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GAS CHROMATOGRAPHY MASS SPECTROMETRIC ANALYSIS OF CARBOXYLIC ACIDS IN THE HERBS OF TWO *DRACOCEPHALUM* L. SPECIES

Shanaida, Mariia[1*]; Pryshlyak, Antonina[1]; Shanaida, Volodymyr[2]
[1]I. Horbachevsky Ternopil National Medical University, Voli 1, 46001, Ternopil, Ukraine
[2]Ternopil Ivan Puluj National Technical University, Ruska 56, 46001, Ternopil, Ukraine

*shanayda@tdmu.edu.ua

Abstract

The genus *Dracocephalum* L. (*Lamiaceae* Martinov family) includes 72 species of essential oil bearing plants with promising biological activities. They belong to the category of unofficial medicinal plants. The most studied group of the bioactive compounds of *Dracocephalum* species are essential oils and polyphenols, while the other components have been investigated much less. The aim of the study was the gas chromatography mass spectrometric (GC-MS) analysis of carboxylic acids in the herbs of two *Dracocephalum* species (*D. moldavica* L. and *D. grandiflorum* L.). The plants were grown under the cultivation conditions of Ukraine. There were identified 14 short-chained carboxylic acids in the *D. moldavica* herb and 16 ones in the *D. grandiflorum* herb. Ferulic acid possessing prominent healing properties prevailed among the found aromatic acids in both species. 14 fatty acids were found in the *D. moldavica* herb and 13 of them in the *D. grandiflorum.* Unsaturated α-linolenic acid (ω3) dominated among the long-chained carboxylic acids in the raw materials of both plants. Thus, the studied species could be regarded as the promising sources of some carboxylic acids.

Keywords: *Dracocephalum moldavica*, *Dracocephalum grandiflorum*, herb, short-chained carboxylic acid, fatty acid, GC-MS analysis

Introduction

Plants produce a diverse number of specialized secondary metabolites which are biosynthetically originated from the primary metabolites [1]. Secondary metabolites play decisive roles in plant adaptation to different environmental factors as well as they are widely used in medicine and human nutrition. The secondary metabolites are synthesized from the certain primary compounds (precursors), such as sugars, amino acids, and carboxylic acids, which are essential for the life of whole plant organisms. Many of the components of primary synthesis as well as secondary ones possess the proven therapeutic activity [2–7].

Short-chained carboxylic acids are quite diverse in structure and biological properties [3, 4]. They can exist in the free state or as the salts, esters, etc. Certain organic acids such as citric, succinic, ferulic, benzoic, and salicylic were defined as the pharmacologically active substances. Some of them are used in the food industry (malic, lemon, benzoic, fumaric acids) as well as in perfume manufacturing (for instance, esters of benzoic and phenyl acetic acids) [3–5].

Polyunsaturated fatty acids play an important role in functioning of the human body [4, 7]. Their deficiency leads to the dysfunction of cell membranes and cell homeostasis at all. So, the appropriate intake of polyunsaturated fatty acids can prevent various health disorders such as diabetes mellitus, chronic inflammation, atherosclerosis [8–13]. Polyunsaturated fatty acids are not synthesized in the human body. Thus, they must be constantly supplied with food or dietary supplements.

The genus *Dracocephalum* L. (*Lamiaceae* Martinov family) includes 72 species of essential oil bearing herbaceous plants and subshrubs distributed in temperate and sub-tropical regions [13, 14]. They belong to the unofficial medicinal plants with promising biological activities. The most studied group of their bioactive compounds are essential oils and polyphenols, while the other components have been investigated much less [14, 16–18].

There is a relatively small portion of scientific information on the contents of carboxylic acids in the raw materials of the *Lamiaceae* representatives, e.g. *Dracocephalum* species [8, 19, 20]. Thus, this aspect of their chemical composition deserves much more attention.

The aim of the study was the gas chromatography mass spectrometric (GC-MS) analysis of carboxylic acids in the herbs of two *Dracocephalum* species (*D. moldavica* L. and *D. grandiflorum* L.) which are cultivated in Ukraine.

Methods

The herbs of investigated plants were harvested from the experimental plots in Ternopil region (Ukraine) and shade dried for 6–8 days.

The sample preparation for GC-MS started from the weighing of an exact portion of grinded raw material (0.05 g). It was placed in a vial (2 mL). Then the internal standard (50 µg of tridecane in hexane) and 1.0 mL of methylating agent (14% boron trichloride in methanol, Supelco 3-3033) were added. The mixture was kept for 8 h in a hermetically sealed vial at a temperature of 65°C for the extraction of fatty oil, its hydrolysis and methylation. The reaction mixture was drained from the precipitate of plant material and diluted by adding 1.0 mL of distilled water. To remove the fatty acid methyl esters, 0.2 mL of dichloromethane was added and it was gently shaken several times during 1 h. Then the resulting mixture of methyl esters was chromatographed.

The component compositions of the obtained methyl esters of carboxylic acids were detected by GC-MS method according to [21]. Chromatograph Agilent Technologies 6890 N with mass spectrometric detector 5973 N was used in the analysis. The chromatographic capillary column was Innowax (30 m × 0.25 mm). The rate of helium as a carrier gas was 1.2 mL/min. The temperature of the thermostat have been programmed in the range 50–250 °C with a rate growing 4 °C/min. The library of mass spectra NIST 05 and WILEY 2007 was taken into accounte for the identification of components.

Results

The GC-MS method was used for the simultaneous investigation of short-chained carboxylic acids and short-chained fatty acids in the herbs of *D. moldavica* and *D. grandiflorum* (Tables 1, 2 and Figures 1, 2).

There were identified 14 short-chained carboxylic acids in the *D. moldavica* herb and 16 ones in the *D. grandiflorum* herb (Table 1). The total amount of the short-chained carboxylic acids was higher in the *D. moldavica* herb (8818 mg/kg) comparatively to *D. grandiflorum* (4977 mg/kg).

As can be seen from the data of Table 1, the aliphatic carboxylic acids prevailed over aromatic acids both quantitatively and in a variety of composition. The malonic, oxalic, malic and citric acids dominated In the aliphatic group. As it is known, the detected acids play an important role in human life [4, 6, 19, 22]. For instance, citric acid is used in medicine as a part of agents that improve energy metabolism; malic and oxalic acids are used in food industry; malonic acid is a precursor in the biosynthesis of flavonoids.

The hydroxycinnamic ferulic acid with prominent nootropic and antioxidant properties [23] prevailed among the found aromatic acids. Found benzoic acid is often used as a food additive due to its antioxidant activity [24]. Accumulation of aromatic acids in the raw materials of plants is of great interest for their further using as antioxidants and anti-inflammatory agents [23–25].

The composition of long-chained carboxylic acids known as fatty acids presented in Table 2. There were identified 14 fatty acids in the *D. moldavica* herb and 13 of them in the *D. grandiflorum* herb. The total amount of the fatty acids was similar: 3726 mg/kg in the *D. moldavica* herb and 3790 mg/kg in the *D. grandiflorum.*

As can be seen from Table 2, unsaturated α-linolenic acid (ω3) dominated among the all fatty acids in the raw material of both plants. The detected predominant α-linolenic and linoleic polyunsaturated acids play an important role in the construction of cell membranes, regulation hormone metabolism, and can prevent the deposition of cholesterol on the blood vessels [10, 20]. Researchers also found the dominance of linoleic and linolenic acids in the aboveground parts of several *Lamiaceae* species from the *Mentha, Salvia, Satureja, Ocimum* genera [8, 19, 20]. The content of carboxilic acids in plant raw materials may vary dependently on the genetic pecularities of plant, geographical location, cultivation season, method of harvesting, etc. [26, 27].

Conclusion

The conducted GC-MS analysis revealed the chromatographic profiles of carboxylic acids in the *Dracocephalum* herbs. There were detected 14 short-chained carboxylic acids in the *D. moldavica* herb and 16 ones in the *D. grandiflorum* herb. Ferulic acid prevailed among the found aromatic acids in both species. 14 fatty acids were found in the *D. moldavica* herb and 13 of them in the *D. grandiflorum.* Unsaturated α-linolenic acid dominated among the fatty acids in the raw materials of both plants. Thus, the herbs of the studied species could be regarded as promising sources of new food additives and medicines with preventing and healing properties.

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Table 1. Composition and contents of the short-chained carboxylic acids in the herbs of two *Dracocephalum* species, mg/kg

Acid	Retention time, min	*D. moldavica*	*D. grandiflorum*
Dimethoxyacetic	8.88	49	37
Oxalic	9.30	245	195
Malonic	11.59	4835	3067
Fumaric	12.34	172	107
Furfurylic	13.02	13	19
Succinic	13.42	159	54
Benzoic*	13.91	27	39
Phenylacetic*	16.88	10	4
Salicylic*	17.13	18	17
Malic	21.68	636	420
Azelaic	24.21	21	15
Citric	29.0	2525	895
Vanillic*	31.97	29	19
Syringic*	37.40	-	31
p-Hydroxycinnamic*	38.94	-	5
Ferulic*	39.80	79	53
Total amount		8818	4977

Note: * – aromatic acid; "-" – component was not detected.

Table 2. Composition and contents of the fatty acids in the herbs of two *Dracocephalum* species, mg/kg

Acid	Retention time, min	*D. moldavica*	*D. grandiflorum*
Myristic	21.92	124	114
Palmitic	25.86	1439	1400
Palmitoleic*	26.18	127	95
7- Hexadecenic*	26.69	8	-
14-Methylpalmitic	26.90	21	-
Heptadecanic	27.54	18	15
Stearinic	29.31	141	116
Oleic*	29.59	76	227
11-Octadecenic*	29.72	26	34
Linoleic*	30.37	381	327
α-Linolenic*	31.48	1275	1379
Arachidic	32.59	31	31
2-Oxipalmitic	32.65	35	17
Behenic	35.66	24	13
Lignoceric	38.52	-	9
Total amount		3726	3790

Note: * – unsaturated fatty acid; "-" – the component was not detected.

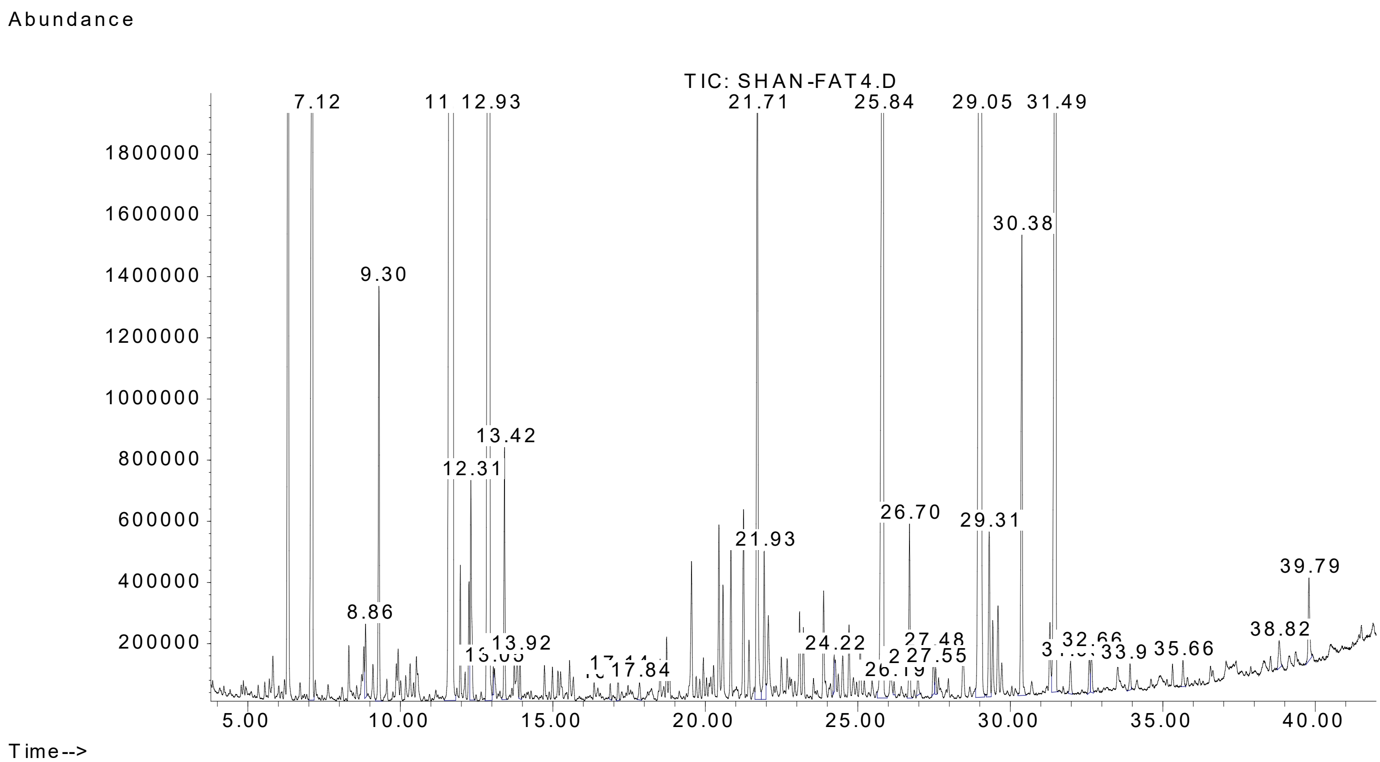

Figure 1. The GC-MS chromatogram of carboxylic acids methyl esters in the *D. moldavica* herb

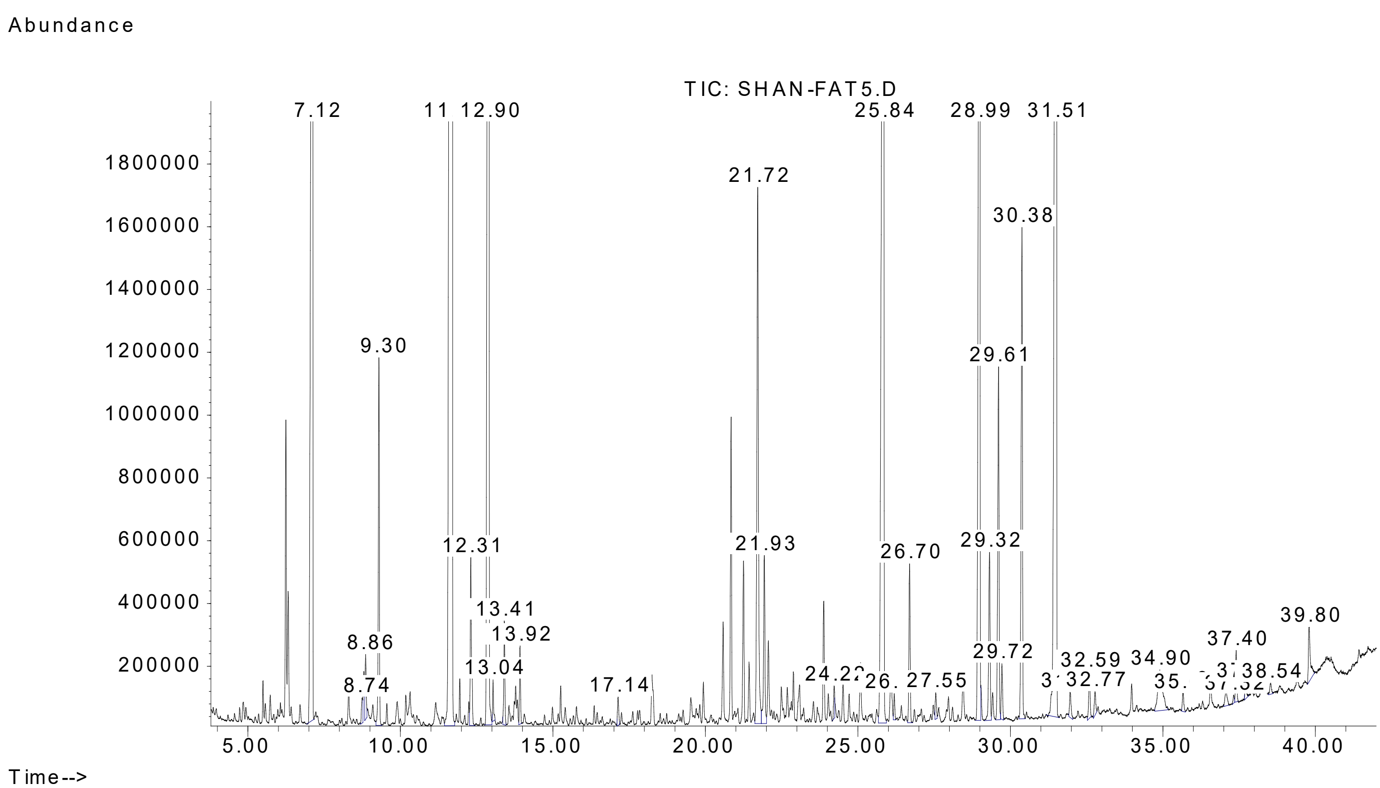

Figure 2. The GC-MS chromatogram of carboxylic acids methyl esters in the *D. grandiflorum* her