PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2019, **53**(1), p. 23–28

Biology

SOME LAMIACEAE FAMILY PLANT ESSENTIAL OIL CHEMICAL COMPOSITION AND THEIR POTENTIAL AS ANTIMICROBIAL AGENTS AGAINST ANTIBIOTIC-RESISTANT BACTERIA

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The antimicrobial activity of essential oils (EO) from several plants of Lamiaceae family (*Ziziphora clinopodioides* Lam., *Thymus vulgaris* L., *Ocimum × citriodorum*, *O. basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora*, *Origanum vulgare* L.), spread in Armenia, was studied using agar diffusion method. As test-organisms the ampicillin-resistant *Escherichia coli* dp5α-pUC18 and non-resistant *E. coli* VKPM M-17 were used. Dimethyl sulfoxide was used as the solvent and negative control; gentamicin was applied as the positive control. *O. × citriodorum*, *O. basilicum* var. *purpureum* and *T. vulgaris* EO expressed the strongest activity against *E. coli* dpα-pUC18; minimal inhibitory concentrations (MIC) was 6.25 $\mu L \cdot mL^{-1}$. MIC of the *O. basilicum* var. *thyrsiflora* EO was 12.5 $\mu L \cdot mL^{-1}$. *Z. clinopodioides* and *O. vulgare* EO MIC was 50 $\mu L \cdot mL^{-1}$. Thus, EO from the Lamiaceae family plants could be recommended as antimicrobial agents against antibiotic-resistant bacteria.

Keywords: essential oils, antibiotic resistance, antibacterial activity.

Introduction. The antimicrobial resistance is a growing worldwide problem, it represents for both human and veterinary medicine. Some key facts are mentioned in World Health Organization last reports: antibiotic resistance is one of the biggest threats to global health, food security, and development today; antibiotic resistance can affect anyone, of any age, in any country; antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process; a growing number of infections, such as pneumonia, tuberculosis, gonorrhoea and salmonellosis, are becoming harder to treat, as the antibiotics used to treat them become less effective; antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality [1].

Although several strategies have been proposed to overcome and control this situation, still a clear solution has not been found due to the antibiotic resistance complexity and consequences and also considering the side effects of classic antimicrobial drugs. Thus herbal origin preparations are of interest, because of their high therapeutic value added by the low level of side effects [2].

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The Lamiaceae family plants are most diverse and widespread in terms of ethnomedicine, and its medicinal value is mainly based on the volatile oils concentration and chemical composition [3]. It is well documented that some plants belonging to this family have antimicrobial activity against the wide spectrum of bacteria and fungi [4, 5]. It is known, that they also possess antioxidant activity [4, 6].

The aim of this investigation was to study the antibacterial activity of essential oils (EO), extracted from some Lamiaceae family plant species spread in Armenia.

Materials and Methods.

Plant material. The investigated five plants from Lamiaceae family (*Ziziphora clinopodioides* Lam., *Thymus vulgaris* L., *Ocimum* × *citriodorum*, *O. basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora*, *Origanumvulgare* L.) were cultivated at the altitude of 1700–1800 *m* above the sea level (Aragyugh, Kotayk Region, Armenia) and harvested during the blossoming period (July–August, 2015). Plant materials were also collected during blossoming period (July–August, 2014). The plant materials were identified at the Institute of Botany, NAS of RA. The samples are available at the Department of Biotechnology, Microbiology and Biotechnology, Yerevan State University, Armenia.

Essential Oil Extraction. EO was extracted from air dried plant material (aerial parts only) by hydro-distillation using a Clevenger-type apparatus and lasted 3 *h*. The distilled essential oils had been dehydrated with anhydrous sodium sulphate and stored at 4°*C* in dark airtight bottles until further analysis [7].

Determination of EO Chemical Composition. The gas chromatography (GC), mass selective (MS) analysis of the EO was performed using a Hewlett–Packard 5890 Series II gas chromatograph ("Hewlett-Packard Comp.", "Agilent Technologies", USA), fitted with a fused silica HP-5MS capillary column (30 *m* × 0.25 *mm* in thickness 0.25 μm). The oven temperature varied from 40–250°*C* with the scanning rate of $3°C{\cdot}min^{-1}$. Helium (purity 5.6) was used as a carrier gas at a flow rate of $1\ mL{\cdot}min^{-1}$. The GC was equipped with Hewlett–Packard 5972 Series MS detector. The MS operating parameters were ionization voltage 70 *eV* and ion source temperature 250°*C*. The diluted samples of EO (1/100, v/v in HPLC methanol) of 1 μL had been injected manually. To avoid overloading the GC column, EO were diluted 1:100 (v/v) in methanol. The identification of peaks was tentatively carried out based on library search using National Institute of Standards and Technology (NIST)-2013. Relative Retention Index (RRI) was calculated for HP-5MS column. For RRI calculation a mixture of homologues *n*-alkanes (C9-C18) was used under the same chromatographic conditions as for analysis of EO.

Investigation of Antimicrobial Activity by Agar Diffusion Method. The antibacterial activity of EO was determined by the agar diffusion method [8]. This method was preferred over the dilution method, because of low solubility of EO in water and in meat peptone broth. The following concentrations of EO were used: 150, 100, 50, 25, 12.5, 6.25 $\mu L{\cdot}mL^{-1}$; dimethyl sulfoxide (DMSO) was used as the solvent. The 100 μL of each oil solution was introduced to the wells in the agar with test microorganisms. Gram-negative ampicillin-resistant *E. coli* dh5α-pUC18 and non-resistant *E. coli* VKPM M-17 were used. Bacterial culture was grown on

Mueller-Hinton agar. Gentamicin (25 $\mu g{\cdot}mL^{-1}$) as a positive control and DMSO as a negative control were used.

The selected pieces of nutrient medium from the zones of microorganism growth absence were transferred to the nutrient medium corresponding to each microorganism and then they were incubated for 2–3 days at appropriate temperature to determine the bacteriostatic or bactericidal action of the oils. The action of oils is evaluated as bacteriostatic in case of renewed growth of test-microorganisms after the re-cultivation.

*Data Processing***.** Experimental data ($n = 3$) were expressed as means with standard errors. The latter did not exceed 3% (if not indicated). The validity of differences between experimental and appropriate control data were evaluated by Student's criteria using Microsoft Excel 2010 with the help of *t*-test function; $p< 0.05$ (if not indicated).

Results and Discussion.

The Chemical Composition of Essential Oils. The results from the quantitative and qualitative analysis of EO constituents are presented in Table: the average yield of the EO reached 0.2–1.0%. More than 180 compounds were isolated, detected and most of them identified for each EO sample. The dominant components for *Ocimum* species were identified to be belonging to methyl chavicol, linalool or citral and nerolchemotypes. According to our data, *O. Basilicum* var. *purpureum* contains 57.3% methyl chavicol. *O. Basilicum* var. *thyrsiflora* belongs to linalool-rich chemotype with concentrations of linalool and methyl chavicol being 68 and 20%, respectively. These data are in a good accordance with the results reported by Sishu et al. [9]. The essential oil of *O.× citriodorum*is rich with citral (21%) and nerol (23%), therefore, it could not be classified as belonging to any of the chemotypes mentioned above.

The data on *O.× citriodorum* are somewhat consistent with the similar results published by Carović-Stanko et al. [10] on essential oil distilled from the plant of the same species, except for the fact that there were more than 45 constituents of *O.× citriodorum* EO identified in the present study, as opposed to 20 components identified by Carović-Stanko et al. [10]. The *Z. clinopodioides* EO is pulegone-rich, which concentration is 42.1%. Pulegone-rich EOs are known to have antibacterial, antioxidant, insecticidal activities [11]. The 20 substances were identified in the *T. vulgaris* EO, among which aromatic monoterpenes (carvacrol/thymol (36.9%), p-cymene (18.5%)) and isomeric hydrocarbons (γ-terpinene, 15.3%) were the main constituents. According to the literature data, these substances possess antimicrobial activity [12]. Using of the hydrodistillation method allows reaching 1% of oregano EO yield. According to GC data, EO extracted from Armenian oregano contains more than 180 types of substances basically of terpenoid or flavonoid nature (see Table). The greatest part of them was different terpenes (β-caryophyllene-epoxide – 13.3%; β-caryophyllene – 8.2%; o-cymene – 5.2%). The other components, which are ordinary typical to the oregano EO, are represented as the minor part: carvacrol, which is described by the huge number of authors as the main component of oregano EO [13, 14], is accounted for only 2.9%, terpineol – 2.3%, eucalyptol – 2.9% of EO distilled from Armenian oregano.

The main components (%) of essential oils of some Lamiaceae family plants cultivated at high altitude Armenian landscape

Chemical components	RRI[a]	*O. basilicum* var. *purpureum*	*O. basilicum* var. *thyrsiflora*	*O.× citrio-dorum*	*Z. clino-podioides*	*T. vulgaris*	*O. vulgare*
Sabinene	897	–	–	–	–	–	3.1
3-Octanone	952	–	–	–	–	–	2.6
β-Ocimene	978	–	–	–	–	–	2.6
3-Octanol	979	–	–	–	–	–	2.4
p-Cymene	1025	–	–	–	–	18.5	–
1-8-Cineole	1035	1.40	3.50	–	8.22	4.0	–
o-Cymene	1045	–	–	–	–	–	2.5
Eucalyptol	1059	–	–	–	–	–	2.0
Linalool	1100	18.0	68.0	9.42	–	0.1	2.9
Isomenthone	1163	–	–	–	9.70	–	–
Neomenthol	1166	–	–	–	5.90	–	–
DL-Menthol	1172	–	–	–	3.90	–	–
γ-Terpinene	1078	–	–	0.22	0.14	15.3	–
L-4-terpineol	1137	–	–	–	–	–	2.3
Methyl chavicol	1203	57.3	20.0	9.45	–	–	–
Nerol	1231	–	–	23.0	–	–	–
Pulegone	1237	–	–	–	42.1	–	–
Neral	1244	–	–	4.93	–	–	–
Geraniol	1259	–	–	5.20	–	–	–
Geranial	1274	–	–	15.7	–	–	–
Carvacrol/thymol	1298	–	–	–	0.35	36.9	2.4
Dihydroedulan II	1342	–	–	–	–	–	2.0
Piperitenone	1370	–	–	–	7.35	–	–
β-Elemene	1387	3.62	0.67	0.53	–	0.6	–
β-Caryophyllene	1419	1.72	–	7.80	–	–	8.2
α-Bergamotene	1433	4.34	1.34	3.52	–	–	–
α-Humulene	1455	0.55	0.28	1.52	–	0.2	–
Humulene	1456	–	–	–	–	–	2.7
β-Cubebene	1497	–	0.75	2.26	–	–	–
β-Caryophyllene epoxide	1517	–	–	–	–	–	13.3
Ent–Spathulenol	1536	–	–	–	–	–	3.2
α-Bisabolene	1561	–	–	2.29	–	–	–
β-Bisabolenene	1572	–	–	8.31	–	–	3.2
Palmitic acid	1968	–	–	–	–	–	2.5

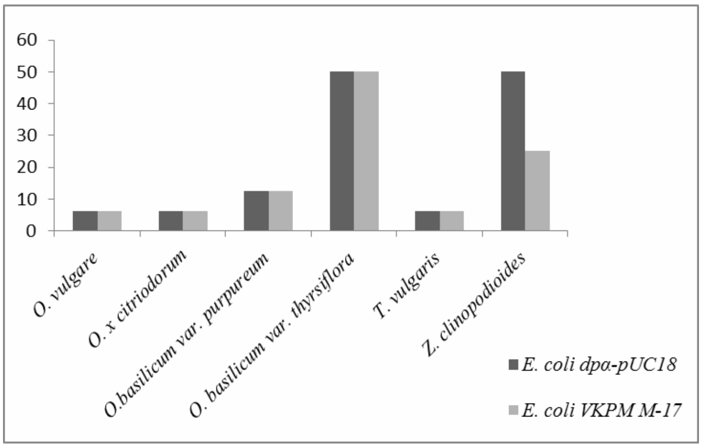
[a] for HP-5 MS capillary column.

Antibacterial Activity. A great number of antibiotics are known against different pathogenic bacteria. But increased multidrug resistance of bacterial strains led to the increased severity of diseases caused by them. Moreover, the ability of bacteria to form biofilm-associated drug resistance has further increased the bacterial infections [1]. In addition, usage of antibacterial agents at higher doses may cause toxicity in humans. In this regard, plant extracts and EOs are potential candidates as antimicrobial agents [15].

The present investigation revealed that the ampicillin-resistant *E. coli* dh5α-pUC18 bacteria, as the non-resistant strain, displayed sensitivity against EO tested:

especially, the MIC values of *O.× citriodorum*, *O. basilicum* var. *purpureum* and *T. vulgaris* against those bacteria were 6.25 $\mu L\cdot mL^{-1}$, while *O. basilicum* var. *thyrsiflora* displayed MIC of 12.5 $\mu L\cdot mL^{-1}$. Antibacterial activity of *O. vulgare* EO against ampicillin-resistant strain of *E. coli* was also almost similar to the activity against non-resistant *E. coli* VKPM M-17 with MIC values of 50 $\mu L\cdot mL^{-1}$. In case of *Z. clinopodioides* EO the MIC value against non-resistant *E. coli* was 25 $\mu L\cdot mL^{-1}$, whereas against resistant strain this value reached 50 $\mu L\cdot mL^{-1}$ (see Figure).

Thus, the MIC values determined are acceptable as effective, and the action of EO in this study was evaluated as bactericidal.



EO MIC values against test-bacteria.

Conclusion. The qualitative and quantitative composition of the essential oils of Lamiaceae plant species (*O. basilicum* var. *thyrsiflora*, *O. asilicum* var. *purpureum, O.× citriodorum*, *T. vulgaris, Z. clinopodioides, O. vulgare*) was quite different, but most of the components are of terpenoid nature. These oils had marked antibacterial effects. Being the very common spices, these plants could be also important in both prevention and treatment of bacterial infections, including caused by the antibiotic-resistant bacteria. So, this potential should be further evaluated for application in medicine, food industry and cosmetics due to their naturally high content of substances with antibacterial activity.

This study was done in the frame of Basic research support from SCS MES of RA as well as with the cooperation with "Nairian" CJSC (Kotayk Region, Armenia).

Received 07.02.2018
Reviewed 07.09.2018
Accepted 05.03.2019

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