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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *MENTHA ARVENSIS* L. HARVESTED AT HIGH ALTITUDE ARMENIAN FLORA

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Currently it is relevant to reveal possible new sources of natural antibacterial components, the mechanisms of antibiotic resistance formation in bacteria, and the possible ways to overcome the problem of microbial resistance to antibiotics. Thus, our research aimed to study the chemical composition and antibacterial activity of the essential oil (EO) isolated from the herb *Mentha arvensis* harvested at high altitude Armenian landscape.

Menthol constitutes a significant part of the EO components of the *M. arvensis* plant, reaching approximately 70%. The investigated EO showed high antimicrobial activity against several Gram-positive and Gram-negative bacterial strains. In particular, the EO equally suppressed the growth of both wild-type and kanamycin-resistant strains of *Escherichia coli* and manifested the minimal inhibitory concentration against all tested microorganisms of 0.18 mg/mL. So, *M. arvensis* EO is relevant for application in the food and cosmetic industry as a natural aromatizer and preservative.

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Keywords: Mentha arvensis, GC-MS, antibacterial activity, antibiotic resistance.

Introduction. Structurally and functionally diverse metabolites are substances of plant origin that are crucial for human activities as well as for the adaptation and survival of plants. These metabolic by-products serve a variety of roles in plant growth, development, and reaction to environmental conditions [1–4]. They exhibit a wide range of biological activities in different test systems, including antibacterial and antioxidant ones [5, 6].

Lamiaceae family plants are the most diverse and widespread in ethnomedicine and are among the most thoroughly studied ones [7, 8]. They possess a broad spectrum of biological activity, including antibacterial and antioxidant, and are known for their application in traditional medicine in many countries, including Armenia [8–15].

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Mentha arvensis L. is an aromatic plant of the Lamiaceae family, widely growing in table fields, damp places, wood-clearings, fields, and pond sides. The main components of M. arvensis essential oil (EO) are menthol, isomenthone, neomenthyl acetate, menthone, and piperidone. According to some literature data, the concentration of menthol can rich 80%, and this plant's EO considered extremely menthol rich. However, its concentration can vary depending on the growing conditions of the plant [9]. Due to its terpenoid-rich composition, this EO possesses a wide range of biological activities, including antibacterial activity [16, 17].

The main aim of this investigation was to study the possible effect of *M. arvensis* EO extracted from plants cultivated at the high-altitude Armenian landscape on some of the antibiotic-resistant *Escherichia coli* strains as well as to reveal some antimicrobial action mechanisms of menthol – the main component of the investigated EO.

Materials and Methods. The plant material was harvested from the Kotayk Region of Armenia at 1650 *m* above sea level during the blossoming period (August 2020). The plant samples were identified at the Institute of Botany, NAS of RA.

Essential Oil extraction. EO has been hydro-distilled from the freshly harvested above-ground part of the plant using Clevenger-type apparatus for 3 h. Sodium sulfate anhydrate was used to dehydrate the distilled EO, which was then kept at 4°C in opaque hermetic bottles for further testing [9].

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 fitted with a fused silica HP-5MS capillary column (30 $m \times 0.25$ mm, in thickness 0.25 μm). The oven temperature varied from 40°C to 250°C with a scanning rate of 3°C/min. Helium (purity 5.6) was used as a carrier gas at a 1 mL/min flow rate. The GC was equipped with Hewlett-Packard 5972 Series MS detector. The MS operating parameters were an ionization voltage of 70 eV at an ion source temperature of 250°C. The diluted samples of EO (1/100, v/v in HPLC methanol) of 1 μL were injected manually. In order to avoid overloading the GC column, the EO were diluted 1:100 (v/v) in methanol to avoid overloading the GC column. The identification of peaks was tentatively carried out based on a library search using the National Institute of Standards and Technology (NIST)-2013. The Relative Retention Index (RRI) was calculated for the HP-5MS column. For RRI calculation, a mixture of homologues series of n-alkanes (C9-C18) was used under the same chromatographic conditions as for analysis of the EO [9].

Investigation of Antimicrobial Activity by Agar Diffusion Method. The antibacterial activity of M. arvensis EO was determined by the agar diffusion method. Different Gram-positive and Gram-negative bacteria were used as test-organisms such as Escherichia coli BW 25113, ampicillin-resistant E. coli dh5α-pUC18, kanamycin-resistant E. coli pARG-25, Staphylococcus aureus WDC 5233, Salmonella typhimurium MDC 1754 (Microbial Depository Center, Armbiotechnology Scientific and Production Center, Armenia; laboratory control strain), Bacillus subtilis WT-A17 (isolated from metal-polluted soils of Kajaran Town, RA) and Enterococcus hirae ATCC 9790 [18, 19].

The test was performed using Mueller-Hinton agar and 0.18–1.4 mg/mL of EO. Ethanol (96%) was used as a negative control and the antibiotic solutions

(kanamycin (50 $\mu g/mL$), ampicillin (50 $\mu g/mL$)) was used as a positive control. Plates were incubated for 24 h at 37°C, then the antimicrobial activity of EO was evaluated by the growth inhibition zone diameter (mm) measurement. Data were expressed in minimal inhibitory concentrations (MIC) values.

The selected pieces of nutrient medium from the zones of microorganism growth absence were transferred to the nutrient medium corresponding to each microorganism, then incubated for 2–3 days at the appropriate temperature to determine the bacteriostatic or bactericidal action of the oils.

Chemicals, Reagents and Statistical Analysis. All applied chemicals and reagents were purchased from "Sigma-Aldrich Co. Ltd" (Germany).

All data presented represent averaged results of 3 independent biological replicates. The standard deviation of the data was determined according to the Student's t-test, and it was less than 5%; p values were calculated by two-tail unpaired t-test using GraphPad Prism 8.0.3 data analyzing tool and p < 0.05 (if not indicated).

Results and Discussion. EO isolated from *M. arvensis* is a mixture of volatile compounds. Manifestations of their biological activity are due to the phenolic compounds in their chemical composition, which may also be responsible for the smell characteristic of EO [9]. The hydro-distillation technique to obtain the EO resulted in a 0.1–0.5 % yield comparable to literature data [20].

Our investigations have discovered that the EO of *M. arvensis* contains over 30 terpenoid compounds, with monoterpenes being the most abundant in terms of quantity. These monoterpenes include menthol (comprising 69.75% of the oil), menthone (7.1%), isomenthone (7.13%), and neomenthyl acetate (7.05%) (Tab. 1).

Moreover, according to our research results, this plant belongs to the menthol chemotype, as its content reaches almost 70%, and other compounds do not exceed 8%. Other literature sources published in recent years document similar results. The studied literature documents up to 80% menthol content, depending on the research methods used, the place of growth of the plant, and the harvesting conditions [21, 22]. However, some other researchers document the absence of menthol in the *M. arvensis* EO, depending on the growing conditions of this plant and other influencing factors [23]. The chemical composition of the EO studied in this article shows a significant difference in the quantitative and qualitative composition of minor compounds compared to other literature sources [20, 24].

The variability in chemical composition could be responsible for the differences in the biological activity of EO extracted from the same plant species growing in different environmental conditions [9, 25].

According to the literature data almost all of the identified compounds possess a strong antimicrobial activity against a range of various Gram-positive and Gram-negative bacteria and other microorganisms, including those which are resistant to antibiotics [26].

Our investigations of the antibacterial activity of the *M. arvensis* EO using the disk-diffusion method gave the following result: all tested bacterial strains were susceptible to the *M. arvensis* EO extracted from plants growing at the high-altitude Armenian landscape. Moreover, Gram-negative antibiotic-resistant strains showed more expressed susceptibility against the high doses of investigated EO components (Fig. 1, Tab. 2).

Table 1
Chemical composition of M. arvensis L. EO

No	Retention time, <i>min</i>	Component name	%
1	11.61	β-Pinene 0.08	
2	12.45	Isopulegol	0.34
3	13.87	Limonene	0.38
4	13.96	Eucalyptol	0.05
5	16.62	Linalool oxide	0.03
6	17.22	Linalool	0.15
7	19.37	Isopulegol	0.98
8	19.82	Isomenthone	7.13
9	20.29	Menthone	7.1
10	21.53	Menthol	69.75
11	21.86	α-Terpeniol	0.48
12	22.24	Cyclopentanone	0.1
13	22.74	Hexanoic acid	0.08
14	23.84	Pulegone	0.9
15	24.54	Piperitone	2.1
16	25.44	Menthyl acetate	0.1
17	26.11	Cyclopentanone	0.05
18	26.37	Neomenthyl acetate	7.05
19	26.93	Isomenthyl acetate	0.12
20	27.11	Isopulegol acetate	0.09
21	29.09	Eugenol	0.21
22	30.27	β-Bourbonene	0.32
23	31.75	Caryophyllene	0.23
24	34.33	Germacrene	0.15
25	34.96	Bicyclogermacrene	0.16
26	36.04	Cadinene	0.12
27	37.62	Nerolidol	0.07
28	32.21	Spathulenol	0.23
		others	1.45

Table 2
Growth inhibition zones of test-microorganisms under the influence of different concentrations of M. arvensis EO

	Tested concentrations of M. arvensis EO, mg/mL			
Test-microorganisms	0.144	0.72	0.36	0.18
	Bacterial growth inhibition zones, mm			
Salm. typhimurium MDC 1754	16.6±5.1	13.0±3.0	8.6±3.0	8.6 ± 3.0
E. coli BW25113	9.6±1.5	12.0±1.0	8.0±1.1	8.0±1.1
E. coli DH5α-pUC18	16.6±6.2	13.6±4.0	8.0±0.01	7.0 ± 0.01
E. coli pARG-25	21.6±1.5	15.6±1.5	14.3±3.7	8.6±1.1
St. aureus WDC 5233	12.0±1.0	10.5±1.5	11.0±1.5	7.0 ± 1.0
B. subtilis WT-A17	15.0±2.6	11.0±4.3	9.0±3.0	8.0±1.7
E. hirae ATCC 9790	9.3±0.5	8.6±0.5	8.6±0.5	8.3±0.5

The suppressing activity of the investigated EO against ampicillin- and kanamycin-resistant *E. coli* strains increases the research's value. Various authors have similarly also mentioned such kind of activity of *M. arvensis* EO against

multidrug-resistant bacterial strains. Moreover, they mentioned that minimum inhibitory concentration (MIC) and minimal bactericide concentration (MBC) values were the same and were $\geq 1 \ mg/mL$ for the *E. coli* strains used in the investigation [27, 28]. In our case, the MIC of EO against all tested microorganisms was 0.18 mg/mL.

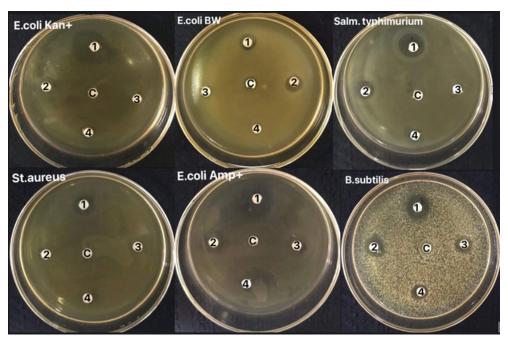


Fig. 1. Antibacterial activity of different concentrations of *M. arvensis* EO against different microbial test-microganisms: 1 – 0.144 mg/mL; 2 – 0.72 mg/mL; 3 – 0.36 mg/mL; 4 – 0.18 mg/mL; c – negative control (the microbial strains are mentioned in figure).

The action of oil evaluated to be bactericide in case of renewed growth of test-microorganisms after the re-cultivation and the MBC value was equal to MIC value.

Conclusion. Based on our investigation data, it was possible to conclude that essential oil extracted from *M. arvensis* cultivated at high altitude Armenian landscape is rich with menthol, with the overall concentrations of other minor components not exceeding 8%. The investigated EO possesses remarkable antibacterial activity against all tested bacteria, including antibiotic-resistant strains. Possibly, as is the case with other menthol-rich EOs, the odor and the antibacterial activity of investigated EO are due to the presence of this monoterpene component. The results obtained in this investigation allow us to recommend the *M. arvensis* EO for application in the food and cosmetic industry as a natural aromatizer and preservative.

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REFERENCES

- Trchounian A., Petrosyan M., Sahakyan N. Plant Cell Redox Homeostasis and Reactive Oxygen Species. In book: *Redox State as a Central Regulator of Plant-Cell Stress Responses* (Eds. Gupta D.K., et al.). Cham (Switzerland), Springer International Publishing (2016), 25–50. https://doi.org:10.1007/978-3-319-44081-1
- Yang L., Wen K.S. et al. Response of Plant Secondary Metabolites to Environmental Factors. *Molecules* 23 (2018). Article number 762. https://doi.org:10.3390/molecules23040762
- Sahakyan N., Petrosyan M., et al. The Caucasian Flora: a Still-to-be-Discovered Rich Source of Antioxidants. Free Radic. Res. 53 (2019),1153–1162. https://doi.org:10.1080/10715762.2019.1648799
- Godlewska-Żyłkiewicz B., Świsłocka R., et al. Biologically Active Compounds of Plants: Structure-Related Antioxidant, Microbiological and Cytotoxic Activity of Selected Carboxylic Acids. *Materials (Basel)* 13 (2020). Article number 4454. https://doi.org:10.3390/ma13194454
- Zhao Y., Wu Y., Wang M. Bioactive Substances of Plant Origin In: Cheung P., Mehta B. (eds) Handbook of Food Chemistry. Springer, Berlin, Heidelberg (2015). https://doi.org/10.1007/978-3-642-36605-5_13
- 6. Sahakyan N., Petrosyan M., Trchounian A. Comparative Analysis of Chemical Composition and Biological Activities of *Ajuga genevensis* L. in *in vitro* Culture and Intact Plants. *Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng.* 10 (2016), 322–326.
- Moumni S., Elaissi A., et al. Correlation between Chemical Composition and Antibacterial Activity of Some Lamiaceae Species Essential Oils from Tunisia. BMC Complement Med. Ther. 2 (2020). Article number 103. https://doi.org/10.1186/s12906-020-02888-6
- Karpiński T.M. Essential Oils of Lamiaceae Family Plants as Antifungals. *Biomolecules* 10 (2020). Article number 103. https://doi.org/10.3390/biom10010103
- Avetisyan A., Markosian A., et al. Chemical Composition and Some Biological Activities of the Essential Oils from Basil Ocimum Different Cultivars. BMC Complement. Altern. Med. 17 (2017). Article number 60. https://doi.org/10.1186/s12906-017-1587-5
- Moghrovyan A., Sahakyan N., et al. Essential Oil and Ethanol Extract of Oregano (Origanum vulgare L.) from Armenian Flora as a Natural Source of Terpenes, Flavonoids and other Phytochemicals with Antiradical, Antioxidant, Metal Chelating, Tyrosinase Inhibitory and Antibacterial Activity. Curr. Pharm. Des. 25 (2019),1809–1816. https://doi.org/10.2174/1381612825666190702095612
- Sahakyan N., Petrosyan M., Trchounian A. The Activity of Alkanna Species in vitro Culture and Intact Plant Extracts against Antibiotic Resistant Bacteria. Curr. Pharm. Des. 25 (2019):1861–1865. https://doi.org/10.2174/1381612825666190716112510
- Sahakyan N., Petrosyan M., Trchounian A. Some Lamiaceae Family Plant Essential Oil Chemical Composition and Their Potential as Antimicrobial Agents against Antibiotic-Resistant Bacteria. *Proc. YSU. Chem. and Biol. Sci.* 53 (2019), 23–28. https://doi.org/10.46991/PYSU:B/2019.53.1.023
- Mamadalieva N.Z., Akramov D.Kh., et al. Extractives and Biological Activities of Lamiaceae Species Growing in Uzbekistan. *Holzforschung* 74 (2020), 96–115. https://doi.org/10.1515/hf-2018-0296
- Ramos da Silva L.R., Ferreira O.O., et al. Lamiaceae Essential Oils, Phytochemical Profile, Antioxidant, and Biological Activities. Evid. Based Complement. Alternat. Med. 2021 (2021) Article number 6748052. https://doi.org/10.1155/2021/6748052
- Moghrovyan A., Parseghyan L., et al. Antinociceptive, Anti-Inflammatory and Cytotoxic Properties of Origanum Vulgare Essential Oil, Rich with β-Caryophyllene and β-Caryophyllene Oxide. Korean J Pain. 35 (2022), 140–151. https://doi.org/10.3344/kjp.2022.35.2.140

- Mancuso M. The Antibacterial Activity of Mentha. In: Akram M., Ahmad R.S. (eds) Herbs and Spices. London, IntechOpen (2020). https://doi.org/10.5772/intechopen.92425
- Wei H., Kong S., et al. Mentha arvensis and Mentha×Piperita-Vital Herbs with Myriads of Pharmaceutical Benefits. *Horticulturae* 9 (2023). Article number 224. https://doi.org/10.3390/horticulturae9020224
- 18. Patel J.B., Cockerill F.R., et al. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. *Clinical and Laboratory Standards Institute, Document M100-S24*. Wayne, PA (2014).
- Petrosyan M., Shcherbakova Y., et al. Alkanna orientalis (L.) Boiss. Plant Isolated Cultures and Antimicrobial Activity of Their Extracts: Phenomenon, Dependence on Different Factors and Effects on Some Membrane-Associated Properties of Bacteria. Plant Cell Tiss. Organ. Cult. 122 (2015), 727–738. https://doi.org/10.1007/s11240-015-0806-3
- Makkar M.K. Sharma S., Kaur H. Evaluation of *Mentha arvensis* Essential Oil and Its Major Constituents for Fungitoxicity. *J. Food Sci. Technol.* 55 (2018), 3840–3844. https://doi.org:10.1007/s13197-018-3291-y
- Bui-Phuc T., Nhu-Trang T., Cong-Hau N. Comparison of Chemical Composition of Essential Oils Obtained by Hydro-Distillation and Microwave-Assisted Extraction of Japanese Mint (Mentha Arvensis L.) Grown in Vietnam. IOP Conf. Ser.: Mater. Sci. Eng. 991 (2020). Article number 012039. https://doi.org/10.1088/1757-899X/991/1/012039
- Singh P., Pandey A. Prospective of Essential Oils of the Genus Mentha as Biopesticides: A Review. Front. Plant Sci. 9 (2018). Article number 1295. https://doi.org/10.3389/fpls.2018.01295
- Benabdallah A., Boumendjel M., et al. Chemical Composition, Antioxidant Activity and Acetylcholinesterase Inhibitory of Wild Mentha Species from Northeastern Algeria. S. Afr. J. Bot. 116 (2018), 131–139. https://doi.org/10.1016/j.sajb.2018.03.002
- Alnuqaydan A.M., Rah B. Comparative Assessment of Biological Activities of Different Parts of Halophytic Plant *Tamarix articulata* (*T. articulata*) Growing in Saudi Arabia. *Saudi J. Biol. Sci.* 27 (2020), 2586–2592. https://doi.org/10.1016/j.sjbs.2020.05.028
- Chaves Rd.S.B., Martins R.L., et al. Evaluation of Larvicidal Potential against Larvae of *Aedes aegypti* (Linnaeus, 1762) and of the Antimicrobial Activity of Essential Oil Obtained from the leaves of *Origanum majorana* L. *PLoS ONE* 15 (2020), e0235740. https://doi.org/10.1371/journal.pone.0235740
- Kalemba D., Synowiec A. Agrobiological Interactions of Essential Oils of Two Menthol Mints: Mentha piperita and Mentha arvensis. Molecules 25 (2020). Article number 59. https://doi.org/10.3390/molecules25010059
- Coutinho H.D., Costa J.G., et al. Enhancement of the Antibiotic Activity against a Multiresistant *Escherichia coli* by *Mentha arvensis* L. and Chlorpromazine. *Chemotherapy* 54 (2008), 328–330. https://doi.org:10.1159/000151267
- Kamatou G.P., Vermaak I., et al. Menthol: a Simple Monoterpene with Remarkable Biological Properties. *Phytochemistry* 96 (2013), 15–25. https://doi.org:10.1016/j.phytochem.2013.08.005

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ՀԱՅԱՍՏԱՆԻ ԲԱՐՁՐԼԵՌՆԱՅԻՆ ՖԼՈՐԱՅԻՑ ՀԱՎԱՔՎԱԾ MENTHA ARVENSIS L.-Ի ԵԹԵՐԱՅՈՒՂԻ ՔԻՄԻԱԿԱՆ ԿԱՁՄԸ ԵՎ ՀԱԿԱԲԱԿՏԵՐԻԱՅԻՆ ԱԿՏԻՎՈՒԹՅՈՒՆԸ

Ներկայումս կարևոր է բացահայտել բնական հակաբակտերիային միացությունների հնարավոր նոր աղբյուրները, բակտերիաներում հակաբիոտիկկայունության ձևավորման մեխանիզմները և հակաբիոտիկների նկատմամբ մանրէների կայունության խնդրի հաղթահարման հնարավոր ուղիները։ Այսպիսով, մեր նպատակն էր ուսումնասիրել Հայաստանի բարձրլեռնային լանդշաֆտներում կուլտիվացվող *Mentha arvensis* խոտաբույսից ստացված եթերայուղի քիմիական կազմը և հակաբակտերիային ակտիվությունը։

Պարզվեց, որ մենթոլը կազմում է M. arvensis բույսի եթերայուղի բաղադրիչների զգալի մասը՝ հասնելով մոտավորապես 70%-ի։ Ուսումնասիրված եթերայուղը բարձր հակամանրէային ակտիվություն է ցուցաբերել մի շարք գրամ-դրական և գրամ-բացասական բակտերիաների շտամերի նկատմամբ։ Մասնավորապես, այն հավասարապես ճնշել է $Escherichia\ coli\$ ինչպես վայրի տիպի, այնպես էլ՝ կանամիցին-կայուն շտամների աճը, իսկ նվազագույն արգելակող կոնցենտրացիան բոլոր փորձարկված միկրոօրգանիզմների նկատմամբ կազմել է $0.18\ dq/d_l$ ։ Այսպիսով, $M.\ arvensis$ եթերայուղը կարելի է կիրառել սննդի և կոսմետիկայի արդյունաբերությունում՝ որպես բնական բուրավետիչ և կոնսերվանտ։

С. К. ТАДЕВОСЯН, А. А. ШИРВАНЯН, А. А. МАРКОСЯН, М. Т. ПЕТРОСЯН, Н. Ж. СААКЯН

ХИМИЧЕСКИЙ СОСТАВ И АНТИБАКТЕРИАЛЬНАЯ АКТИВНОСТЬ ЭФИРНОГО МАСЛА MENTHA ARVENSIS L., СОБРАННОГО ИЗ ВЫСОКОГОРНОЙ ФЛОРЫ АРМЕНИИ

В настоящее время актуально выявление возможных новых источников природных антибактериальных соединений, механизмов формирования антибиотикорезистентности у бактерий и возможных путей преодоления проблемы микробной резистентности к антибиотикам. Целью нашего исследования было изучение химического состава и антибактериальной активности эфирного масла (ЭМ), выделенного из травы *Mentha arvensis*, собранной на высокогорных ландшафтах Армении.

Было обнаружено, что ментол составляет значительную часть компонентов ЭМ *М. arvensis*, достигая примерно 70%. Исследуемое ЭМ проявляло высокую противомикробную активность в отношении ряда грамположительных и грамотрицательных штаммов бактерий. Так ЭМ одинаково подавляло рост как дикого типа, так и резистентных к канамицину штаммов *Escherichia coli*, а минимальная ингибирующая концентрация в отношении всех тестируемых микроорганизмов составила 0,18 *мг/мл*. Следовательно, ЭМ *М. arvensis* годно для применения в пищевой и косметической промышленности в качестве натурального ароматизатора и консерванта.