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Organic acids of vegetative and generative organs of black horehound

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Abstract

By use of chromatography-mass spectrometry the content of organic acids in leaves, stems, corollas and calyces of black horehound (*Ballota nigra* L.) was researched. 34 compounds, including 26 aliphatic and 8 aromatic, were detected. The highest total content of organic acids (4.39%) was observed in leaves, the highest content of aromatic acids, including hydroxycinnamic – in corollas.

Keywords: Black horehound, organic acids, chromatography-mass spectrometry.

1. Introduction

Black horehound (*Ballota nigra* L.) – a perennial herb of the family *Lamiaceae* L. Black horehound herb has a sedative effect, it is used for treatment of depression, insomnia. The main biologically active substances of this plant material are phenolcarbonic and hydroxycinnamic acids, coumarins, flavonoids, tannins, essential oil components^[2,3].

Previously we have studied the component composition of essential oil and aromatic acids of black horehound herb^[1, 4]. The aim of presented study was chromatography-mass spectrometric research of organic acids of vegetative and generative organs of black horehound. The objects of study were vegetative and generative organs of *B. nigra* L. herb, harvested in the summer 2013 in the Kharkiv region, namely leaves, stems, corollas and calyces.

2. Materials and methods

Analysis of fatty and organic acids was performed at the National Institute for Vine and Wine "Magarach". Fatty acids composition was analyzed by gas-liquid chromatographer Agilent Technologies 6890 with mass spectrometric detector 5973. Conditions of chromatography: capillary chromatographic column INNOWAX (internal diameter of 0.25 mm and length of 30 m). Carrier gas – helium, gas flow rate – 1.2 ml/min. The temperature of the input heater – 250 °C. The thermostat temperature is programmed from 50 to 250 °C at a speed of 4 °C / min. For a detailed analysis of acids we have conducted preliminary preparation of raw material which consisted in methylation of acids by 140 g/l boron chloride solution in methanol to obtain volatile derivatives with a low boiling point. The mixture was kept in a tightly corked vial for 8 hours at 65 °C. Acid methyl esters were extracted with methylene chloride. To identify the acids we used mass spectra libraries NIST05 and WILEY 2007 in conjunction with programs AMDIS and NIST. For quantitative calculations we used the method of internal standard.

3. Results and Discussion

Chromatographic profiles of organic acids of vegetative and generative organs of *B. nigra* L. herb are shown in Fig. 1-4.

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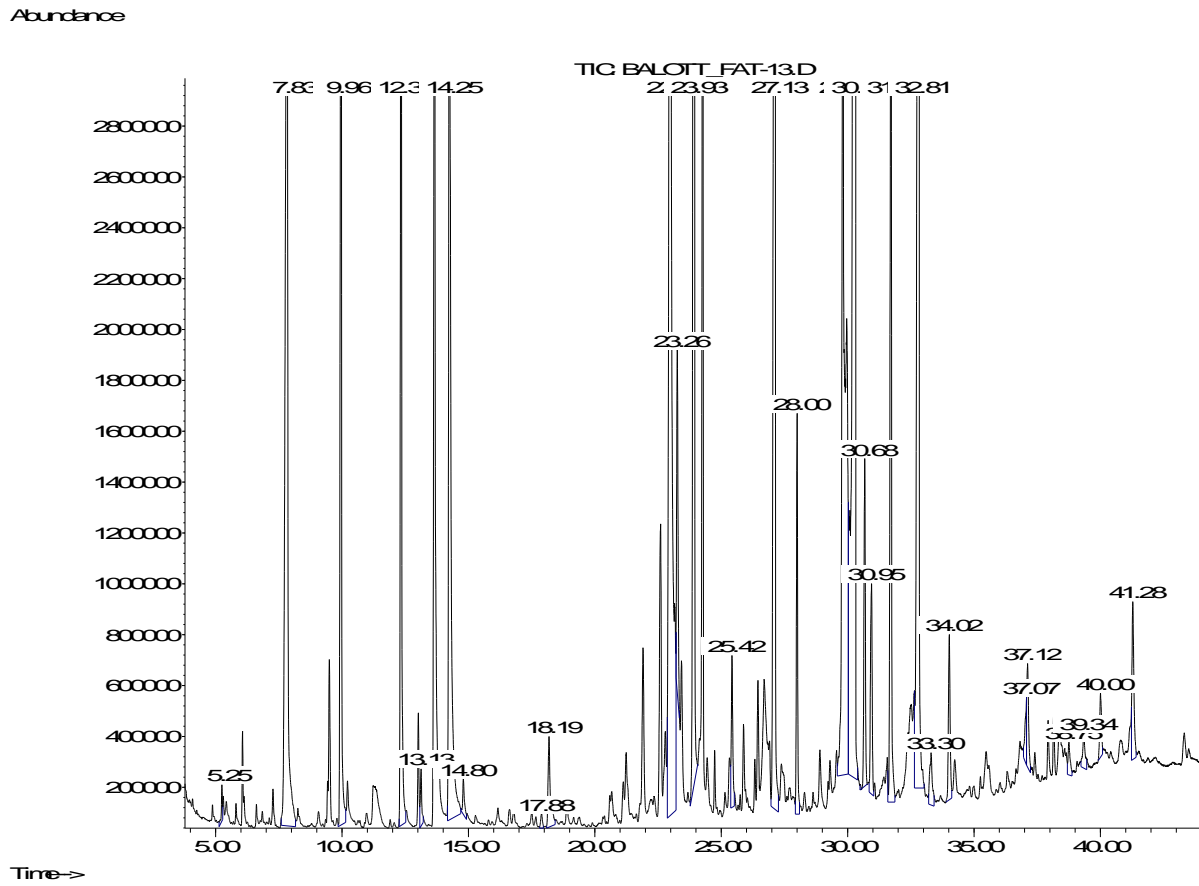


Fig 1: Chromatographic profile of organic acids of *B. nigra* L. Leaves

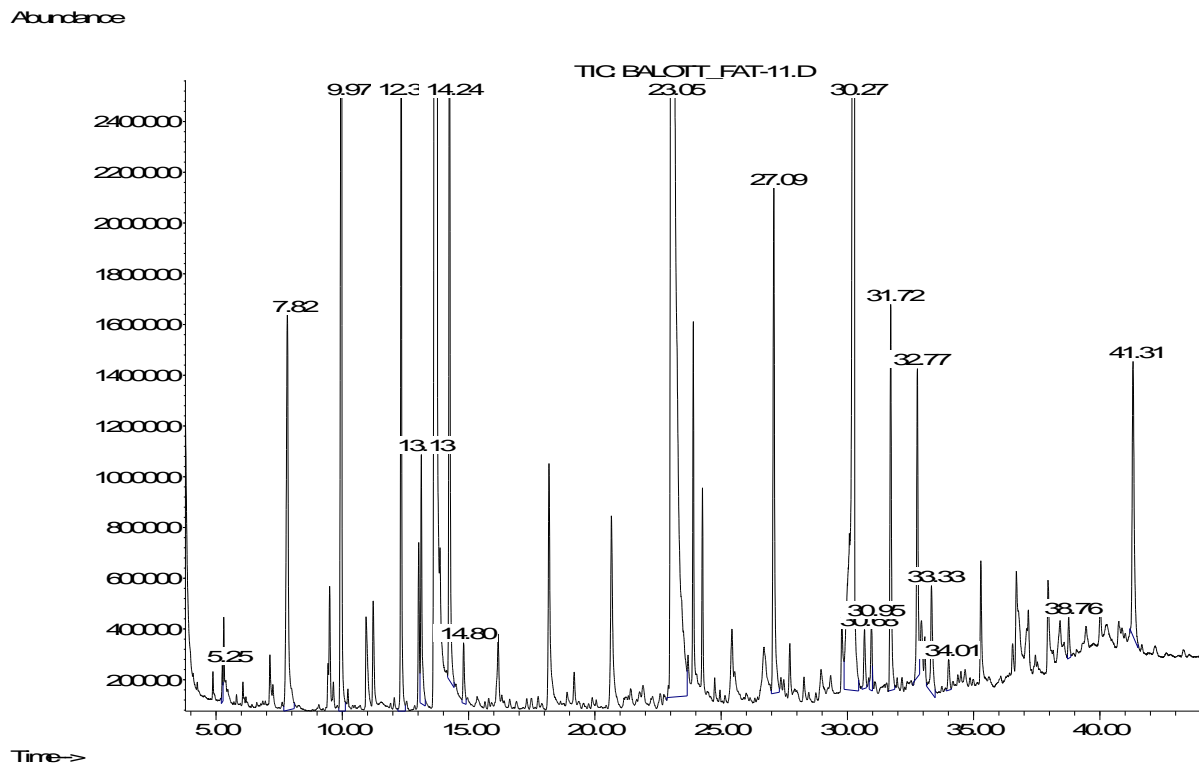


Fig 2: Chromatographic profile of organic acids of *B. nigra* L. Stems

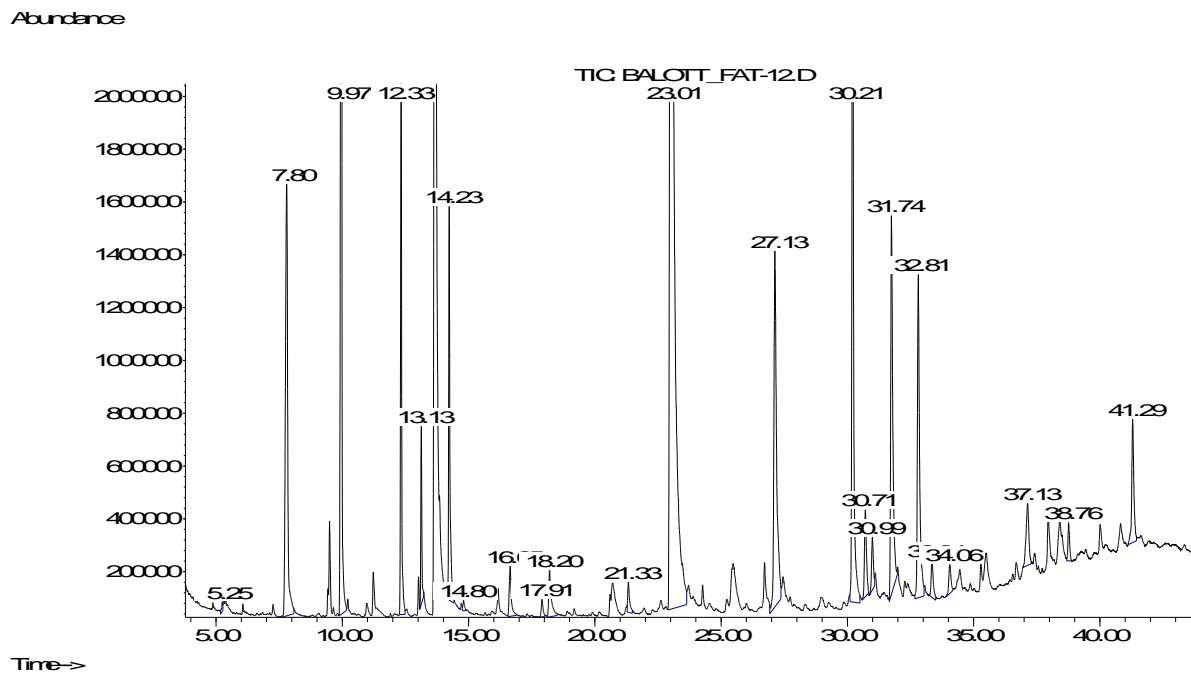


Fig 3: Chromatographic profile of organic acids of *B. nigra* L. Corollas

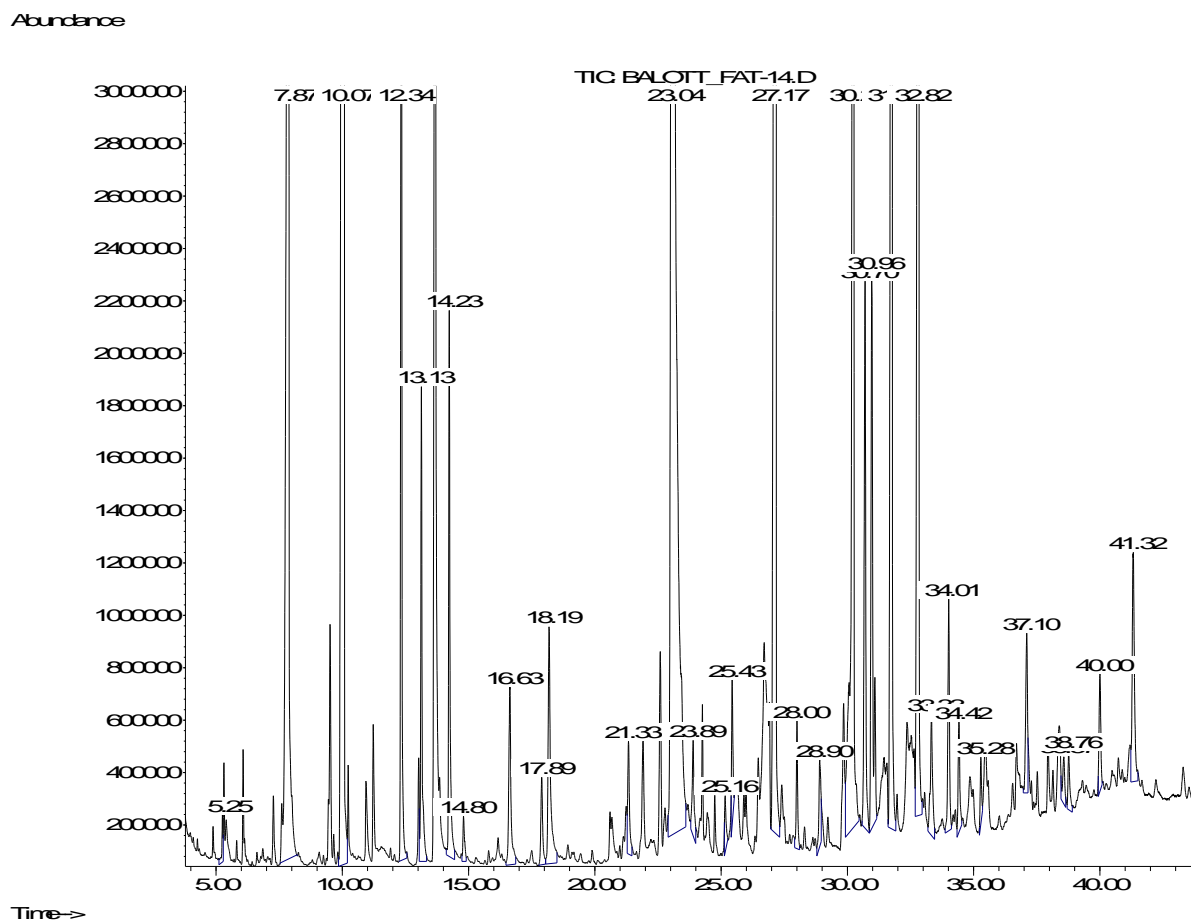


Fig 4: Chromatographic profile of organic acids of *B. nigra* L. calyces

In the studied organs of *B. nigra* L. herb 34 organic acids were detected, including 26 aliphatic and 8 aromatic compounds (Tab. 1).

Among the aliphatic acids dominate higher fatty acids – they are 14 compounds, including 9 saturated (caproic, pentadecanoic, palmitic, margaric, stearic, arahidic, behenic, tricosanoic, tetracosanoic), 3 monounsaturated (palmitoleic, oleic, gondoic acid), 2 polyunsaturated (linoleic and linolenic)

acids. 9 aliphatic dicarboxylic acids: oxalic, malonic, succinic, glutaric, azelaic, unsaturated fumaric, hydroxyacids malic and 3-hydroxy-2-methylglutaric, oxoacid 2-oxo-glutaric; 3 tricarboxylic – 2 isomers of 1,1,2-ethane-tricarboxylic and citric acids were identified.

Among the aromatic acids benzoic acid, phenolic acids – salicylic, vanillic, syringic and hentisinic, phenyl carbonic acid – phenylacetic, hydroxycinnamic acids – *para*-coumaric and

ferulic were identified.

In the leaves of black horehound 20 aliphatic acids were identified, predominant among them are citric (10784.5 mg/1,000 g), isomers of 1,1,2-ethane-tricarboxylic (total content of 7561.6 mg/1,000 g), linolenic (5009.7 mg/1,000 g), malic (4759.4 mg/1,000 g) and palmitic (3903.0 mg/1,000 g). 8 aromatic acids were identified, the highest contents among which are ferulic (348.9 mg/1000 g) and salicylic (245.1 mg/1,000 g) acids. It should be noted that among the studied parts of *B. nigra* L. herb only in leaves 2-oxo-glutaric acid and hentisinic acids have been founded. Also specific for this part of the plant is the high content of the 1,1,2-ethane-tricarboxylic acid isomers.

In the stems of the investigated plant 13 aliphatic acids, predominant among them are malic (7140.1 mg/1,000 g) and citric (5782.2 mg/1,000 g), and 5 aromatic, the most of which are ferulic (557.8 mg/1,000 g) and vanillic acids (223.5 mg/1,000 g), were founded.

In the corollas of *B. nigra* L. 15 aliphatic acids with the significant predominance of malic (11381.6 mg/1,000 g), and

7 aromatic with the highest content of ferulic, *para*-coumaric and salicylic acid (445.0, 299.8 and 201.3 mg/1,000 g, respectively) were detected.

Among the 24 aliphatic acids identified in calyces of black horehound predominate oxalic (4385.7 mg / 1,000 g), malic (4289.2 mg / 1,000 g) and palmitic (3359.2 mg / 1,000 g), among 6 aromatic acids – salicylic (339.8 mg / 1,000 g) and ferulic (301.7 mg / 1,000 g). Specific for this part of plant were pentadecanoic, margaric, gondoic and tricosanoic. Specific for generative organs are glutaric and 3-hydroxy-2-methylglutaric acid.

Leaves considerably exceeds among all studied organs by the total content of organic acids – 4.39% in comparison with the stems (1.98%), the calyces (2.46%) and the corollas (2,54%), mainly due to the relatively high content of aliphatic (malonic, succinic, citric, isomers of 1,1,2-ethane-tricarboxylic) and fatty (palmitic, linolenic) acids. At the same time the highest content of aromatic acids was founded in corollas – 1274.8 mg/1000 g (0.13%), including hydroxycinnamic – 744.8 mg/1000 g (0.07%).

Table 1: The content of organic acids in raw materials of *Ballota nigra* L.

N	Acid	Content, mg/1000 g			
		leaves	stems	corollas	calyces
Aliphatic acids					
1	Caproic	45,9	31,6	21,0	30,6
2	Oxalic	1910,5	1677,8	3094,9	4385,7
3	Malonic	1909,2	806,5	1052,9	891,5
4	Fumaric	93,6	321,1	314,2	372,4
5	Succinic	2490,6	1017,8	847,7	433,9
6	Malic	4759,4	7140,1	11381,6	4289,2
7	Glutaric	-	-	181,0	228,9
8	2-Oxo-glutaric	559,8	-	-	-
9	3-Hydroxy-2-methylglutaric	-	-	97,2	115,1
10	1,1,2-Ethane-tricarboxylic (isomer 1)	4021,9	-	-	104,8
11	Pentadecanoic	-	-	-	47,3
12	Azelaic	257,1	-	-	108,5
13	Palmitic	3903,0	817,6	1658,0	3359,2
14	Palmitoleic	559,8	-	-	103,6
15	1,1,2-Ethane-tricarboxylic (isomer 2)	3539,7	-	-	-
16	Margaric	-	-	-	94,4
17	Citric	10784,5	5782,2	2342,5	2799,3
18	Stearic	540,6	95,6	301,4	514,0
19	Oleic	423,5	99,4	166,8	632,2
20	Linoleic	1659,7	555,2	1372,7	2101,5
21	Linolenic	5009,7	450,3	1224,1	2318,4
22	Arahidic	329,4	54,0	109,4	213,7
23	Gondoic	-	-	-	110,6
24	Behenic	217,7	-	-	164,4
25	Tricosanoic	-	-	-	58,4
26	Tetracosanoic	133,6	-	-	119,4
Sum of aliphatic acids		42797,9	18849,2	24165,4	23597,0
Aromatic acids					
27	Benzoic	72,3	88,6	27,0	43,6
28	Phenylacetic	30,2	-	55,0	95,6
29	Salicylic	245,1	-	201,3	339,8
30	Vanillic	157,8	223,5	139,6	159,7
31	<i>para</i> -Coumaric	105,3	-	299,8	-
32	Syringic	80,1	60,1	107,1	62,8
33	Hentisinic	100,7	-	-	-
34	Ferulic	348,9	557,8	445,0	301,7
Sum of aromatic acids		1140,4	930,0	1274,8	1003,2
Total		43938,3	19779,2	25440,2	24600,2

4. Conclusions

By use of chromatography-mass spectrometry the content of organic acids in leaves, stems, corollas and calyces of black horehound (*Ballota nigra* L.) was researched. 34 compounds, including 14 higher fatty acids, 9 aliphatic dicarboxylic acids, 3 tricarboxylic acids, and 8 aromatic acids, were detected.

The highest total content of organic acids was observed in leaves, the highest content of aromatic acids, including hydroxycinnamic – in corollas.

Obtained data are of great practical importance, because many organic acids have the biological activity and contribute to the cumulative effect of the herbal drugs.

5. References

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