty acids occurs. Free organic and phenolcarboxylic acids were methylated simultaneously. The reaction mixture was elutriated from the plant material and diluted with 1.0ml of distilled water. Methyl ethers were extracted with 0.2 ml of methylene chloride, carefully shaken up several times within an hour and then the obtained extract was chromatographed^{23, 24)}.

Chromatographic conditions

Sample introduction (2.0 µl) was carried out into a chromatographic column in the splitless mode without the split ratio within 0.2 minute. A capillary column HP-INNOWAX (30 m × 250 µm × 0.50 µm) was used for separation. The mobile phase: helium, gas flow rate: 1.2 ml/min. Temperature of the sample injection heater: 250 °C. Temperature of furnace is programmable from 50 to 320 °C with the rate of 4 degree/min. For component identification, the data from the mass-spectra libraries NIST05 and WILEY 2007 with the total number

of spectra of more than 470,000 were used combined with identification programs AMDIS and NIST^{25,26)}.

For quantitative calculations, the internal standard method was used²⁷⁾. Calculation of components content C (mg/kg) was carried out using the formula:

$$C = P_1 \cdot 50 \cdot 1000 / P_2 \cdot M,$$

where P_1 – peak area of the tested substance, P_2 – peak area of the standard, 50 – mass of the internal standard (µg) injected into the sample; m – sample mass (g).

The relative content of carboxylic acids was determined in % of their sum.

Results and discussion

The analysis of carboxylic acid composition of the rhizomes of *I. carthaliniae* and *I. medwedewii* showed the presence of 35 common acids and 2 acids specific only for certain species.

The analysis of the content in the rhizomes revealed the presence of the total content of the carboxylic acids for *I. carthaliniae*, 13447.54 mg/kg or 1.34%, including (%) – 0.65 fatty; 0.36 mono-, di- and tri-carboxylic; 0.33 phenol carboxylic acids. The yield of the carboxylic acids for *I. medwedewii* was 15829.61 mg/kg or 1.58%, including (%) – 0.73 fatty; 0.38 mono-, di- and tri-carboxylic;

Table 1. Lower carboxylic acids of the rhizomes of *I. carthaliniae* and *I. medwedewii*

| № | RT, min | Empirical formula | Acid | Content of carboxylic acids | | | |
|----|---------|--|-------------------------|-----------------------------|-------|----------------------|-------|
| | | | | <i>I. carthaliniae</i> | | <i>I. medwedewii</i> | |
| | | mg/kg | % | mg/kg | % | | |
| 1 | 10.851 | $(\text{COOH})_2$ | oxalic | 594.16 | 4.42 | 14.08 | 0.09 |
| 2 | 13.087 | $\text{C}_3\text{H}_4\text{O}_4$ | malonic | 22.13 | 0.16 | 230.12 | 1.45 |
| 3 | 13.957 | $\text{C}_4\text{H}_4\text{O}_4$ | fumaric | 1.55 | 0.01 | 13.26 | 0.08 |
| 4 | 14.866 | $\text{C}_5\text{H}_8\text{O}_3$ | levulinic | 2129.49 | 15.84 | 4652.25 | 29.39 |
| 5 | 15.485 | $\text{C}_4\text{H}_6\text{O}_4$ | succinic | 85.17 | 0.63 | 271.81 | 1.72 |
| 6 | 15.971 | $\text{C}_7\text{H}_6\text{O}_2$ | benzoic* | 9.98 | 0.07 | 18.03 | 0.11 |
| 7 | 18.714 | $\text{C}_8\text{H}_8\text{O}_2$ | phenylacetic* | 0.79 | 0.01 | 4.31 | 0.03 |
| 8 | 19.133 | $\text{C}_7\text{H}_6\text{O}_3$ | salicylic* | 1.36 | 0.01 | 1.87 | 0.01 |
| 9 | 22.284 | $\text{C}_6\text{H}_{10}\text{O}_5$ | 2-oxi-3-methyl glutaric | 73.14 | 0.54 | 67.45 | 0.43 |
| 10 | 23.856 | $\text{C}_4\text{H}_6\text{O}_5$ | malic | 486.37 | 3.62 | 522.13 | 3.30 |
| 11 | 24.425 | $\text{C}_8\text{H}_{14}\text{O}_4$ | suberic | – | – | 22.20 | 0.14 |
| 12 | 26.444 | $\text{C}_9\text{H}_{16}\text{O}_4$ | azelaic | 48.54 | 0.36 | 136.98 | 0.87 |
| 13 | 31.173 | $\text{C}_6\text{H}_8\text{O}_7$ | citric | 252.42 | 1.88 | 4015.41 | 25.37 |
| 14 | 34.291 | $\text{C}_8\text{H}_8\text{O}_4$ | vanillic* | 123.98 | 0.92 | 203.63 | 1.29 |
| 15 | 38.401 | $\text{C}_9\text{H}_8\text{O}_3$ | p-coumaric* | 18.13 | 0.13 | 61.64 | 0.39 |
| 16 | 38.518 | $\text{C}_6\text{H}_{10}\text{O}_4$ | adipinic | 15.84 | 0.12 | 29.08 | 0.18 |
| 17 | 39.371 | $\text{C}_7\text{H}_6\text{O}_3$ | p-hydroxybenzoic* | 7.51 | 0.06 | 291.34 | 1.84 |
| 18 | 39.723 | $\text{C}_9\text{H}_{10}\text{O}_5$ | syringic* | 17.92 | 0.13 | 4.62 | 0.03 |
| 19 | 40.286 | $\text{C}_7\text{H}_6\text{O}_4$ | gentianic* | 5.07 | 0.04 | 27.14 | 0.17 |
| 20 | 42.634 | $\text{C}_{10}\text{H}_{10}\text{O}_4$ | ferulic* | 326.45 | 2.43 | 382.47 | 2.42 |

*phenol carboxylic acids; – means that the substance was not identified

Abundance

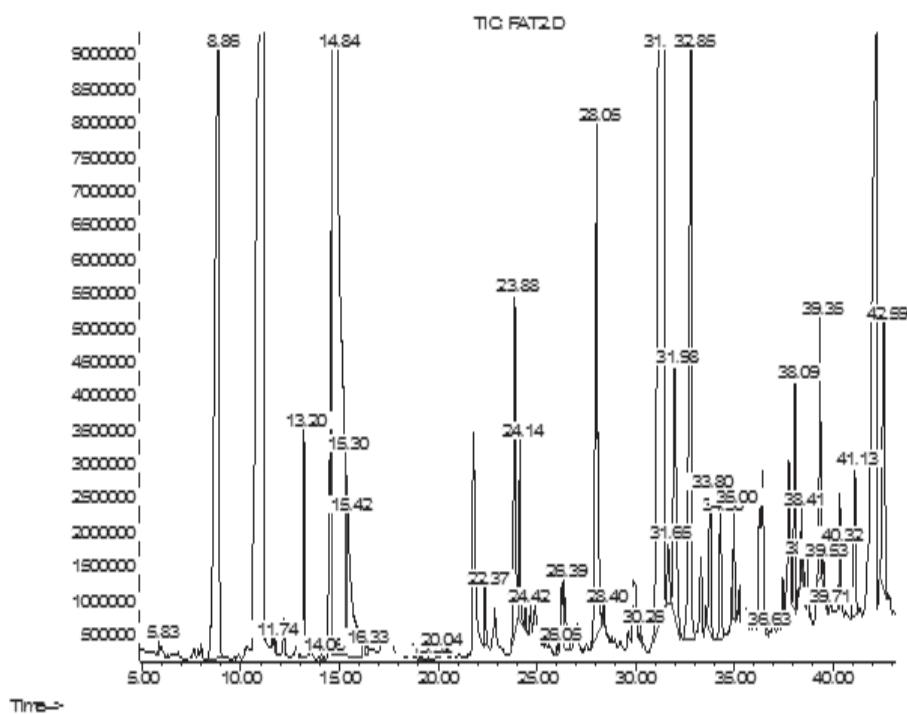


Fig. 2. GC-MS chromatogram of carboxylic acids of the rhizomes of *I. carthaliniae*

0.47 phenol carboxylic acids. Qualitative composition of the carboxylic acids varied: there are lower and higher carboxylic acids; mono-, di- and tricarboxylic acids by the number of the carboxyl groups; aliphatic and aromatic acids by the nature of the hydrocarbon radical connected with a COOH-group; saturated and unsaturated acids by the level of saturation. The lowest acids are presented by both free organic acids and hydroxyacids. The length of carbon chains in fatty acids

is from 6 to 24 atoms. The results are given in Figs. 2–3 and Tables 1–2 below.

The content of the organic acids for *I. carthaliniae* is 31.38% of the total carboxylic acids content; 9.3% of them are hydroxy acids. Oxalic, malonic, fumaric, levulinic, succinic, 2-oxi-3-methylglutaric, and azelaic acids are common for the both species of irises. 10 aliphatic acids were identified in the rhizomes of *I. carthaliniae*, their total content as 27.58%, relative to the total amount

Abundance

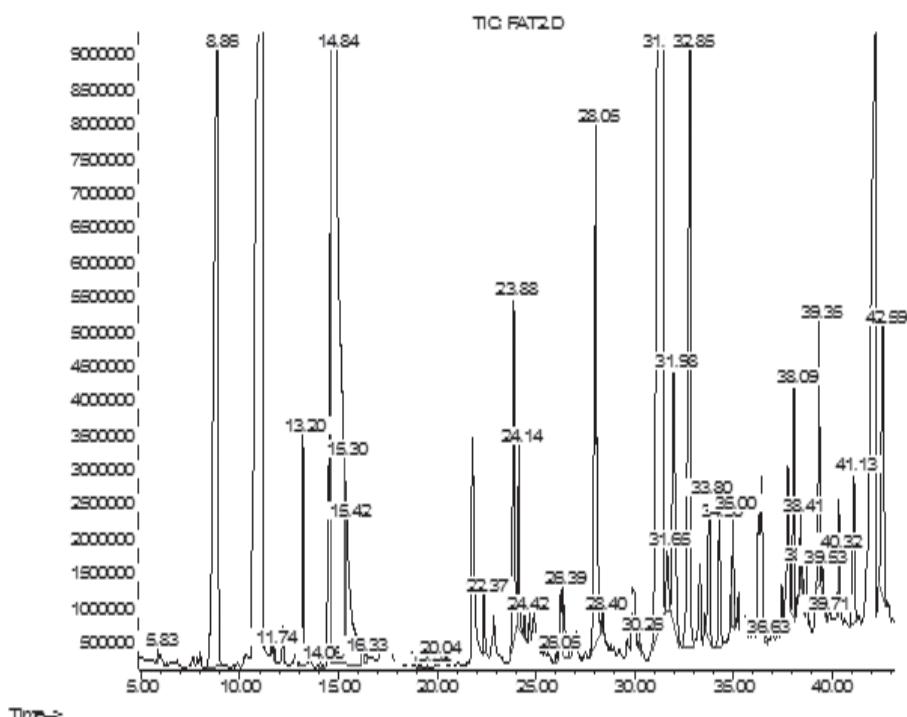


Fig. 3. GC-MS chromatogram of carboxylic acids of the rhizomes of *I. medwedewii*

of acids. Levulinic acid (15.84%), malic acid (3.62%), oxalic acid (4.42%), citric acid (1.88%), succinic (0.63%) prevail among the organic acids in the rhizomes of *I. carthaliniae*. Adipinic acid (0.12%) was found only in the rhizomes of *I. carthaliniae*. In addition, in the raw materials 9 aromatic acids were identified, their content being 3.8%. The content of ferulic acid (up 2%) significantly dominates in the both species of irises.

The yield of the organic acids in the rhizomes of *I. medwedewii* is 69.32% and the total of 20 acids were identified, the hydroxy acids content was 34.96%, which is almost 4 times higher than in the rhizomes of *I. carthaliniae*. Among the aliphatic acids dominated: levulinic (29.39%), citric (25.37%), malic (3.30%) malonic (1.45%), succinic (1.72%); their total content being 63.02% of the total acids. Additionally, in *I. medwedewii* the suberic acid (0.14%) was identified. The content of aromatic acids was 6.29%. Benzoic, phenylacetic, salicylic, malic, citric, vanillic, *p*-coumaric, *p*-hydroxybenzoic, syringic, gentianic, ferulic acids were common hydroxy acids for both *Irises*.

The content of the saturated fatty acids in the rhizomes of *I. carthaliniae* was 58.25%, which far exceeds the amount of the unsaturated (10.38%) ones of the total of carboxylic acids. Among the saturated acids dominated myristic (25%), palmitic (14.41%), stearic (10.51%) acids; lower quantities of lauric (2.03%) and capric (3.80%) acids were found. Caprylic (0.04%) and

2-hydroxy-stearic (0.07%) acids were found only in *I. carthaliniae*.

ω -6 Dienoic linoleic acid (6.05%) prevailed among the unsaturated acids in the rhizomes of *I. carthaliniae* and monoenoic ω -9 oleic acid (2.90%) was second to it. The content of palmitoleic acid and linolenic acid were until 1%.

The composition of the fatty acids in the rhizomes of *I. medwedewii* was almost identical: caprylic and 2-oxy-stearic acid were absent, but tricocyl acid (0.26%) was found. The saturated fatty acids content was 16.61% and the unsaturated represented 14.07% of the total of carboxylic acids. Although, the qualitative composition of the *Irises* was similar, the content of the fatty acids in *I. medwedewii* did not exceed 0.1–2%, but the palmitic (6.51%), olein (3.87%) and linoleic (8.38%) acids made the exception.

Conclusion

1. Qualitative and quantitative composition of the carboxylic acids in the rhizomes of *I. carthaliniae* and *I. medwedewii* was studied at the first time using the method of chromato-mass spectrometry, 37 carboxylic acids being identified.
2. Total content of the carboxylic acids for *I. carthaliniae* was 13447.54 mg/kg or 1.34%, including (%) – 0.65 fatty; 0.36 mono-, di- and tri-carboxylic; 0.33 phenol

Table 2. Higher carboxylic acids of the rhizomes of *I. carthaliniae* and *I. medwedewii*

| № | RT, min | IUPAC formula | Acid | Content of fatty acids | | | |
|----|---------|------------------|---------------|------------------------|-------|----------------------|------|
| | | | | <i>I. carthaliniae</i> | | <i>I. medwedewii</i> | |
| | | | | mg/kg | % | mg/kg | % |
| 1 | 5.77 | C6:0 | caproic | 4.69 | 0.03 | 11.23 | 0.07 |
| 2 | 12.68 | C8:0 | caprylic | 5.02 | 0.04 | – | – |
| 3 | 15.591 | C10:0 | capric | 510.92 | 3.80 | 508.77 | 3.21 |
| 4 | 19.886 | C12:0 | lauric | 272.72 | 2.03 | 9.65 | 0.06 |
| 5 | 24.425 | C14:0 | myristic | 3362.24 | 25.00 | 192.79 | 1.22 |
| 6 | 26.182 | C15:0 | pentadecanoic | 13.26 | 0.10 | 13.32 | 0.08 |
| 7 | 28.457 | C16:0 | palmitic | 1937.90 | 14.41 | 1030.27 | 6.51 |
| 8 | 28.669 | C16:1 ω 7 | palmitoleic | 63.56 | 0.47 | 16.60 | 0.10 |
| 9 | 29.896 | C17:0 | margarine | 49.95 | 0.37 | 12.98 | 0.08 |
| 10 | 32.049 | C18:0 | stearic | 1413.14 | 10.51 | 113.87 | 0.72 |
| 11 | 32.211 | C18:1 ω 9 | oleic | 389.82 | 2.90 | 613.37 | 3.87 |
| 12 | 32.98 | C18:2 ω 6 | linoleic | 812.92 | 6.05 | 1326.05 | 8.38 |
| 13 | 33.811 | C18:3 ω 3 | linolenic | 128.77 | 0.96 | 271.64 | 1.72 |
| 14 | 35.01 | C20:0 | arachic | 97.69 | 0.73 | 146.30 | 0.92 |
| 15 | 36.51 | C21:0 | heneicosanoic | 10.56 | 0.08 | 8.69 | 0.05 |
| 16 | 38.066 | C22:0 | behenic | 83.93 | 0.62 | 286.83 | 1.81 |
| 17 | 38.267 | C18:0 | 2-oxy-stearic | 9.21 | 0.07 | – | – |
| 18 | 39.533 | C23:0 | tricocyl | – | – | 41.63 | 0.26 |
| 19 | 41.039 | C24:0 | lignoceric | 61.24 | 0.46 | 255.80 | 1.62 |

– means that the substance was not identified

carboxylic acids. 10 aliphatic acids and 9 aromatic acids were identified in the rhizomes of *I. carthaliniae*, their total content as 27.58% and 3.8% respectively, relative to the total amount of acids. Myristic acid (25%), palmitic acid (14.41%), stearic acid (10.51%) and linoleic acid (6.05%) prevailed among the fatty acids; levulinic acid (15.84%) and oxalic acid (4.42%) prevailed among the organic acids; ferulic acid (2.43%), citric acid (1.88%) and malic acid (3.62%) prevailed among the hydroxy acids.

3. Yield of the carboxylic acids for *I. medwedewii* was 15829.61 mg/kg or 1.58%, including (%) – 0.73 fatty; 0.38 mono-, di- and tri-carboxylic; 0.47 phenol carboxylic acids. Palmitic acid (6.51%), linoleic acid (8.38%), oleic acid (3.87%) and capric acid (3.21%) prevailed among the fatty acids; levulinic (29.39%), malonic (1.45%), succinic (1.72%) acids prevailed among the organic acids; citric (25.37%), malic (3.30%) and ferulic (2.42%) acids prevailed among the hydroxy acids.
4. The results of this study are significant for the determination of beneficial compounds in the rhizomes of the *Iris* species, so making use of these plant raw materials as a source of new medicines in the future is possible.

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