

Phytochemical study on Ziziphora clinopodioides Lam essential oils wild-growing in the Armenian flora and grown up in the conditions of a hydroponics.

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Phytochemical study on Ziziphora clinopodioides Lam essential oils wild-growing in the Armenian flora and grown up in the conditions of a hydroponics.

Abstract-The objects of this study are the wild-growing grass of the species *Ziziphora clinopodioides* Lam. collected in the flowering phase in April-July 2013 from the mountains of the villages Voghjaberd, Hankavan, Arzakan and the grass *Ziziphora* cultured in the hydroponic conditions.

For the first time, the numerical characteristics of the merchandising quality control for raw material of the *Ziziphora clinopodioides* Lam. were defined from wild-growing plants (Voghjaberd, Hankavan, Arzakan) and grown up in a hydroponics.

The highest value of the essential oil yield differs raw material collected in the condition of hydroponics $1.25 \pm 0.01\%$, for the relative density differs raw material collected in the vicinity of the village Hankavan 0.977 ± 0.001 , for the refractive index differs raw materials collected in the vicinity of the village Arzakan 1.490 ± 0.003 .

The highest values for the total extractive composition /50 ° spirits / and humidity differs raw material collected in Arzakan $29.0 \pm 0.01\%$, $8.8 \pm 0.15\%$, respectively, for the ash - hydroponics $9.1 \pm 0.02\%$.

By the method of gas chromatography-mass spectrometry, the above studied samples revealed for the first time more than 80 components, among which there were the following main components:(±) pulegone (16,62-25,71%), verbenone (7,78-14,33%), eucalyptol (8,94-12,98%), DL (±) menthol (1,48-10,02%), isomenthone (3,42-8,05%), I-menthone (3,53 - 7,02%), D-menthone (5,13-6,85%), DL-carvone (3,18-6,57%), D (±) limonene (1,3-6,47%), thymol (0,73-5,41%).

(Abstract) Keywords-*Ziziphora clinopodioides* Lam., essential oils, density, refractive index, gas chromatography-mass spectrometry (key words)

1. Introduction

In recent years, the arsenal of the medicinal plants and the herbal medicines has been significantly increased on the global pharmaceutical market. The essential oils production is concentrated mainly in the southern countries, which are the main sources of raw materials [1,2].

The plant *Ziziphora clinopodioides* Lam. has the scientific interest among the raw material sources of the essential oils. This plant is used mostly in traditional medicine as sedative and carminative, anti-vomiting and, anti-inflammatory and antiseptic in different foods and also in traditional medicines for treatment of digestive and viral diseases, on the interaction between these compounds and yoghurt starter culture activity [3,4,5].

The analysis of the international scientific literature shows that *Ziziphora clinopodioides* Lam. is one of the most promising plants for the essential oils production as well as the raw source of flavonoids which determine the biological activity of the wild-growing species [6,7,8].

The chemical composition of the essential oil obtained from the aerial parts of *Ziziphora clinopodioides* Lam. was analyzed by the gas chromatography-mass spectrometry (GC-MS) method. Twenty six components representing 97.6% - 97.62% of the total oil were identified. The major constituents were pulegone (34.4%-36.45%), piperitenone (15.1%-19.12%), 1-8- cineole (6.5%), neo-menthol (5.7-4.78%), menth-2-en-1-ol (5.3%-5.31%), menthol (5.2%), carvacrol (5.1%) and menthone (4.5%-4.46%) [9,10,11,12,13,14,15,16].

The study of the domestic plant raw material shows, that the flora of Armenia is the most relevant of identifying perspective essential oil plants, and creating the effective medicines. [17,18,19].

From this point of view, the object of studying the *species* of *Ziziphora clinopodioides* Lam served wild-growing in the flora of Armenia [20].

Ziziphora L.(Labiatae family) includes about 30 species that are wide-spread in the southern part of Europe, in the Mediterranean region and South-East Asia [21].The species *Ziziphora* is one of the most common plants in the flora of Armenia. The raw material resources study indicated that the populations of the wild-growing species *Ziziphora* met in Armenia as a form of the small scattered semi shrubs in small populations which alternate from the rocky slopes of mountain belts to subalpine elevations.

In the traditional Armenian medicine, *Ziziphora clinopodioides* var.*Serpyllacea* is widely used, as the phytoncides, cardiotoxic and hypotensive means to ease nausea during pregnancy, as well as a fragrance ingredient in the soaps manufacturing and in cosmetic purposes [22].

Thus, the study of the species *Ziziphora* growing in the flora of Armenia, as a plant of a valuable raw material oils production as well as a source of the flavonoids, acquires a scientific and practical value.

Material and methods.

The objects of this study are the wildy-growing grass of the species *Ziziphora clinopodioides* Lam. collected in the flowering phase in April-July 2013 from the mountains of the villages Voghjaberd, Hankavan, Arzakan (mountain chain Teksari), adjacent to the eastern and south-western slopes of the ridge Geghama and the grass *Ziziphora* cultured in the hydroponic conditions (Fig.1).

Fig.1. *The grass Ziziphora clinopodioides* Lam. for the first time cultured in the hydroponic conditions.



For the purpose of cultivation, the bushes of *Ziziphora* were collected near the villages of Voghjaberd and Hankavan in the Kotayk region in the middle of April (12.04.13) and were planted on an 5 m² area of the artificial cultivation in the vegetative hydroponics conditions, as well as in the soil per 40 plants. The nutrient black dross in diameter 3-15 mm as a filler was used, which was pre-disinfected by the 0.05% solution of the KMnO₄. As the standard sample, the normal soil culture was used, in which the applicable regulations of agricultural standards were fulfilled [20,23].

The essential oils were obtained by the method of hydrodistillation (World Health Organization 2011) [24].

According to the merchandising analysis, the quantitative characteristics of raw material quality and the physicochemical parameters of essential oils were determined for all the samples. The macroscopic and microscopic characteristics analysis has been carried out according to the quality control methods for herbal materials [24].

The measurements of the relative density (ρ/ρ_0) at 20° C, were performed by a micropipette firm "Pipette" / Germany / and the analytical electron balance firm "Scientech SA80" / Germany /.

A refractive index was measured by refractometer firm RL-2 / Poland / connected to a thermostat at a standard temperature 20°C.

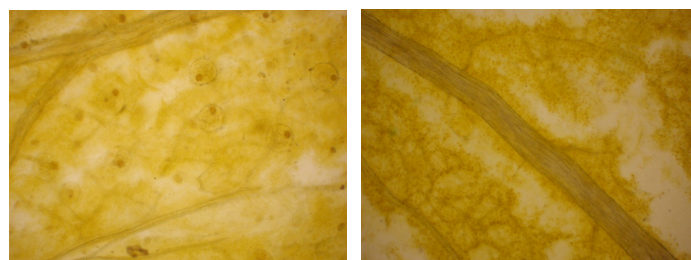
The chemical composition of the components of the essential oil was investigated by the gas chromatography-mass spectrometry method, gas chromatograph with the mass selective detector manufactured by BRUKER, the USA, was used chromatographic column OPTIMA-FFAP-0.25mm, 60m×0.25mm (ID.MACHEREY-NAGEL, Germany), the qualitative analysis based on the comparison of retention times and the total mass spectra with the corresponding reference data components of oils and pure compounds with the mass spectra data of the library catalog NIST, the flow of helium carrier gas - 1.0ml/min, volume of injected sample 2mcl, the evaporator temperature - 220° C, a temperature gradient 50 ° C (2min), heated to 250 ° C (2.5 ° C / min) (retention 5min), split-division 5. The component contents were calculated from the gas chromatographic peak areas.

The components identification was carried out by their retention time and the peak increase by adding a witness. The quantification of the components was determined by the method internal normalization [25].

II. Results and discussions.

The results of the microscopic studies have generated that all the kinds of samples *Ziziphora clinopodioides* Lam., regardless of climatic conditions of germination and processing features, histologically identical: the round essential oil glands, the unicellular hairs, the stomata anomocytic types are specifically for the family Labiatae (Fig. 2.3).

Fig. 2.3.The results of the microscopic studies.



The results showed, that the yield of essential oil obtained from the raw materials grown up in hydroponics was (1.25 ± 0.01%), Vohchabert (0.85 ± 0.02%), Hankavan (0.79 ± 0.02%), Arzakan (0.78 ± 0.03%) (Tab. 1).

The Tab.I demonstrates, that the greatest amount of the essential oil samples of raw materials are collected in hydroponics ($1.25 \pm 0.01\%$), which indicates a favorable effect of growing conditions (black slag) on the intensity of the production of essential oil in plant.

For raw materials collected of the wild-growing plants of the Kotayk region (Vohchabert, Hankavan, Arzakan) was noted almost the same percentage of the essential oil yield ($0.78-0.85\%$). The inauthenticity of difference was statistically noted ($p > 0,05$).

TABLE I
THE YIELD OF THE ESSENTIAL OIL ZIZIPHORA CLINOPODIOIDES LAM. WILD-GROWING AND GROWN IN HYDROPONICS DEPENDS FROM THE GROWTH AREAS (n=5).

Sample number	Places of the growing of the raw materials	Essential oil yield, %
I	Hydroponics (black slag)	1.25 ± 0.01
II	Vohchabert (above the sea level at altitude 1880 meters on the slopes of the Vohchaberts ridge, the dry rocky mountain-steppe rubbly terrain) (40°10'5"N 44°38'40"E.)	$0.85 \pm 0.02^*$
III	Hankavan (above the sea level at altitude 1990 meters on the slopes of the river Marmarik mountain-forest rocky terrain) (40°38'39" N 44°28'53" E.)	$0.79 \pm 0.02^*$
IV	Arzakan (above the sea level at altitude 1700 m and rocky mountain-steppe terrain) (40°26'58" N 44°36'23" E.)	$0.78 \pm 0.03^*$

* $p < 0,05$ as compared to hydroponics.

As it is known, such physicochemical parameters as the density and the refractive index of essential oil assumes the good quality and the timely collection of raw materials [24, 26].

As our studies showed, the largest amount of essential oils accumulation in the sample growing in the hydroponics, the percentage of which exceeded the others were almost 1.6 times. However, the relative density of the sample was inferior to other samples, indicating to a small amount of oxygen-containing terpenes.

A special attention should be paid to the samples collected near the village Hankavan by a higher value of the relative density. The relative density of the oil of this sample is higher than the other almost 1.07-1.17 times, respectively.

The relative density of the samples grown up in hydroponics - (0.857 ± 0.002), is inferior to the other samples, indicating to a small amount of oxygen-containing compounds, as well as the timeliness of the collection of raw materials in a hydroponics (Tab. I,II).

TABLE II
THE PHYSICO-CHEMICAL AND ORGANOLEPTIC CHARACTERISTICS OF THE ESSENTIAL OILS (n = 5).

	Analytical samples of raw materials. Name of the locality	Relative density of essential oils $t=20^\circ C$, $\rho/\rho_0 \pm m$	Refraction indexes essential oils $t=20^\circ C$, $n \pm m$	Organoleptic characteristics	
				Color	Odour
1	Hydroponics (black slag)	0.857 ± 0.002	1.489 ± 0.001	Yellowish	spicy with a touch of floral
2	Vohchabert	0.912 ± 0.001	1.488 ± 0.001	Yellowish	spicy with a touch of floral
3	Hankavan	0.977 ± 0.001	1.485 ± 0.002	Light yellow	spicy with a touch of floral
4	Arzakan	0.911 ± 0.001	1.490 ± 0.003	Yellow	spicy with a touch of floral

The following data were obtained due to the results of the merchandising analysis of the quality of raw material (humidity, ash, the total extractive composition); in hydroponic conditions - humidity $8.6 \pm 0.05\%$, ash $9.1 \pm 0.02\%$, the total extractive composition $22.5 \pm 0.08\%$, for the wild-growing near the villages Vohchabert - the humidity $7.8 \pm 0.18\%$, ash $7.8 \pm 0.07\%$, the total extractive composition $25.0 \pm 0.06\%$, Hankavan - the humidity $8.4 \pm 0.12\%$, ash $7.0 \pm 0.07\%$, the total extractive composition $23.0 \pm 0.06\%$, Arzakan - the humidity $8.8 \pm 0.15\%$, ash $8.0 \pm 0.11\%$, the total extractive composition $29.0 \pm 0.01\%$ (Tab.III).

TABLE III
THE NUMERICAL CHARACTERISTICS OF THE QUALITY CONTROL FOR RAW MATERIAL OF THE ZIZIPHORA CLINOPODIOIDES LAM.

Analytical samples of raw materials	Raw material weight loss % humidity	Total ash %	The total extractive composition /50 ° spirits/ %
Hydroponics	8.6 ± 0.05	9.1 ± 0.02	22.5 ± 0.08
Voghjaberd	7.8 ± 0.18	7.8 ± 0.07	25.0 ± 0.06
Hankavan	8.4 ± 0.12	7.0 ± 0.07	23.0 ± 0.06
Arzakan	8.8 ± 0.15	8.0 ± 0.11	29.0 ± 0.01

TABLE IV.COMPOSITION OF THE ESSENTIAL OIL OF THE ZIZIPHORA CLINOPODIOIDES LAM. (GC-MS ANALYSIS). HYDROPONICS -I, VOGHJABERD-II, HANKAVAN-III, ARZAKAN-IV

№	Retention index (RI) min	Compound	Height of the peak, mm				The peak area , mm ²				Relative percentage			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
1	10.2	l R- α -pinene d- α - pinene (10.55)*	16	2.5	28	12.5	208	12.5	140	43.8	2.36	0.16*	1.41	0.95
2	12.7	β - pinene	73	24	90	71	292	66	337.5	230.8	3.31	0.83	3.41	4.99
3	13.15	Sabinene	50.5	-	42	23.5	214.7	-	126	58.8	2.44	-	1.27	1.27
4	16.6	D(\pm)Limonene L-Limonene (16.9)*	114	29.5	70	29	570	103.3	280	87	6.47	1.3*	2.83	1.88
5	17.7	Eucalyptol	137	140	155	120	787.8	875	930	600	8.94	11.03	9.4	12.98
6	20.4	O-cimene	95	24	60	23	285	54	210	69	3.23	0.68	2.12	1.49
7	20.95	α - Terpinolene	21	45	1.5	-	42	135	2.25	-	0.48	1.7	0.02	-
8	30.5	l-menthone	-	140	-	86.5	-	280	-	324.4	-	3.53	-	7.02
9	30.6	D- menthone	-	128	145	-	-	544	507.5	-	-	6.85	5.13	-
10	32	D(+) Isomenthone	86	-	122.5	93	301	428.8	-	372	3.42	-	4.33	8.05
11	36.8	γ - Terpineol	51.5	44	-	-	450.7	88	-	-	5.11	1.11	-	-
12	36.95	DL(\pm)menthol	-	47	147	46.5	-	117.5	992.3	232.5	-	1.48	10.2	5.03
13	40	(\pm)Pulegone	151	152	153	144	2265	1558	1645	1188	25.7	19.63	16.6	25.70
14	41.6	α - terpineol	6	3.5	80	7.5	12	13.2	200	16.9	0.14	0.17	2.02	0.37
15	41.95	D-Germacrene	99	-	-	-	346.5	-	-	-	3.93	-	-	-
16	41.8	Camphor	16	24	17	47	36	60	38.3	129.3	0.32	0.76	0.39	2.80
17	43.5	DL-Carvone L-Carvone *	102	122	130	-	280.5	396.5	650	-	3.18*	5.00	6.57	-
18	51.9	Verbenone	148.5	143	154	131.5	1262.3	679.3	770	657.5	14.33	8.56	7.78	14.22
19	61.15	Thymol	78	135	29	125	175.5	405	72.5	250	1.99	5.10	0.73	5.41
20	66.5	Mint furanone	15	87	54	32	37.5	261	189	72	0.43	3.29	1.91	1.56
21	75.25	Phytol	78	-	16.5	23	253.5	-	20.7	34.5	2.88	-	0.21	0.75

More than 80 components were found by the gas chromatography-mass spectrometry method in the composition of the essential oils of the samples № I-IV *Ziziphora*(Fig.4-7),(Tab. IV).

Chromatographic studies (GC-MS) revealed that all the samples of essential oils as in the qualitative composition as well as in quantitative contents of the main components were similar. Differences in the quantitative content of some components are caused not only to climatic factors, but also the growing conditions.

The great attention should be given to the fact that the main components in the all 4 samples are the monoterpenes; (\pm) pulegone (16.62-25.71%), verbenone (7.78-14.33%), eucalyptol (8.94 -12.98%), DL (\pm) menthol (1.48-10.02%) , isomenthone (3.42-8.05%), I-menthone (3.53-7.02%), D - menthone (5.13-6.85%), DL-carvone (3.18-6.57%), D (\pm) limonene (1.3-6.47%), thymol (0.73-5.41%).

The study results showed a promising future of the *Ziziphora clinopodioides* Lam. use as a source of essential oils containing biologically active substances.

III. Conclusions.

- For the first time, the numerical characteristics of the merchandising quality control for raw material of the *Ziziphora clinopodioides* Lam. were defined from wild-growing plants (Voghjaber, Hankavan, Arzakan) and grown up in a hydroponics.
- The highest value of the essential oil yield differs raw material collected in the condition of hydroponics $1.25 \pm 0.01\%$, for the relative density differs raw material collected in the vicinity of the village Hankavan - 0.977 ± 0.001 , for the refractive index differs raw materials collected in the vicinity of the village Arzakan 1.490 ± 0.003 .
- The highest values for the total extractive composition /50 ° spirits / and humidity differs raw material collected in Arzakan $29.0 \pm 0.01\%$, $8.8 \pm 0.15\%$, respectively, for the ash - hydroponics $9.1 \pm 0.02\%$.
- By the method of gas chromatography-mass spectrometry, the above studied samples revealed for the first time more than 80 components, among which there were the following main components:(\pm) pulegone (16.62-25.71%),verbenone (7.78-14.33%), eucalyptol (8.94-12.98%), DL (\pm) menthol (1.48-10.02%), isomenthone (3.42-8.05%), I-menthone (3.53 - 7.02%), D-menthone (5.13-6.85%), DL-carvone (3.18-6.57%), D (\pm) limonene (1.3-6.47%), thymol (0.73-5.41%).
- The study results indicated that in the essential oil collected from the plants grown up in hydroponic conditions prevail the bicyclic monoterpenoids (camphene, pinene, sabinene) and sesquiterpenoid D-germacrene, which were absent in the essential oils obtained from the wild growing plants. The dominant monoterpenoids in all the samples are: (\pm) pulegone (16.62-25.71%), verbenone (7.78-14.33%), eucalyptol (8.94-12.98%), thymol (0.73-5.41%).

Fig.4-7. The basic components of the gas liquid chromatography samples I-IV:

- 1.(\pm) pulegone (16.62-25.71%),
2. verbenone (7.78-14.33%),
3. eucalyptol (8.94-12.98%),
- 4.DL (\pm) menthol (1.48-10.02%),
5. D-isomenthone (3.42-8.05%),
6. I - menthone (5.13-6.85%),
7. D - menthone (3.53-7.02%) ,
8. DL-carvone (3.18-6.57%),
- 9.D (\pm) limonene (1.88-6.47%),
- 10.timol (0.73-5.41%),
11. γ - terpineol (1.11-5.11%),
- 12.L- α - terpineol 4.95%
13. β -pinene (0.83-4.99%),
14. o-cymene (0.68-3.23%),
15. camphor (0.32-2.80%),
16. sabinene (1.27-2.44%),
- 17.1R- α -pinene (0.95-2.36%),
18. α -terpineol (0.17-2.02%),
19. levomenthol 8.46%,
20. L-4-terpineneol 5.55% .

Fig.4. Gas- liquid chromatogram of the sample of *Hydroponica*

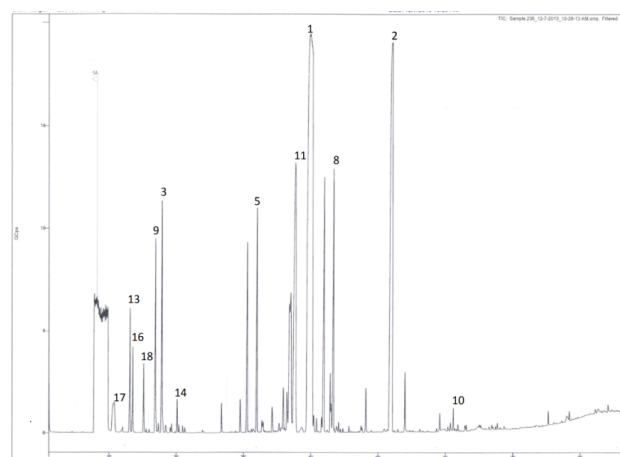


Fig.5. Gaz- liquid chromatogram of the sample of Vokhchaber

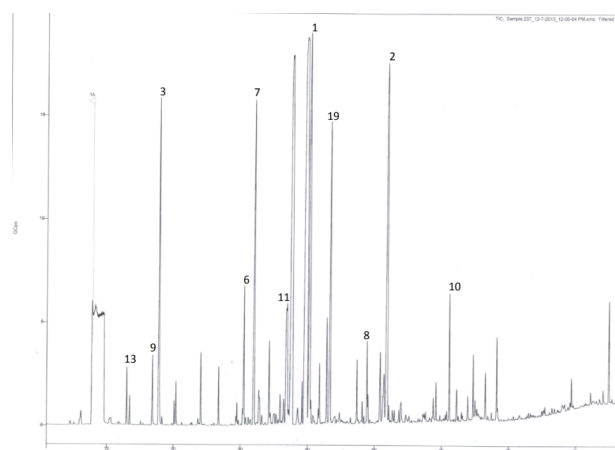


Fig.6. Gaz- liquid chromatogram of the sample of Hankavan

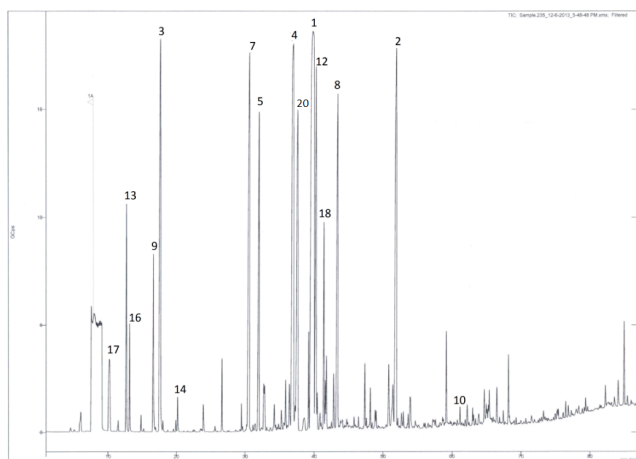
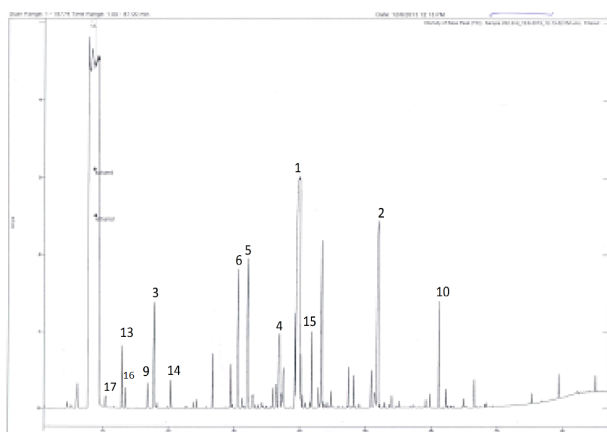


Fig. 7. Gaz- liquid chromatogram of the sample of Arzakan



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