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Article

The study of the biological activities of *Ziziphora clinopodioides*

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The aim of the current study was to determine the chemical constituents of essential oil and to study the antibacterial and antioxidant activities of essential oil and the extracts obtained from the raw material of *Ziziphora* wild growing in the floras of Armenia and Artsakh cultivated in the hydroponic conditions. The essential oils were obtained by the method of hydro-distillation. The determination of the essential oil constituents were performed by the GC-MS method. Agar disk diffusion method was used to study the antimicrobial activity of essential oils. The antioxidant activity determination was carried out DPPH test by the spectrophotometric method, at the same time IC_{50} was determined. The highest values of the essential oils yield (1.25 ± 0.01%) and IC_{50} 13.83±0.218(x10^{-5})g/l) were received for the plant cultivated in hydroponic conditions. For the first time in the above studied samples, by the method of GC-MS more than 70 components were revealed. The results of the study showed that essential oils of *Ziziphora* exhibit antimicrobial activity and the extracts revealed relatively expressed antioxidant activity. The study results show the future prospects of the use of *Ziziphora* not only as the source of flavonoids and essential oils, but also antimicrobial and antioxidant agents.

Keywords: *Ziziphora clinopodioides Lam*. Essential oil**.** Antimicrobial activity. Antioxidant activity. DPPH. GC-MS. Kovats indices. IC_{50}.

INTRODUCTION

Plants are valuable sources of biologically active substances which exhibit specific biological activity and are important components for the development of new medicines.

From this point of view, some endemic plants of the Lamiaceae family which have been used in traditional medicine in different countries from early times are quite interesting. These plants are mainly studied as sources of essential oils and phenolic compounds, especially flavanoids, which determine their biological (antioxidant, anti-inflammatory, cytotoxic, etc.) activity.

In recent decades, scientific articles have focused on the study of the antioxidant effect of flavonoids (Kaur, Kapoor, 2002; Biljana, Djendji, 2019) and their ability to

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inhibit free radicals that cause serious human pathologies (Rice-Evans, Miller, 1996). Many flavonoids have strong antioxidant, antibacterial properties (Ozturk, Ercisli, 2006; Faiza*et al.*, 2015), which also exhibit hypoglycemic (Taj, Ahmed, Elwahab, 2010), hypocholesteremic (Zou, Lu, Wei, 2005) and hepatoprotective (Akachi *et al*., 2015) effects.

The interest to the arsenal of plant raw materials, which are the source of the essential oils and the source of bioflavonoids has increased by world scientific community.

They are widely used in the herbal preparations, drug, cosmetic and food industries, as well as in the medical practice (Sezai *et al.*, 2014).

The international scientific literature analysis showed that the plants of the family Lamiaceae (Labiate) had the special interest as the source of phenolic, flavonoid compounds and essential oils (Tian *et al.*, 2011; Leila, Ali, 2016). One of them is *Ziziphora* that contains bioflavonoids and terpenic compounds as a main group of biologically active substances (Zhou *et al.*, 2012; Ding *et al.,* 2014; Karomatov, 2015), at the same time in the composition of the ethanol extract obtained from the raw material *Ziziphora* phenolic and flavonoid compounds were determined (Shahbazi 2015; Shahbazi, Shavisi, Mohebi, 2017).

In the studies carried out by Guo Dan and co-authors mentioned that the raw material of *Ziziphora* possessed a significant antioxidant capacity. The Bioassay-guided fractionation of antioxidants from the *Ziziphora clinopodioides* raw material by combination of silica gel column chromatography (CC) with high-speed countercurrent chromatography (HSCCC), led to the isolation of 3 active components: methyl rosmorinate, caffeic acid and luteolin. The study showed that the antioxidant potential was due to the flavonoid fraction (Guo *et al.,* 2015).

Ziziphora tenuior is an aromatic herbaceous plant, distributed throughout Iran. The pharmacological studies showed antibacterial, sedative, analgesic and immunostimulant activities of *Z.tenuior.* The phytochemical investigations have established the presence of six flavonoid derivatives, luteolin, apigenin, 5-O-methylapigenin, apigenin-7-O-glucoside and ziziphorins A & B and some triterpenoid derivatives in the plant extracts as well as high amounts of pulegone (71-87%) in its essential oil (Mohammad-Reza *et al.*, 2014).

Hamidreza Khodaverdi–Samani *et al.,* reports that plants of genus *Ziziphora* are widely used as carminative, stomach tonic, expectorant and antiseptic in different parts of Iran (Hamidreza *et al.,* 2015). Pulegone is the major constituent in the essential oils from plants of the Lamiaceae family (Hamidreza *et al.,* 2015; Sharopov, Setzer, 2011).

The investigation of cytotoxic activity of pulegone and its metabolites like piperitenone, piperitone, menthofuran and menthone demonstrated their cytotoxic activity against rat (MYP-3) and human (1T1) urothelial cell lines (Yousefbeyk *et al.,* 2016).

The research by Yasser Shahbazi stated the traditional use of *Ziziphora clinopodioides Lam*. essential oils (collected from different parts of Iran)in the treatments of gastrointestinal diseases based on in vitro antimicrobial and antioxidant activities (Shahbazi, 2017).In the studies by Ozturk reported about in vitro antibacterial activities of essential oil and methanol extract of *Ziziphora persica Bunge*. (Ozturk, Ercisli, 2006).

The investigation carried out by Younes Anzabi showed the essential oils of *Ziziphora clinopodioides* (were collected from the wild region of East Azerbaijan province) had inhibitory effect on the isolated gram positive and gram negative bacteria from woman's urogenital tract infections (Younes, 2016). Also, in the study carried out by Mohammad and co-authors' (Mohammad *et al.,* 2015) was shown insecticidal activity and in Shahbazi and co-authors'.(Shahbazi, Shavisi, Mohebi, 2017)-antibacterial activity of the essential oils of *Ziziphora clinopodioides Lam*..

The study of native plant raw materials revealed that the floras of Armenia and Artsakh are the most relevant in the point of view of defining the plants which are the sources of essential oils and flavonoids (Chichoyan *et al.,* 2015; Ulikhanyan, 2015). One of the prospective plant as a source of a raw material for yielding the essential oil and flavonoids is a wild growing plant *Ziziphora* (Ulikhanyan *et al.,* 2017).

The species of *Ziziphora* is one of the endemic plants in the flora of Armenia. The raw material resources

study indicated that the populations of the wild-growing species of *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 (Chichoyan *et al.,* 2015).

In the traditional Armenian medicine, *Ziziphora clinopodioides var.* Serpyllacea is widely used as the phytoncides, cardiotonic and hypotensive means to ease nausea during pregnancy, as well as a fragrance ingredient in the soaps manufacturing and in cosmetic purposes (Zolotnitskaya, 1965).

For the first time in the 90's, Armenian scientists defined 8 compounds; 5 flavonoids: chrysin-7-rutinoside, linarin, diosmin,7-methyl sudahitin, timonin (which were approximately 2% of the total dry weight), and 3 acids, oleanolic acid, caffeic acid, trimexigal acid in the extracts of the raw material *Ziziphora* collected in the vicinity of Lake Sevan near the village Jiel (Oganesyan *et al.,* 1990). It was shown that the main biologically active substance of the *Ziziphora* is the polyphenolic complex, besides; the antimicrobial activity of the extracts was defined (Oganesyan *et al.,* 1991).

So, the study of the raw material resources, the analysis of physicochemical parameters, as well as the definition of the chemical constituents and the antimicrobial activity of the essential oils and antioxidant activity of the extract derived from the *Ziziphora* of Armenian and Artsakh floras were not carried out.
From this point of view, for the creation of the effective herbal medicines the study of the endemic *Ziziphora* plant growing in Armenian floras a perspective source of biologically active substances, gets scientific and practical value.

MATERIAL AND METHODS

Herbal material

From the vicinity of Armenian villages of Arzakan, Hankavan, Voghjaberd, and from the vicinities of the villages of Surenavan, Nakhijevanik, Berdadzor of Artsakh from the natural populations, several samples (shrub) of *Ziziphora clinopodioides Lam*. were collected (from 14.04.2015 to 20.05.15) for scientific research and then identified by the registry for species identification (*Z.clinopodioides Lam.,* 1791, Tabl. Encycl. Meth. Bot., Illustr.1:63) according to Takhtajyan (Takhtajyan, 1987) and Grossgeym (Grossgeym, 1949), (GACP for medicinal plant; WHO, Geneva, 2003).

The voucher specimen (ERE N194583) of the plant was deposited in the Institute of Botany after A.L.Takhtajyan; (National Academy of Sciences, Republic of Armenia).

Method of cultivation

For the cultivation, nearly 40 plant of *Ziziphora* bushes collected in the area of the village of Voghjaberd and Hankavan, Kotayk region, in mid-April (12.04.15) were planted on ($5m^2$) hydroponic and soil areas. Black slag was used as a nutrient filler in a 3-15 mm diameter, which was previously disinfected with 0.05% solution of $KMnO_4$ (Davtyan, 1980; Chichoyan *et al.,* 2015). In the process of vegetation, the plants were nutriated according to G.S.Davtyan's (Davtyan, 1980) nutrient solution with pH-5.5-6.5, 1-2 times a day (GACP for medicinal plant; WHO, Geneva, 2003).

The first collection of the raw materials was in early July, at the beginning of the flowering phase. This oil was used as a standard sample which was analyzed according to agro-technical norms (GACP for medicinal plant; WHO, Geneva, 2003).

Isolation of the essential oil

The essential oils were obtained by hydro-distillation method. The hydro-distillation was carried out for 3 hours by Clevenger-type apparatus. The distilled essential oils were stored at +4±1°C for the further research (Quality control methods for herbal materials, WHO, Geneva, 2011).

Preparation of extracts

The preparation of the extracts was carried out by the extraction of the air-dried plant raw material of the Ziziphorain 50% methanol during 30 minutes. The extracts were filtered and evaporated to dryness under

the reduced pressure in the rotary evaporator. Then, the dry extracts were weighed and the percentages of the different extractive substances of the Ziziphora were determined with respect to the dried weight raw material.

By thin layer chromatography (TLC) method was identified phenolic compounds in methanol extracts; flavonoid apigenin and phenyl-propanoid glycoside-verbascoside. Qualitative-quantitative composition of phenolic compounds was confirmed by HPLC method (Ulikhanyan *et al*., 2019).

Gas chromatography-mass spectrometry method

The determination of the chemical composition of the essential oil was performed by the gas chromatography-mass spectrometry method in the analytical laboratory "FDA Lab" ("Tonus-Les Pharmaceutical Company"). Gas chromatograph with the mass selective detector manufactured by BRUKER(USA), using chromatographic column OPTIMA-FFAP-0.25mm, 60m×0.25mm ID.MACHEREY-NAGEL, (Germany). For identification of the main components of the essential oil were used relative retention indices. The calculation of relative retention indices (Kovats indices) performed with the help of Van den Dool and Kratz equations (Walraven, 1968). As a standard was used n-alkanes (C_{10}-C_{15}) injection, under the same chromatographic conditions. The qualitative analysis based on the comparison of the retention times and the total mass spectra with the corresponding oils components data and pure compounds with the mass spectra data of the library catalog NIST 21 and WILEY 229, the flow of helium carrier gas - 1.0ml/min, volume of injected sample 2µl, the evaporator temperature - 220° C, a temperature 50°C (2min), heated to 250°C (2.5°C /min) (retention 5min), split-division 5, GC series apparatus fitted with the detector FID.

The contents of the components were calculated with the gas chromatographic peak areas. The identification of the compounds was carried out by their retention time and the peak increase while adding a witness. The relative percentage (area %) amount from the FID chromatograms were defined. The quantification of the components was determined by the method of internal normalization (Guretskiy, Kuznetsov, Kuznetsova, 1987).

Disc diffusion method

To study the antimicrobial activity of essential oils of *ZiziphoraclinopodioidesLam.* raw materials agar disk diffusion method was used (Birger, 1982; Deans, Ritchie, 1987; Marjorie 1999; Vadalazova, Myader, Karpova, 2011; Polly *et al*., 2014).

In the present study, the antimicrobial activity of *Z.clinopodioides es*sential oil was examined against Pseudomonas *aeruginosa MDC 5249, Serratia marcescens MDC 5251, Enterococcus faecalis MDC 5254, Bacillus subtilis MDC 1820, Staphylococcus aureus MDC 5233, Streptococcus faecalis MDC 5242, Mycobacterium sp. MDC 5237, Bacillus coagulans MDC 1906* and *Escherichia coli MDC 5002* test-microorganisms.

In vitro microbial sensitivity to essential oils, was determined by measuring the growth inhibition zones around the disc, including the disc diameter (d = 5 mm) after 24 hours incubation of microbial test cultures in a thermostat at 37 °C. The zones of inhibition were compared with the zones of inhibition produced by benzyl penicillin and ceftriaxone.

DPPH (free radical-scavenging) assay

In the research, the methanol, a stable radical of DPPH ((2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$,

M = (394.33) (Sigma Aldrich GmbH)) and the dry methanol extracts of the *Ziziphora* were used.

The antioxidant activity determination was carried out using the spectrophotometric method, in which the natural antioxidant interacts with the stable chromogen radical DPPH (Ananikyan, Yeribekyan, Mnatsakanyan, 2007). The antioxidant activity (in the percentage) was determined after the interaction of the methanol solution of DPPH with the test solution and was calculated using a calibration graph of the DPPH (the dependence of the optical density from the DPPH concentration). The absorbance of the samples was determined at the wavelength 515 nm.

The optical density definition of the testing solutions was performed by the device Helios Comp Thermoelectron (England). The measurements were carried out in five replicates. The optical density of the DPPH was recorded after 1, 5 and 20 minutes.

The scavenging effect in percentage was defined according to this formula:

Antioxidant Activity (%) = ((C_c-C_{ext}) / Cc) x 100%,

C_c- concentration control absorbance,
C_{ext}-concentration corresponds to the absorbance in the presence of extracts (Ananikyan, Yeribekyan, Mnatsakanyan, 2007; Mnatsakanyan, Yeribekyan, Ananikyan, 2009).

Antioxidant activity was calculated as the effective concentration at which the DPPH radicals were inhibited by 50% (IC_{50}).

Statistical analysis:

Statistical analysis was made by SPSS ® for Windows (Version 16.0, Chicago, IL, USA). The results were presented as a mean ± standard error of mean; $p < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

The results of the study showed that the yield of the essential oil obtained from the raw material of *Ziziphora* cultivated in the hydroponic conditions was 1.25 ± 0.011%, and wildly growing in the vicinity of the villages Voghjaberd0.848 ± 0.014%, Hankavan0.794 ± 0.005%, Arzakan0.788±0.016%, Nakhijevanik 1.064 ± 0.063%, Surenavan1.002 ± 0.034%, Berdadzor1.012 ± 0.032%. The yield of the essential oil of *Ziziphora* from Kotayk region of Armenia (villages Voghjaberd, Hankavan, Arzakan)almost the percentage was identical (0.788 ± 0.016% - 0.848 ± 0.014%). And the yield of essential oils obtained from the plants from Artsakh (villages Nakhijevanik, Surenavan, Berdadzor) almost had the identical percentage (1.064 ± 0.063% - 1.002 ± 0.034%). The results are presented in Table I (Chichoyan *et al.,* 2015; Ulikhanyan, 2015).

TABLE I - Dependence of the essential oil yield (% -v/w based on dry weight) on the places and the conditions of the growing of the raw materials *Ziziphora*(n=5, $\bar{x}$- mean, E_S- standard error of the mean)

Sample number	Places of the growth of the plant	Essential oil yield,% $\bar{x} \pm E_S$
I	Hydroponics (black slag)	1.25±0.01
II	Voghjaberd (Armenia) - above the sea level at altitude 1880 meters on the slopes of the Voghjaberd ridge, the dry rocky mountain-steppe rubbly terrain (40°10’5”N 44°38’40”E.)	0.85±0.02*
III	Hankavan (Armenia) - 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±0.02*
IV	Arzakan(Armenia) - above the sea level at altitude 1700 m and rocky mountain-steppe terrain (40°26’58’’ N 44°36’23’’ E.)	0.78±0.03*
V	Nakhijevanik (Artsakh) - above the sea level at altitude 821 meters and rocky mountain-steppe terrain (39°54’28’’N 46°52’14’’E.)	1.1±0.04*

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TABLE I - Dependence of the essential oil yield (% -v/w based on dry weight) on the places and the conditions of the growing of the raw materials *Ziziphora*(n=5, $\bar{x}$- mean, E_S- standard error of the mean)

Sample number	Places of the growth of the plant	Essential oil yield,% $\bar{x} \pm E_S$
VI	Surenavan (Artsakh) - above the sea level at altitude 1780 meters on the slopes of mountain-forest rocky terrain (40°04'07''N 46°54'21''E.)	0.99±0.02*
VII	Berdadzor(Artsakh)-above the sea level at altitude 2650 meters, the dry rocky mountain-steppe rubbly terrain (39° 39' 30''N 46° 36' 7''E.)	1.0±0.04*

*P<0.05 as compared to hydroponics.

Based on the experimental data of the GC-MS analysis of n-alkanes, Kovats indices were defined and presented in Table II, Figure 1.

TABLE II - Essential oil composition of *Ziziphora*identified by GC-MS

	KI** RRI exp	Compound	Compotition (%)						
			I	II	III	IV	V	VI	VII
1	937	1 R-a-Pinene d-a- Pinene (10,55)*	2.36	0.16*	1.41	0.95	0.46	0.34	1.23
2	**971**	**Sabinene**	**2.44**	**-**	**1.27**	**1.27**	**1.33**	**0.78**	**1.34**
3	**976**	**β- Pinene**	**3.31**	**0.83**	**3.41**	**4.99**	**2.47**	**1.19**	**2.97**
4	**1018**	**D(±)Limonene L-Limonene (16,9)***	**6.47**	**1.3***	**2.83**	**1.88**	**0.22**	**0.23***	**0.41**
5	**1029**	**Eucalyptol**	**8.94**	**11.03**	**9.4**	**12.98**	**8.19**	**11.03**	**7.62**
6	1037	O-Cimene	3.23	0.68	2.12	1.49	1.07	0.36	0.88
7	1041	a- Terpinolene	0.48	1.7	0.02	-	-	-	-
8	**1143**	**I-menthone**	**-**	**3.53**	**-**	**7.02**	**3.96**	**-**	**6.01**
9	**1145**	**D- menthone**	**-**	**6.85**	**5.13**	**-**	**-**	**-**	**-**
10	**1157**	**D(+)Isomenthone**	**3.42**	**-**	**4.33**	**8.05**	**16.8**	**8.89**	**12.61**
11	1161	ϒ–Terpineol	5.11	1.11	-	-	1.16	-	-
12	**1168**	**DL(±)menthol**	**-**	**1.48**	**10.2**	**5.03**	**-**	**8.06**	**5.21**
13	1234	(±)Pulegone	25.71	**19.63**	**16.62**	**25.70**	**23.97**	**20.49**	**21.92**
14	1273	a- Terpineol	0.14	0.17	2.02	0.37	3.03	0.29	1.04
15	1467	D-Germacrene	3.93	-	-	-	0.23	2.85	-
16	1484	Camphor	0.32	0.76	0.39	2.80	1.61	2.13	-
17	**1495**	**DL-Carvone L-Carvone ***	**3.18***	**5.00**	**6.57**	**-**	**5.78***	**5.51***	**6.01***

(continues on the next page...)

TABLE II - Essential oil composition of *Ziziphora*identified by GC-MS

	KI** RRI exp	Compound	Compotition (%)						
			I	II	III	IV	V	VI	VII
18	1503	Verbenone	14.33	**8.56**	**7.78**	**14.22**	**7.35**	**3.07**	**5.52**
19	**1516**	**Thymol**	**1.99**	**5.10**	**0.73**	**5.41**	**5.18**	**0.81**	**1.58**
20	1524	(-)-spathulenol	0.54	0.5	0.95	0.26	0.48	0.27	0.52
21	1532	Caryophyllene oxide	0.32	0.69	0.39	1.17	0.53	0.66	0.59
22	1547	a-Cadinol	0.44	0.17	0.55	0.33	0.38	0.6	0.73
		Total	81.12	67.79	74.71	92.97	77.96	61.48	68.95

I-hydroponics; II-Voghjaberd; III-Hankavan;IV-Arzakan;V-Nakhijevanik;VI-Surenavan; VII-Berdadzor
Expressed as the percentage of the total peak area and the dominant compounds are indicated in bold
KI**-Kovats index (RRI- relative retention index).

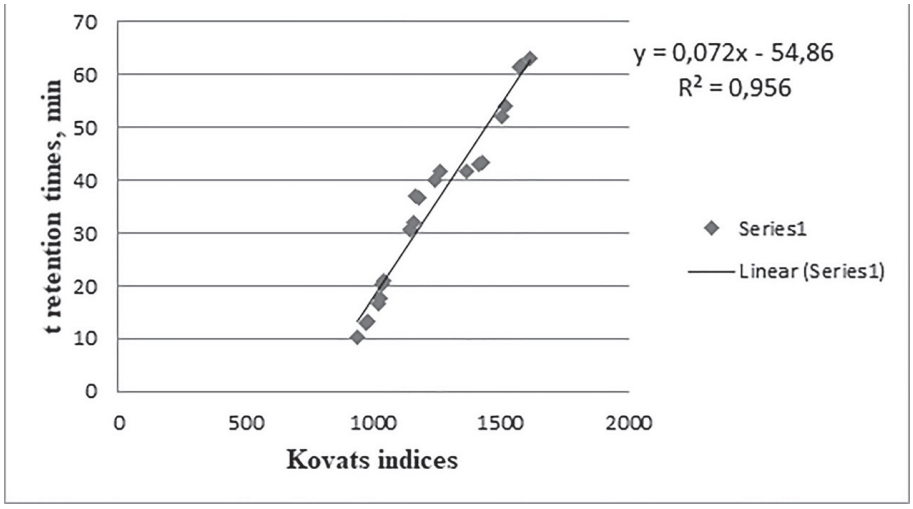
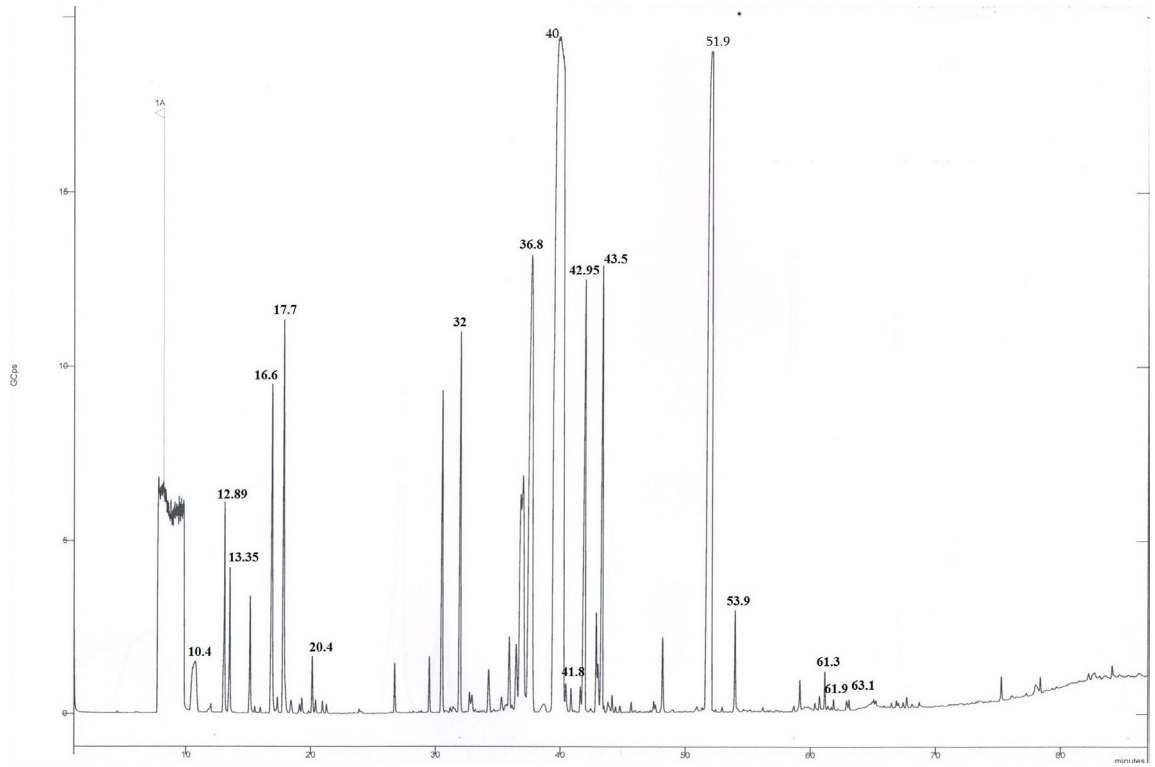


FIGURE 1 - Dependence retention times of alkanes (C_{10}-C_{15}) from Kovats indices for essential oils obtained from the raw material *Ziziphora* wildly growing and in hydroponic conditions.

By the method of GC-MS in the above mentioned samples, for the first time more than 70 components were revealed, the dominants were the following; (±) Pulegone (16.62-25.71%), Verbenone (3.07-14.33%), Eucalyptol (7.62-12.98%), DL(±)menthol (1.48-10.2%), D(+)Isomenthone(3.42-16.8%), I-menthone(3.53–7.02%), D-menthone(5.13-6.85%),DL(L)-Carvone(3.18-6.57%),Thymol(0.73-5.41%), D(±)(L)-Limonene (0.22-6.47%), β-Pinene(0.83-4.99%), Sabinene (0.78-2.44%).

In Table II were presented twenty one components with the total percentage 62.48-92.97% (see Figures 2-8).

The study results indicated that in the essential oils obtained from the plants cultivated in hydroponic conditions prevailed the monoterpenoids (D(±)(L)-Limonene, Pinene, Sabinene) and sesquiterpenoid D-Germacrene, which were absent in some essential oils obtained from the wild growing plants of the Armenian flora. The dominant monoterpenoids in all samples were: (±)Pulegone (16.62-25.71%),Verbenone (3.07-14.33%), Eucalyptol (7.62-12.98%), DL(±)menthol (1.48-10.2%) (TableII, Figures 2-8).



Figures 2 - Gas- liquid chromatogram and retention times of the sample of hydroponics.:

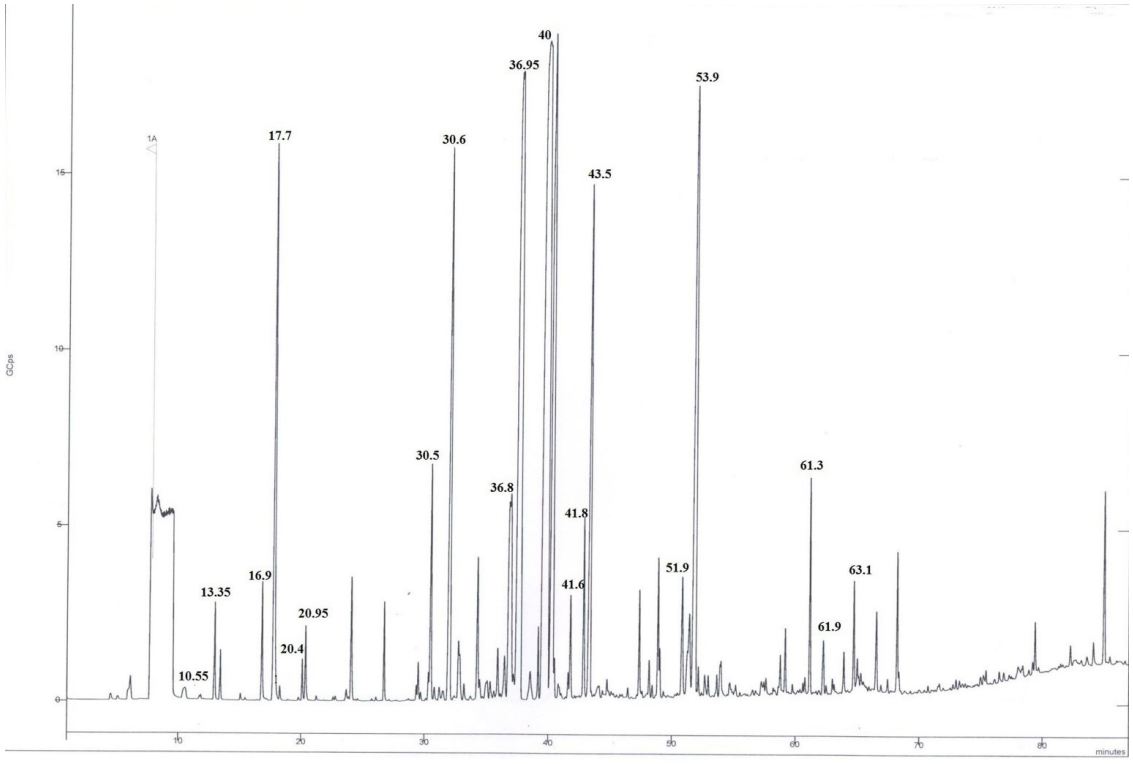


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FIGURE 3 - Gas-liquid chromatogram and retention times of the sample of Voghjaberd.

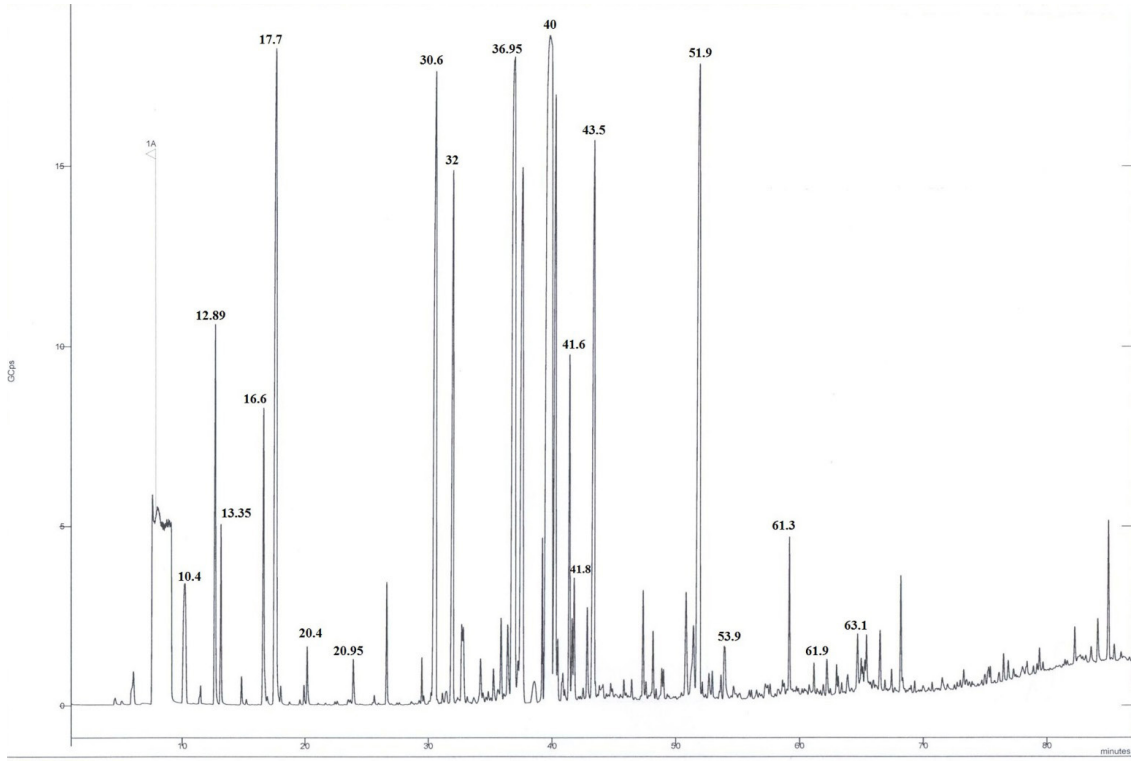

FIGURE 4 - Gas-liquid chromatogram and retention times of the sample of Hankavan.

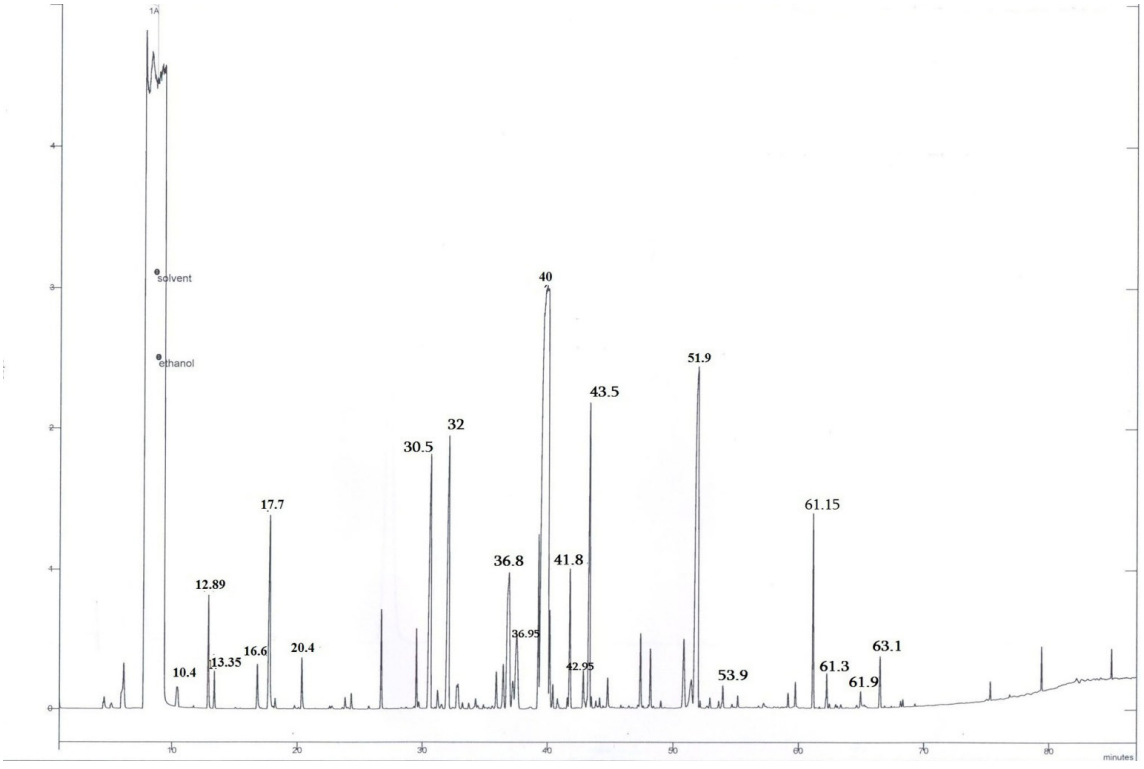

FIGURE 5 - Gas- liquid chromatogram and retention times of the sample of Arzakan.

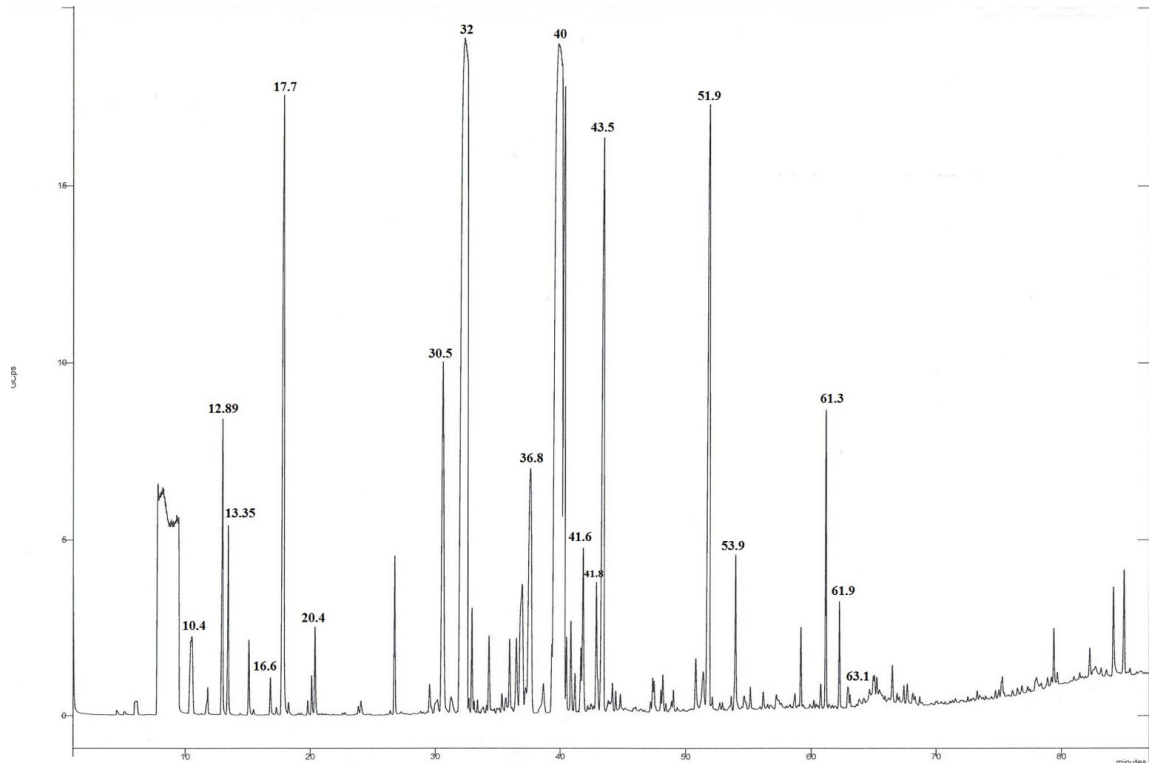

FIGURE 6 - Gas-liquid chromatogram and retention times of the sample of Nakhijevanik.

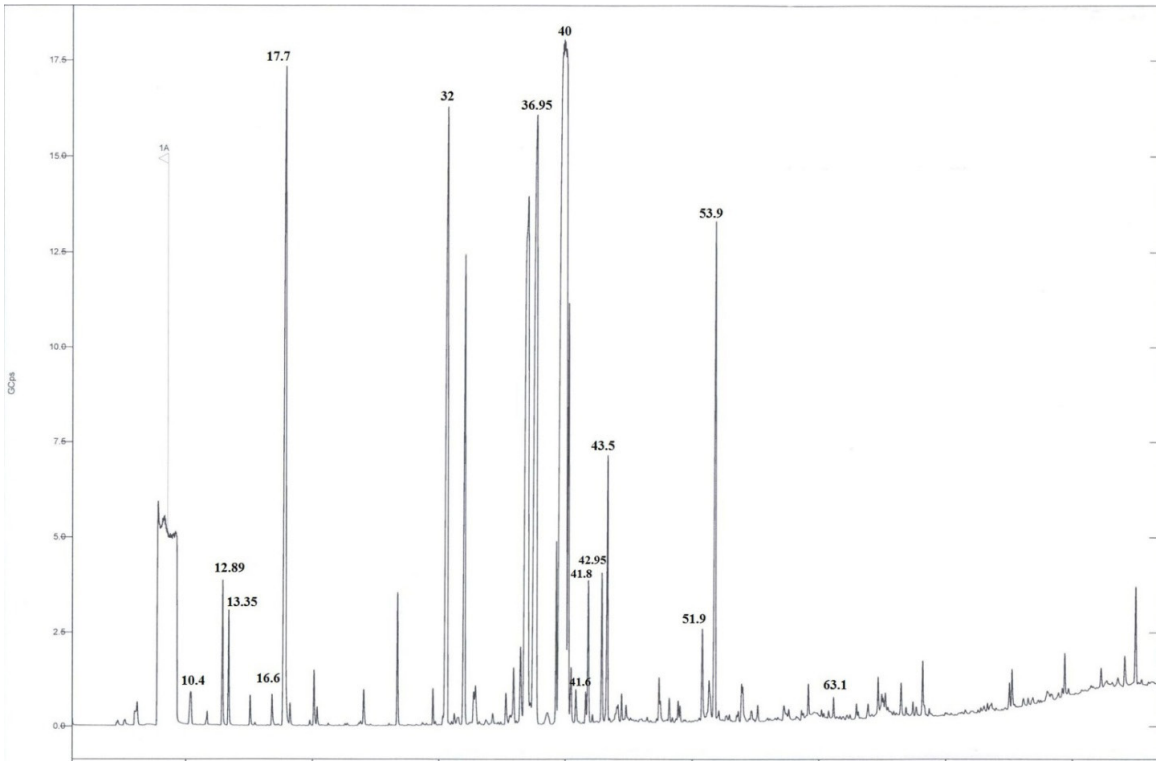

FIGURE 7 - Gas-liquid chromatogram and retention times of the sample of Surenavan.

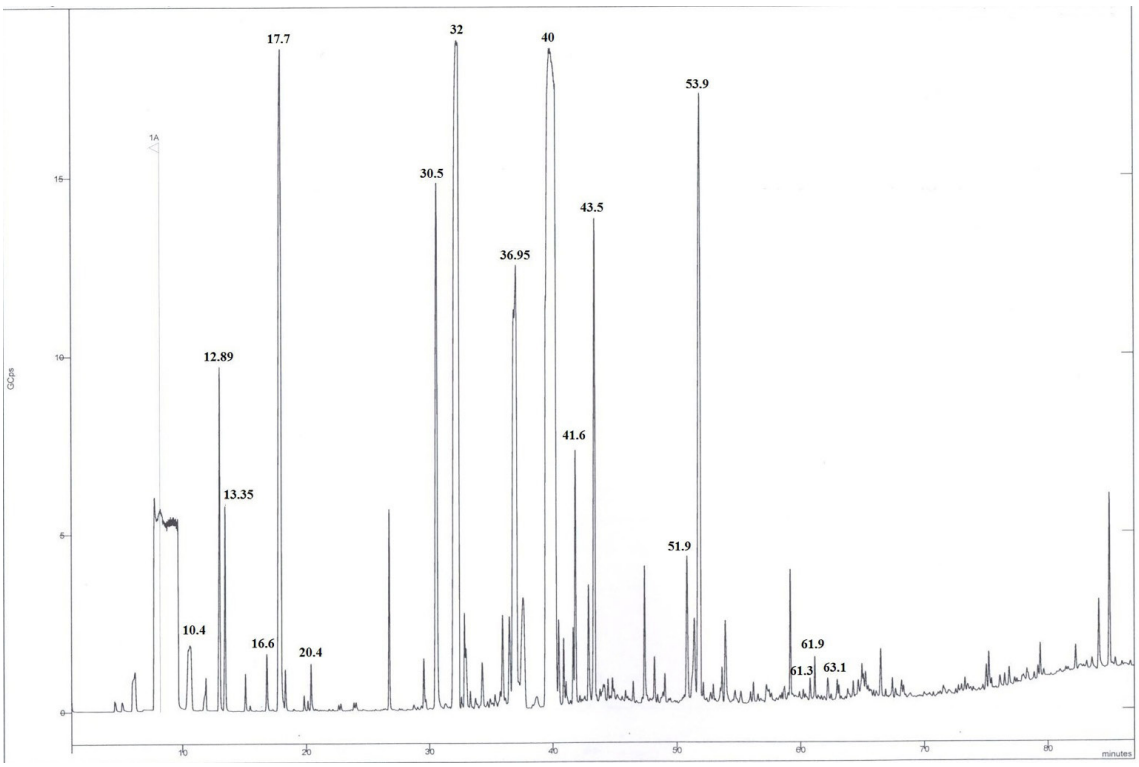

FIGURE 8 - Gas-liquid chromatogram and retention times of the sample of Berdadzor.

Antimicrobial activity

As shown in tables (Table I, II) the amount of essential oils derived from the plant material *Ziziphora*, depends on natural-climatic, ecological and several factors. It is not surprising that the percentage of the active components in the essential oils, due to growing and cultivation conditions, will be different and as a result the antimicrobial activity will be also different against various types of bacteria's.

The data of essential oils antimicrobial activity in vitro obtained from the plant material *Ziziphora*, wildly-growing in the flora of Artsakh (Surenavan), and cultivated in the hydroponic conditions are given below in Table III.

TABLE III - In vitro antimicrobial activity of *Ziziphora* essential oils by agar disc diffusion method

Bacteria	Diameter of zone of inhibition in mm (disc diameter: d=5mm,) $\bar{x} \pm E_s$			
	1	2	3	4
Pseudomonasaeruginosa MDC 5249	-	7.08 ±0.086	-	-
Mycobacterium sp. MDC 5237	20.44 ±0.458	9 ±0.158	15.08 ±0.124	15.06 ±0.121
Bacillus subtilis MDC 1820	15.04 ±0.172	12.12 ±0.128	14 ±0.141	30.94 ±0.266

(continues on the next page...)

TABLE III - In vitro antimicrobial activity of *Ziziphora* essential oils by agar disc diffusion method

Bacteria	Diameter of zone of inhibition in mm (disc diameter: d=5mm,) $\bar{x} \pm E_S$			
	1	2	3	4
Streptococcus faecalis MDC 5242	18.02 ±0.128	30.14 ±0.225	10.06 ±0.157	20.1 ±0.274
Bacillus coagulans MDC 1906	8.1 ±0.182	10.12 ±0.222	16.04 ±0.15	19.8 ±0.228
Enterococcus faecalis MDC 5254	11.98 ±0.296	13.96 ±0.256	12 ±0.272	33.02 ±0.215
Serratiamarcescens MDC 5251	6.94 ±0.163	9.14 ±0.194	-	-
Escherichia coli MDC 5002	7.08 ±0.163	9.04 ±0.144	-	-
Staphylococcus aureus MDC 5233	25 ±0.179	28.14 ±0.254	19.02 ±0.193	20.06 ±0.108

(n=5, $\bar{x}$- mean, E_S- standard error of the mean)
1. Essential oils obtained from plant material *Ziziphora* wildly-growing in the flora of Artsakh (Surenavan); 2.Essential oils obtained from plant material *Ziziphora* cultivated in the hydroponic conditions; 3.Benzylpenicillin; 4.Ceftriaxone.

The study results of the antimicrobial activity of the essential oils obtained from the raw material *Ziziphora*, showed the relatively pronounced antimicrobial activity against *Staphylococcus aureus MDC 5233, Enterococcus faecalis MDC 5254, Bacillus subtilis MDC 1820, Streptococcus faecalis MDC 5242, Mycobacterium sp, MDC 5237, Bacillus coagulans MDC 1906*. Their antimicrobial activities slightly differ from benzylpenicillin and ceftriaxone in some cases, particularly in the case of *Staphylococcus aureus MDC 5233, Streptococcus faecalis MDC 5242* and *Mycobacterium sp., MDC 5237*, even slightly superior, forming a more pronounced zone of inhibition.

Pyrogenic microbe *Staphylococcus aureus MDC 5233* has a pronounced sensitivity to the test essential oils, and taking into account the fact that this microbe exhibits an expressed drug resistance, the essential oils obtained from the plant *Ziziphora*, could be an alternative for prevention and treatment of infectious diseases.

The demonstrated activity of the essential oils of this plant against *Mycobacterium sp*., *MDC 5237* makes it possible candidate for the fight against mycobacteria causing tuberculosis, taking into consideration the fact that the latter are highly resistant to anti-TB medicines. The non-effective influence of the essential oils on the *Pseudomonas aeruginosa strain MDC 5249* can be explained by the fact that this microbe has a gram-negative cell wall containing of lipopolysaccharide, which makes the penetration of essential oils difficult. On the other hand, it is known that the porin channels of the cell wall of this microbe have a very narrow lumen, which also limits the passage of various active substances into the cell, and explains the nature of multiple drug resistance.

To assess the antimicrobial effect of the essential oils obtained from *Ziziphora,* the fact should be taken into account that this study was conducted in vitro conditions. The results of the study differ from the antimicrobial effect in vivo conditions, though in this case, the disk diffusion method has been used, and the hydrophobic nature of the essential oils prevents the diffusion process in the agar medium, and limits the diameter of the inhibition zone of the microbial growth.

Free Radical Scavenging Activity (% DPPH inhibition)

The results of the study showed that all the samples of the raw material are manifested a positive antioxidant activity, determined by the rate of the DPPH inhibition.

A correlation between the inhibition concentration of the DPPH and the optical density absorbance of the extracts was determined. Figure 9shows the correlation graphs of the inhibition concentration of the DPPH extracts of the plants cultivated in the hydroponic conditions (Z3), wild growing *Ziziphora* in the floras of Armenia (Z2) (from Voghjaberd) and Artsakh (Berdadzor) (Z1). Figure10showsthe relationship between the optical density (D) and concentration (C) of the experimented herbal extracts for corresponding concentrations a-6mg and b-12mg; 1a plants from Berdadzor- the concentration 6mg, 1b-12mg; 2a plants from Voghjaberd the concentration 6mg, 2b-12mg; 3a plants from hydroponics the concentration 6mg, 3b-12mg. the results of which are given in Tables IV, V, VI.

TABLE IV - Correlation of the DPPH for the (plants from the hydroponics) Z3 and (plants from Berdadzor) Z1 (for line Ziziphora1)

Optical density for the Z1 and Z3 (D)	Concentration (C)
C=33.962*D-1.226415	
0.625	**20**
0.095	**2**

TABLE V - Correlation of the DPPH for the (plants from Voghjaberd) Z2(for line Ziziphora2)

Optical density for the Z2(D)	Concentration (C)
C=35.088*D-1.368	
0.609	**20**
0.096	**2**

TABLE VI -The relationship between the optical density (D) and concentration (C) [(x 10^{-5}) g/l] of the inhibition DPPH(n=5, $\bar{x}$ - mean, E_S- standard error of the mean)

The areas of the plants collected	Extract (mg)	The time of the inhibition DPPH (min)		
		1	5	20
Hydroponics (black slag)	*D-6	0.446±0.001	0.352±0.001	0.327±0.001
	D-12	0.277±0.001	0.155±0.002	0.123±0.002
Hydroponics (black slag)	**C-6	14.287±0.048	10.975±0.045	10.119±0.042
	C-12	8.358±0.05	4.084±0.04	2.947±0.049
Voghjaberd	*D-6	0.409±0.001	0.301±0.002	0.277±0.002
	D-12	0.302±0.001	0.117±0.001	0.08±0.002
Voghjaberd	**C-6	12.975±0.038	9.207±0.057	8.358 ±0.037
	C-12	9.228±0.04	2.751±0.042	1.453±0.043
Berdadzor	*D-6	0.393±0.002	0.318±0.001	0.275±0.001
	D-12	0.227±0.002	0.088±0.001	0.07±0.002

(continues on the next page...)

TABLE VI -The relationship between the optical density (D) and concentration (C) [(x 10^{-5}) g/l] of the inhibition DPPH(n=5, $\bar{x}$ - mean, E_S- standard error of the mean)

The areas of the plants collected	Extract (mg)	The time of the inhibition DPPH (min)		
		1	5	20
Berdadzor	**C-6	12.41±0.057	9.8±0.043	8.295±0.05
	C-12	6.61±0.06	1.733±0.038	1.091±0.053

*D-6, D-12- the values of the optical densities of the extracts with concentrations of 6 mg and 12 mg, respectively
** C-6, C-12-the values of the concentrations of experimented herbal extracts6 mg and 12 mg, respectively

TABLE VII - The antioxidant activity of the dry 50% methanol extracts of *Ziziphora*,

The areas of the plants collected	Extract (mg)	The time ofthe inhibition DPPH, min and antioxidant activity (%) $\bar{x} \pm E_S$			IC_{50}(x 10^{-5}) g/l
		1	5	20	
Hydroponics (black slag)	6	28.56±0.238	45.12±0.225	49.4±0.212	13.83±0.218
	12	67.51±0.277	79.58±0.212	85.26±0.248	10.02±0.213
Voghjaberd	6	36.67±0.181	54.85±0.194	59.06±0.181	10.22±0.17
	12	54.95±0.276	86.3±0.21	92.47±0.21	10.26±0.22
Berdadzor	6	39.46±0.277	52.06±0.205	59.37±0.244	10.23±0.212
	12	67.52±0.278	91.12±0.183	94.28±0.265	10.227±0.241

(n=5, $\bar{x}$- mean, E_S - standard error of the mean)

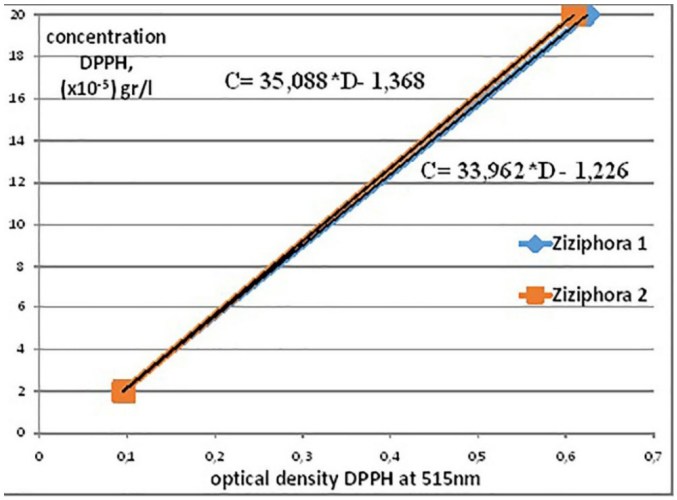

FIGURE 9 - Correlation graph of the DPPH for Z1 (plants from Berdadzor) and Z3 (plants from the hydroponics) (the line Ziziphora1), Z2 (plants from Voghjaberd) (the line Ziziphora 2).

The results of the studies presented in Tables VI, VII and Figure 10 indicated that the extracts of the raw material from the hydroponics and wild growing had a pronounced antioxidant activity, differing from each other in the rate of the DPPH scavenging and corresponding values of IC_{50}.

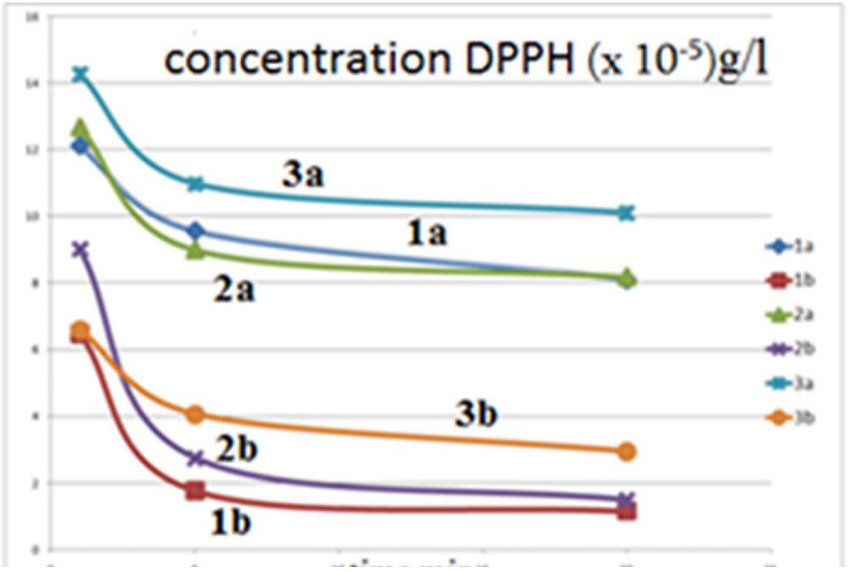

FIGURE 10 - The relationship between the optical density (D) and concentration (C) of the experimented herbal extracts for corresponding concentrations a-6mg and b-12mg;

1a plants from Berdadzor- the concentration 6mg, 1b-12mg;
2a plants from Voghjaberd- the concentration 6mg, 2b-12mg;
3a plants from hydroponics- the concentration 6mg, 3b-12mg.

The antioxidant activity of the methanol extracts of *Ziziphora* was carried out for the first time (Table VII) and came to the following conclusions; the extracts of the raw material *Ziziphora* collected in the vicinities of the villages Voghjaberd and Berdadzor showed a positive antioxidant activity, and the highest antioxidant activity noticed in the extracts of the raw material from Berdadzor, at the 20th minute for the concentration of 12 mg, reached 94.28 ± 0.265%,for the Voghjaberd -reached 92,47±0.21%, for the extract of the raw material cultivated in the hydroponic conditions -reached 85.26± 0.248%.IC_{50} values were in the range from 10.02±0.213 to 13.83±0.218(x 10^{-5})g/l. The highest value of IC_{50} 13.83±0.218(x 10^{-5}) g/l was received for extract of the raw material cultivated in the hydroponic conditions for the concentration of 6mgwhich had the smallest of antioxidant activity.

CONCLUSIONS

The current study revealed that the essential oils compositions of the *Ziziphora* are varied not only due to natural climatic conditions, but also the growing conditions. The great attention should be given to the fact that the main components in all 7 samples are the monoterpenes, sesquiterpenes and their oxygenated derivatives, especially Pulegone, Verbenone, Eucalyptol, DL(±)menthol.

Essential oils derived from raw material of *Ziziphora* wild-growing in Artsakh flora (Surenavan) and cultivated in hydroponic conditions showed a relatively pronounced antimicrobial activity against *Staphylococcus aureus MDC 5233, Enterococcus faecalis MDC 5254, Bacillus subtilis MDC 1820, Streptococcus faecalis MDC 5242, Mycobacterium sp, MDC 5237, Bacillus coagulans MDC 1906*.

The methanol extracts of the raw material of *Ziziphora* wildly growing and cultivated hydroponic conditions showed a pronounced radical scavenging activity.

The results of the research showed that *Ziziphora* wildly growing in Armenian and Artsakh floras, as well as the plants cultivated in the hydroponic conditions, are the sources of the essential oils and flavonoids. In the future not only wildly growing *Ziziphora* (the stocks of which are limited in the nature), but the raw material cultivated in the hydroponic conditions could be used in the herbal preparations because of its antimicrobial and antioxidant activities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akachi T, Shiina Y, Ohishi Y, Kawaguchi T, Kawagishi H, Morita T, et al. Hepatoprotective effects of flavonoids from shekwasha (Citrus depressa) against D-galactosamine-induced liver injury in rats. J NutrSciVitaminol (Tokyo). 2010;56(1):60-7.

Ananikyan V, Yeribekyan M, Mnatsakanyan V. Accelerated method for the detecting the antiradical activity of organic compounds. Globe Sci. 2007;7:31-33.

Biljana K, Djendji V. Flavonoids and Phenolic Acids as Potential Natural Antioxidants. Intech Open. 2019 Feb, DOI: 10.5772/intechopen.83731

Birger M. Handbook of microbiological and virological research methods. Moscow: Medisina; 1982. 464 p.

Chichoyan N, Ulikhanyan G, Galstyan A, Ulikhanyan Gh. Phytochemical study on Ziziphora clinopodioides Lam essential oils wild-growing in the Armenian flora and cultivated in the conditions of a hydroponics. In: Proceedings of the International conference on Biochemistry and molecular biology. J Med Bio Sci. 2015 April 22-24. Paris. Paris: Cité Internationale Universitaire de Paris France. 2015;2:p73-79.

Davtyan G. Reference book on the chemicalization of the agriculture. Moscow: Kolos; 1980.p. 382-385.

Deans S, Ritchie G. Antibacterial properties of plant essential oils. Inter J of Food Microbiol.1987; 165–180.

Ding W, Yang T, Liu F, Tian S. Effect of different growth stages of Ziziphora clinopodioides Lam. on its chemical composition. Pharmacogn Mag. 2014 Jan;10(Suppl1):S1-5. doi: 10.4103/0973-1296.127329.

Faiza M, Aissa L, Khellaf R, Lotfi B, Amina B, Bounab S, et al. Antioxidant and Antibacterial Activities of Essential oil and extract of Zizyphora Hispanica L, of M'sila, AENSI. J Adv in Environ Biology. 2015; 9(14): 90-96.

Grossgeym AA. Plant Identifier of the Caucasus. Moscow: Sovetskaya Nauka; 1949. 376p.

Guo D, Rahima A, Luo Y, Zou G. Antioxidants from Ziziphora clinopodioides Lam, by combination of chromatographic techniques. In: Proceedings of the11-th International symposium on the chemistry of natural compounds. 2015 October1-4. Antalia. 2015.p 232.

Guretskiy IY, Kuznetsov VV, Kuznetsova LB. Practicum on physical and chemical methods of the analysis. Moscow: Khimiya; 1987.248p.

Hamidreza KhS, Abdollah GhP, Hamzeh ASh. Chemical composition of essential oils of Ziziphora clinopodioides Lam.(endemic Iranian herb) collected from different natural habitats. Indian J Trad Know. 2015,1(1): 57-62.

Karomatov ID. Ziziphora as a medicinal remedy (Review of the literature). Apriory, Series: Nat TechnSci. 2015;3:1-8.

KaurCh, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol. 2002;37(2):153-161.

Leila Sh, Dastjerdiand AM. Antioxidant activity and total phenols content of different solvent extracts of ziziphora

clinopodioides from three geographical locations in Iran. J Chem Pharm Res. 2016;8(11):243-248.

Marjorie CM. Plant Products as Antimicrobial Agents. Clin Microbiol Rev.1999;12(4):564–582.

Mnatsakanyan VA, Yeribekyan MI, Ananikyan VV. Comparative evaluation of the antiradical activity of some isoquinoline alkaloids. Globe Sci. 2009;8:31-33.

Mohammad Kh, Vahid Gh, Asghar K, Samaneh R. Chemical composition and insecticidal activity of essential oil from Ziziphora clinopodioides Lam, used against the Mediterranean flour moth, Ephestia kuehniella Zeller. J Plant Protec. Iran. 2015;55(3):260-265.

Mohammad-Reza D, Farzin B, Sepideh S, Mona Sh, FaribaGh, Saeed T, et al. Essential oil composition, antioxidant activity and total phenolic content of some lamiaceae taxa growing in Northwest of Iran. Pharm Sci. 2014, 20(1): 22-28.

Oganesyan GB, Galstyan AM, Gach-Baits E, Mnatsakanyan VA. Oleanolic acid and flavonoid glycosides from Ziziphora clinopodioides. Arm Khim Zhurn.1990;43:210–1.

Oganesyan GB, Galstyan AM, Mnatsakanyan VA, Paronikyan RV, Ter-Zakharyan YZ. Phenolic and flavonoid compounds of Ziziphora clinopodioides. Chem Nat Comp. 1991;27:247.

Ozturk S, Ercisli S. The chemical composition of essential oil and in vitro antibacterial activities of essential oil and methanol extract of Ziziphora persica Bunge. J Ethno Pharmacol. 2006;106(3):372-6.

Polly SX, Beow CY, Hu CP, SweeHua EL. Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol J. 2014;8:6–14.

Rice-Evans CA, Miller NJ. Antioxidant activities of flavonoids as bioactive components of food. BiochemSoc Trans. 1996;24(3):790−795.

Sezai E, Hulya D, Tunde J, Elma T, Alma L, Alisa H. Chemical Constitutions and Antioxidant Activity of Ziziphora clinopodioides Lam. Ecotypes from Turkey. In: Proceedings of the International Conference on Food and Nutrition Technology. IPCBEE. IACSIT Press, Singapore. 2014. doi:10/7763. IPCBEE. 2014.72(16):72-76.

Shahbazi Y. Chemical composition and in vitro antibacterial effect of Ziziphora clinopodioides essential oil. Pharm Sci. 2015;21:51-56.

Shahbazi Y. Chemical compositions, antioxidant and antimicrobial properties of Ziziphora clinopodioides Lam. essential oils collected from different parts of Iran. J Food Sci Technol. 2017;54(11):3491-3503. doi 10.1007/s13197-017-2806-2.

Shahbazi Y, Shavisi N, Mohebi E. Antibacterial activity of Ziziphora clinopodioides essential oil and nisin against bacillus subtilis and salmonella typhimurium in commercial barley soup. Bulgarian J Vet Med. 2017;20(1):65-72.

Sharopov FS, Setzer WN.Chemical diversity of Ziziphora Clinopodioides: composition of the essential oil of Ziziphora from Tajikistan. Nat Prod Com. 2011;6(5):695-8.

TajEldin IM, Ahmed EM, Elwahab H M A. Preliminary Study of the Clinical Hypoglycemic Effects of Allium cepa (Red Onion) in Type 1 and Type 2 Diabetic Patients. Environ Health Insights. 2010;4:71-77. doi:10.4137/EHI.S5540

TakhtajyanT. Flora of Armenia. Volume 8 of NAS RA: Yerevan; 1987.p.127-134.

Tian S, Shi Y, Zhou X, Ge L, Upur H. Total polyphenolic (flavonoids) content and antioxidant capacity of different Ziziphora clinopodioides Lam, extracts. Pharmacogn Mag.2011;7(25):65-8.

Ulikhanyan GR, Chichoyan NB, Galstyan AM, Ulikhanyan Gh. Development method of Ziziphora clinopodioides Lam, raw material standardization by flavonoids. In: Proceedings of the Scientific Conference. 2017 Nov27-Dec1; Yerevan. Yerevan: YSMU Scientific week. The New Arm Med J. 2017;11(3):p 43.

Ulikhanyan GR. Dynamics of the accumulation biologically active substances and component composition of the essential oils of Ziziphora clinopodioides Lam. Armenian flora. New Arm Med J. 2015;9(3):31.

Ulikhanyan GR., Galstyan HM., Tsaturyan AH., Chichoyan NB.. Standardization of the raw material Ziziphora clinopodioides Lam. by HPLC and spectrophotometric methods. Abstracts / Toxicon. 159 2019. S1e-S32 P-06.

Vadalazova SV, Myader MA, Karpova MR, Saranchina YuV. Antimicrobial activity of essential oils and aquatic extracts from the medicinal plants Khakassia. Sib Med J. 2011;26(2):54-58.

Walraven J.J. Identification in complex volatile organic mixtures; Eindhoven: Technische Hogeschool Eindhoven. 1968. doi:10.6100/IR71820.

World Health Organization. WHO. Guidelines on good agricultural and collection practices (GACP) for medicinal plants. 2003. Geneva. World Health Organization. 2003.72p.

World Health Organization. WHO. Quality control methods for herbal materials 2011.Geneva: World Health Organization; 2011. 173p.

Younes A. In Vitro Comparison of Antibacterial Effects of Ziziphora clinopodioides L. and Some Standard Antibiotics on Bacteria Isolated From Women's Urogenital Tract Infections. Int J Women's Health Reprod Sci. 2016,4(1):38-41.

Yousefbeyk F, Tabaside J, Ostad SN, Salehi Sourmaghi MH, Amin GR. Investigation of chemical composition and cytotoxic activity of aerial parts of Ziziphora clinopodioides Lam. Res J Pharmacogn. 2016,3(2):47-51.

Zhou X, Yu Q, Gong H, Tian S.GC-MS analysis of Ziziphora clinopodioides essential oil from North Xinjiang. Nat Prod Commun China. 2012;7(1):81-2.

Zolotnitskaya SYa. Medicinal resources of flora of Armenia. Yerevan: AN publishing house Arm.S.S.R.. 1965;(2):327p.

Zou Y, Lu Y, Wei D. J Hypocholesterolemic effects of a flavonoid-rich extract of Hypericum perforatum L. in rats fed a cholesterol-rich diet. J Agric Food Chem. 2005;53(7):2462-6.

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Erratum

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