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CHROMATOGRAPHIC PROFILES OF CARBOXYLIC ACIDS IN THE RAW MATERIALS OF SOME *MENTHEAE* DUMORT. SPECIES

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Abstract

The *Mentheae* Dumort. is the largest tribe in the *Nepetoideae* Burnett subfamily of the *Lamiaceae* Marinov family. The unofficial medicinal plants of the *Mentheae* tribe are of great interest for pharmacy and food industry. The representatives of the *Hyssopus* L., *Agastache* Gronov, *Monarda* L., and *Satureja* L. genera are gradually spreading in Ukraine as spicy-aromatic and honey-bearing plants. Such secondary metabolites of these representatives as terpenoids and polyphenols were studied much better than primary ones such as carboxylic acids, amino acids, carbohydrates, etc. The chromatographic profiles of carboxylic acids in the herbs of four *Mentheae* representatives (*Agastache foeniculum, Hyssopus officinalis*, *Monarda fistulosa*, and *Satureja hortensis*) were revealed by the GC-MS analysis. There were detected from 12 to 16 short-chained carboxylic acids and from 11 to 16 fatty acid in their raw materials. *p*-Hydroxycinnamic acid prevailed among the identified aromatic acids in all the herbs and the most significant amount of it was found in the *Hyssopus officinalis* (632 mg/kg). Unsaturated *α*-linolenic acid noticeably dominated among the fatty acids in the raw material of *Satureja hortensis* (3372 mg/kg) and *Monarda fistulosa* (2620 mg/kg). Generally, the founding of this study can regard the investigated species as prospective sources of some carboxylic acids.

Keywords: *Agastache foeniculum, Hyssopus officinalis*, *Monarda fistulosa*, *Satureja hortensis,* GC-MS analysis, herb, carboxylic acid

Introduction

The *Mentheae* Dumort. tribe is the largest one in the *Nepetoideae* Burnett subfamily of the *Lamiaceae* Marinov family [1]. Many of its representanives such as mint, melissa, rosemary, oregano, sage, thyme, etc. are economically important essential oil-bearing plants. The unofficial medicinal plants of the *Mentheae* tribe are of great interest for pharmacy and food industry [2–4]. The species of the genera *Agastache* Gronov, *Monarda* L., and *Satureja* L. are not included to any Pharmacopoeia. The *Hyssopus officinalis* herb was included to the British Herbal Pharmacopoeia [5].

The genus *Hyssopus* includes 7 species of drought-tolerant herbaceous plants and shrubs which are naturally distributed in Asia and in the Mediterranean [6, 7]. They have been used in folk medicine of European and Asian countries mainly for the treatment of colds and bronchitis [7].

The genus *Agastache* counts 22 species of herbaceous plants distributed as wild in North America and Southeast Asia [6, 8]. *Agastache spp.* are used in different countries as immunostimulants, for the treatment of colds, fever, inflammatory skin diseases, *etc*.

There are 20 species of herbaceous plants in the *Monarda* genus [6], which are native to North America [9, 10]. The Indians classified the *Monarda* representatives as the most important sacred plants, which were often used in the treatment of fever, cough, wounds, *etc*. [9, 10].

The genus *Satureja* comprises 52 species of herbaceous or semi-woody plants common in the wild in Asian countries and in the Mediterranean area [6, 11]. The aerial parts of many *Satureja* species are used as analgesic, antihypertensive, expectorant and wound healing agents [11, 12].

The representatives of the abovementioned genera are gradually spreading in Ukraine as spicy-aromatic and honey-bearing plants. Secondary metabolites of the representatives of this tribe such as terpenoids and polyphenols were within the scope of many scientists [2–12]. Nevertheless, many of primary metabolites of plants including carboxylic acids, carbohydrates, nucleotides, and amino acids also possess the valuable pharmacological properties [13] and deserve the considerable attention.

As it is known, the appropriate intake of polyunsaturated fatty acids and other essential compounds can prevent various health disorders such as chronic inflammation, atherosclerosis, diabetes mellitus, obesity, neurodegeneration, etc. [14–22]. The contents of carboxylic acids in the raw materials of the *Mentheae* tribe representatives were studied only sporadically [23–26].

The aim of the present study was the chromatographic analysis of carboxylic acids in the raw materials of four *Mentheae* representatives (*Agastache foeniculum, Hyssopus officinalis*, *Monarda fistulosa*, and *Satureja hortensis*) under their cultivation in Ukraine.

Methods

The aerial parts of the studied plants were harvested in the flowering period from the experimental plots in Ternopil region, Ukraine. The herbs were dried for several days in a shade. The samples were identified by the authors.

As it is known, the choice of solvent and extraction technique plays a crucial role in the analysis of carboxylic acids [13, 27, 28]. The preparation of the investigated raw materials for the gas chromatography - mass spectrometric (GC-MS) analysis included placing the grinded herbs (0.05 g) in a vial (2 mL) and adding the internal standard (50 µg of tridecane in hexane) followed by 1.0 mL of 14% boron trichloride in methanol as a methylating agent.

The obtained mixture was kept in a hermetically sealed vial at a temperature of 65°C for 8 h to extract the fats as well as their hydrolysis and methylation. The reaction mixture was drained from the precipitate and diluted by adding 1.0 mL of purified water. 0.2 mL of dichloromethane was added and the mixture was gently shaken during 1 h to remove the fatty acid methyl esters. The compositions of the resulting mixture of methyl esters was chromatographed.

Chromatograph Agilent Technologies 6890 N equipped with mass spectrometric detector 5973 N was used in the analysis. The chromatographic column was Innowax (30 m × 0.25 mm); the rate of helium as a carrier gas was 1.2 mL/min. The library of mass spectra WILEY 2007 and NIST 05 was used for the identification of carboxylic acids.

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Results

The compositions and contents of short-chained carboxylic acids in the investigated herbs are represented in Table 1. The typical GC-MS chromatogram of the carboxylic acids methyl esters is given on the example of *Hyssopus officinalis* (Figure 1).

As can be seen from the data of Table 1, the total amount of the short-chained carboxylic acids was the highest in the *Hyssopus officinalis* herb (9691 mg/kg). Three aliphatic acids (citric, malonic, and malic) prevailed in the herbs of all species.

It should be mentioned that short-chained aromatic acids such as *p*-hydroxycinnamic, ferulic, benzoic, salicylic (Figure 2) possess the proven therapeutic properties [29–34]. They are used in the food industry as well as in perfume manufacturing mainly as antiseptics and antioxidants.

p-Hydroxycinnamic acid dominated among the all found aromatic acids in the *Hyssopus officinalis* herb (632 mg/kg). It possesses the significant antioxidant and anticancer potential [34]. Ferulic acid as well as benzoic acid possessing prominent antioxidant effects [32, 33] were also revealed in the most abundant amount in the *Hyssopus officinalis* raw material (361 mg/kg and 466 mg/kg, respectively). Generally, *Agastache foeniculum* was characterized by the greatest variety of detected aromatic acids (7 acids) in comparison with other species: *Hyssopus officinalis* (6), *Monarda fistulosa* (5), and *Satureja hortensis* (4).

Alexa et al. [30] reported that rosmarinic and ferulic acids were the predominant phenolic compounds of such *Mentheae* tribe species as *Mentha × piperita* L. and *Lavandula angustifolia* Mill. extracts.

It was revealed the composition of long-chained carboxylic (fatty) acids in the studied *Mentheae* representatives (Table 2). The largest total amounts of fatty acids were found in the *Satureja hortensis* (8007 mg/kg) and *Monarda fistulosa* (6960 mg/kg) herbs.

It should be mentioned that unsaturated *α*-linolenic acid belonging to ω3 was the predominant short-chained acid of *Satureja hortensis* (3372 mg/kg) and *Monarda fistulosa* (2620 mg/kg) aerial parts. It is worth noting that the raw materials of other investigated species also accumulate a significant contents of unsaturated fatty acids. As it is known, *α*-linolenic, linoleic and other polyunsaturated fatty acids play an important role in the structure of cell membranes, hormone metabolism, *etc*. [14, 35–37]. These acids were aslo found in the significant amounts in the aboveground parts of *Mentheae* species belonging to the *Mentha, Salvia, Melissa, etc.* genera [23, 37].

Conclusion

The chromatographic profiles of carboxylic acids in the herbs of some *Mentheae* representatives were revealed by the GC-MS analysis. There were detected from 12 to 16 short-chained carboxylic acids and from 11 to 16 long-chained ones in the studied raw materials. *p*-Hydroxycinnamic acid prevailed among the all found aromatic acids and the most significant amount of it was detected in the *Hyssopus officinalis* herb (632 mg/kg). Unsaturated *α*-linolenic acid crucially dominated among the fatty acids in the raw material of *Satureja hortensis* (3372 mg/kg) followed by *Monarda fistulosa* (2620 mg/kg).

Thus, the founding of this study can regard the investigated species as prospective sources of new medicines and food additives.

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

Acid	Retention time, min	*Agastache foeniculum*	*Hyssopus officinalis*	*Monarda fistulosa*	*Satureja hortensis*
Dimethoxyacetic	8.88	61	-	-	-
Oxalic	9.30	370	84	131	420
3,3-dimethoxypropionic	9.55	-	-	-	17
Malonic	11.59	3578	3731	631	652
4,4-dimethoxybutyric	12.24	-	-	-	26
Fumaric	12.34	59	71	40	22
Furfurylic	13.02	14	-	15	29
Succinic	13.42	214	82	221	298
Benzoic*	13.91	101	466	-	14
Phenylacetic*	16.88	7	-	6	10
Salicylic*	17.13	57	47	30	-
Malic	21.68	590	946	64	856
Dimethylmalonic	23.09	-	-	-	288
Azelaic	24.21	32	104	79	-
10-oxo-8-decenic	27.64	-	-	-	49
Citric	29.0	1500	3063	1842	2195
Vanillic*	31.97	53	26	45	55
Syringic*	37.40	10	78	-	-
p-Hydroxycinnamic*	38.94	31	632	-	104
Ferulic*	39.80	87	361	134	-
Total amount		6764	9691	3238	4969

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

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Table 2. Composition and contents of the long-chained carboxylic (fatty) acids in the herbs of some *Mentheae* species, mg/kg

Acid	Retention time, min	*Agastache foeniculum*	*Hyssopus officinalis*	*Monarda fistulosa*	*Satureja hortensis*
Laurinic	17.84	-	20	-	-
Myristic	21.92	102	133	168	87
13-methylmyristic	23.86	-	-	-	38
14-methylpenthadecanoic	24.86	22	-	-	-
Palmitic	25.86	1562	1536	2256	2114
Palmitoleic*	26.18	158	99	355	36
7- Hexadecenic*	26.69	20	30	41	296
7,10-Hexadecadienic*	26.85	-	30	-	-
14-Methylpalmitic	26.90	-	-	-	-
15-Methylhexadecenic	26.96	30	-	-	41
Heptadecanic	27.54	32	21	-	33
7,10,13-Hexadecatrienic	27.97	-	35	-	-
Stearinic	29.31	162	90	285	319
Oleic*	29.59	147	89	312	167
7-Octadecenic*	29.71	-	-	-	50
11- Octadecenic*	29.72	27	16	29	-
Linoleic*	30.37	541	217	791	1028
α-Linolenic*	31.48	1085	713	2620	3372
Arachidic	32.59	21	32	78	217
2-Oxipalmitic	32.65	-	29	-	-
Behenic	35.66	19	29	25	94
Tricocylic	37.10	-	-	-	20
Lignoceric	38.52	-	-	-	95
Total amount		3927	3103	6960	8007

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

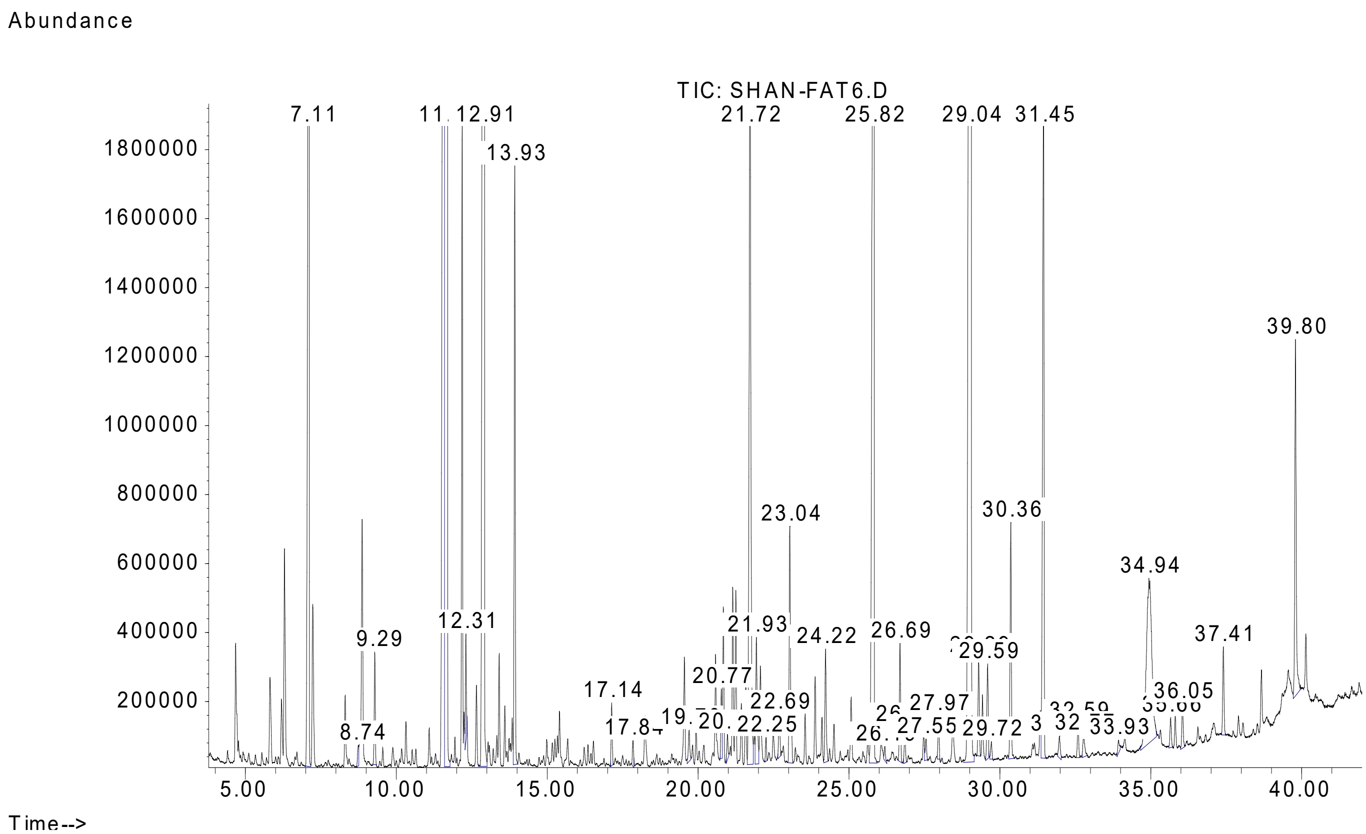

Figure 1. Typical GC-MS chromatogram of carboxylic acids methyl esters in the *Hyssopus officinalis* herb

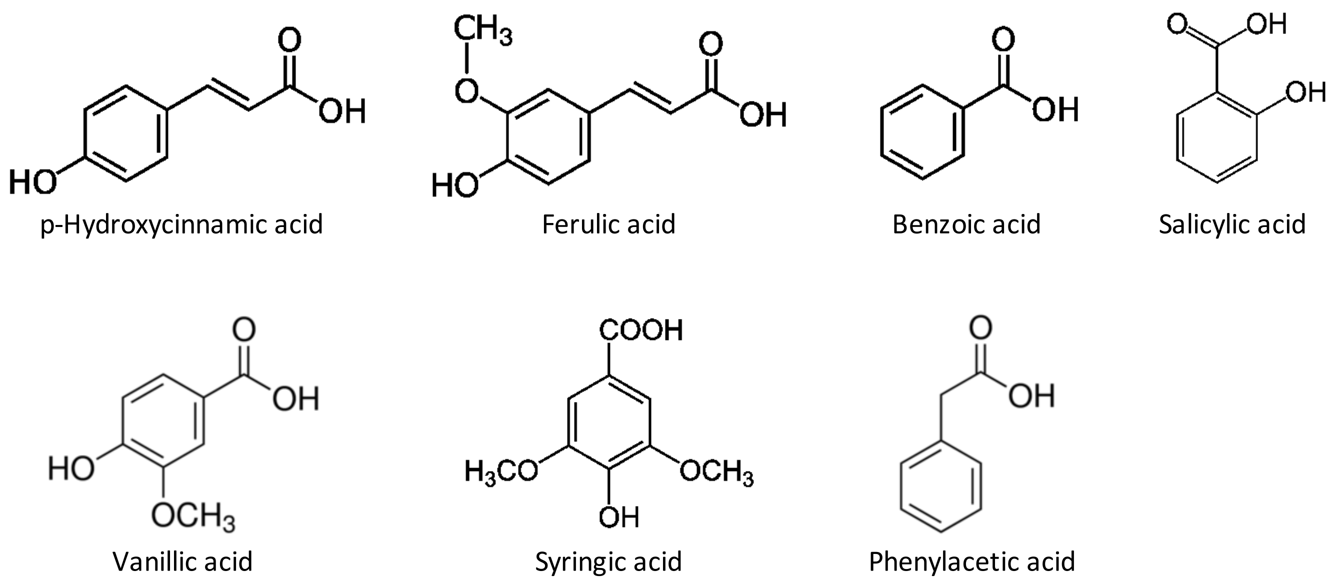

Figure 2. Structural formulas of the aromatic carboxylic acids found in the herbs of *Mentheae* species

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