INTRODUCTION

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

Keywords: Ziziphora clinopodioides Lam. Essential oil. Antimicrobial activity. Antioxidant activity. DPPH. GC-MS. Kovats indices. IC_{50}.
inhibit free radicals that cause serious human pathologies (Rice-Evans, Miller, 1996). Many flavonoids have strong antioxidant, antibacterial properties (Ozturk, Ercisli, 2006; Faizaet 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 Ziziphs 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, pipertone, menthofuran and menthone demonstrated their cytotoxic activity against rat (MYP-3) and human (IT1) urothelial cell lines (Yousef beyk et al., 2016).

The research by Yasser Shahbazi stated the traditional use of Ziziphora persica Bunge 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 persica Bunge (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
The 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, Berdazdor 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 (5m²) 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 KMnO₄ (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 *Ziziphora* 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 essential 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_{2}O_{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.
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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:

\[ \text{Antioxidant Activity (\%)} = \left( \frac{(C_c - C_{\text{ext}})}{C_c} \right) \times 100\% \]

\(C_c\) - concentration control absorbance,
\(C_{\text{ext}}\) - 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%, Surennavan1.002 ± 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, Surennavan, 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 Marmorik 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 exp | 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    | γ-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* |

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

| KI** | RRI exp | Compound          | I (%) | II | III | IV | V | VI | VII |
|------|---------|-------------------|-------|----|-----|----|----|----|-----|
| 18   | 1503    | Verbenone         | 14.33 |    |     | 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|

I-hydroponics; II-Voghjaberd; III-Hankavan; IV-Arzakan; V-Nakhijevanik; VI-Surenvan; 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%) (Table II, Figures 2-8).
**Figures 2** - Gas-liquid chromatogram and retention times of the sample of hydroponics.

**FIGURE 3** - Gas-liquid chromatogram and retention times of the sample of Voghjaberd.
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**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.
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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: \(d=5\text{mm}\)) \(\bar{x} \pm E_s\) |
|-------------------------------|----------------------------------------------|
|                               | 1                                 | 2                      | 3                      | 4                      |
| Pseudomonas aeruginosa        | -                                 | 7.08 ±0.086            | -                      | -                      |
| MDC 5249                      |                                   |                         |                         |                         |
| 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           |

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**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.
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.

### 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) ±ES |
|---------------------------------|---------------------------------------------------------------|
|                                 | 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, x- mean, ES-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.
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 9 shows 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). Figure 10 shows 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 results of which are given in Tables IV, V, VI.

TABLE VI - The relationship between the optical density (D) and concentration (C) [(x 10^-5) g/l] of the inhibition DPPH (n=5, 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                                   |
| 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 |
|----------------------------------|--------------|--------------------------------------|---|---|----|
| silky | **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 extracts 6 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 of the inhibition DPPH, min and antioxidant activity (%) | \(\bar{x} \pm E_s\) | IC\(_{50}\) (x 10\(^{-5}\)) g/l |
|----------------------------------|--------------|-------------------------------------------------|----------------|------------------|
| Hydronponics (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 Ziziphora 1), 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 6mg which 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 widely 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.

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Where it was written:
Ulikhanyan Greta¹,²*, Galstyan Hasmik¹,⁵, Dumanyan Karine¹,
Beglaryan Margarita³, Poghosyan Gayane⁴, Shekoyan Vladimir⁴,
Ananikyan Hrachya⁶, Ananikyan Violeta⁶, Goginyan Vigen⁷,
UlikhanyanGhukas⁸, Chichoyan Naira¹, Taschyan Sona⁹

Should read:
Ulikhanyan Greta¹,²*, Galstyan Hasmik¹,⁵, Dumanyan Karine¹,
Beglaryan Margarita³, Poghosyan Gayane⁴, Shekoyan Vladimir⁴,
Ananikyan Hrachya⁶, Ananikyan Violeta⁶, Goginyan Vigen⁷,
UlikhanyanGhukas⁸, Taschyan Sona⁹, Chichoyan Naira¹