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CHEMICAL COMPOSITION AND ANTIMICROBIAL POTENTIAL OF ESSENTIAL OIL OF *ARTEMISIA DRACUNCULUS* L., CULTIVATED AT HIGH ALTITUDE ARMENIAN LANDSCAPE

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The gas chromatography-mass spectrometry setup was used to reveal the chemical composition of essential oil (EO) of A. dracunculus cultivated at the altitude of 1700–1800 *m* above sea level (Aragyugh, Armenia) and harvested during the blossoming period (July, 2015). Estragol (methyl chavicol) in EO reached 84.9%. The other components were linalool (5.09%), trans-beta-ocimen (4.00%), limonene (1.63%), (Z,E)-alloocimene (2.29%), 3-caren (0.81%) and beta-ocimen (0.61%). Antimicrobial activity of EO from *A. dracunculus* L. was determined by agar diffusion method. Gram-positive, gram-negative and ampicillin-resistant bacteria, as well as some yeasts were tested. The concentrations of EO from 1.5625 to 150 *mg*/*mL* were used. The minimal inhibitory concentration (MIC) of *A. dracunculus* EO was 6.25 *mg*/*mL* against *St. aureus* and *B. subtilis*. MIC value against *E. coli* VKPM-M17 was 50 *mg*/*mL*, but *P. aeruginosa* was less susceptible to EO components and MIC value reached 150 *mg*/*mL*. The antibiotic resistant *E. coli* dhpα-pUCl8 strain possessed high sensitivity against the EO with 6.25 *mg*/*mL* MIC value. The action of EO was bactericidal. Tested yeasts were more susceptible against oil component, MIC=1.56 *mg*/*mL*. The obtained results show that *A. dracunculus* EO can be useful for cosmetics, medicine and food as antimicrobial natural agent.

Keywords: *Artemisia dragunculus*, essential oil, estragol, antimicrobial activity.

Introduction. Within genus Artemisia there are approximately 500 known plant species, commonly occurring in Asia, Europe and North America, distinguished by characteristic aroma and biological activity. Artemisia species are frequently used in treating such diseases as malaria, liver disease, neoplasms, inflammatory diseases and infections caused by microorganisms.

Tarragon (*Artemisia dracunculus* L., *Asteraceae* family) is a plant having several diverse common names such as estragon, dragon sage-wort, false tarragon and dragon wormwood. It is a shrubby perennial herb. The plant is cultivated in many countries including Armenia. Key biologically active secondary metabolites are essential oils (EO) (0.15–3.1%), coumarins (>1%), flavonoids, and phenol-carbonic acids. It contains also phenolic compounds, carotenoids, tannins, bitter tastes, and mineral compounds [1]. *In vivo* studies have shown the presence of anti-inflammatory, hepatoprotective and antihyperglycemic effects.

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The main compound of EO of this plant is mentioned to be methylchavicol (estragol) [2, 3], but some scientists pointed that there are also different chemotypes containing *p*-allylanisole [4], capillene [5], (Z)-anethole [6] and others. The chemical composition of tarragon oil is significantly differentiated, which is caused by different kinds of variability. Six main groups were distinguished: methyl chavicol-rich, methyl eugenol-rich, α-terpinolene-rich, capillene-rich, 5-phenylo-1,3-pentadine-rich and (E)-β-ocimene/(Z)-β-ocimene-rich, representing different chemical profile of the oils [7]. Inspite of the concerns about the toxic effects of two Artemisia EO main constituents, estragol and methyleugenol, no acute toxicity or mutagenic activity have been reported at doses relevant for human consumption. Water extracts of *A. dracunculus* contain very low level of estragol and methyleugenol, so there is a very limited risk [8]. It was approved that some plants EOs possess strong antibacterial power against food-borne pathogens [2].

The main objective of this study was to evaluate the antibacterial effect of essential oil of tarragon on different microbial strains.

Materials and Methods. The investigated plant *Artemisia dragunculus* L. was cultivated at the altitude of 1700–1800 *m* above sea level (Aragyugh, Kotayk Region, Armenia) and harvested during the blossoming period (July, 2015). The plant materials were identified at the Institute of Botany, National Academy of Sciences of Armenia. The samples are available at the Department of Biotechnology, Microbiology and Biotechnology, Yerevan State University, Armenia.

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 [9].

Determination of Essential Oil Chemical Composition. The gas chromatography (GC) mass selective (MS) analysis 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* min^{-1}. Helium (purity 5.6) was used as a gas carrier at a flow rate of 1 *mL*/*min*. 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 and EO was 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 agar diffusion method [10]. This method was preferred to 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 a solvent. 100 μL of each oil solution was introduced to the wells in agar with test microorganisms. Gram-positive (*Bacillus subtilis* WT-A; *Staphylococcus aureus* MDC 5233)

and gram-negative (*Escherichia coli* VKPM-M17; *Pseudomonas aeruginosa* GRP3, *Salmonella thyphimurium* and ampicillin-resistant *E. coli* dhpα-pUCl8) bacteria, *Candida guillermondii* WT and *Debariomyces hansenii* WT yeasts were tested. Microbial culture was grown on meat-peptone agar. Gentamicin, ampicillin and fluconazole (25 *μg*/*mL*) as 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 were expressed as the minimal inhibitory concentrations (MIC).

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 (P) using Microsoft Excel 2010 with the help of T-test function; $p< 0.05$ (if not indicated).

Results and Discussion.

Chemical Composition of Essential Oils. The results of quantitative and qualitative analysis of EOs constituents are presented in Table. The dominant components were identified and *A. dragunculus* is considered to be belonging to methyl chavicol (estragol) chemotype. Estragol is one of the main components of various plant EO and according to literature data its concentration in EO of *A. dracunculus* different chemo-types varied between 0.172–75.0% [11]. Our investigation revealed that estragol concentration of EO of *A. dracunculus* growing at high altitude reached 84.9% (see Table). Estragol is a volatile terpenoid, which has several pharmacological and biological activities. Estragol blocks neuronal excitability by direct inhibition of Na^+-channels [12]. According to literature data estragol is carcinogenic and mutagenic [13], but no acute toxicity or mutagenic activity has been reported at doses relevant for human consumption [8]. It also possesses anti-inflammatory and antimicrobial activity [14].

The main components of essential oils of A. dragunculus cultivated at high altitude Armenian landscape

Chemical components	RRI[a]	Amount, %
Beta-ocimene	978	0.61
Trans-beta-ocimene	1097	4.00
Linalool	1100	5.09
Estragol	1203	84.9
Limonene	1205	1.63
(Z,E)-alloocimene	1282	2.29
3-caren	1726	0.81

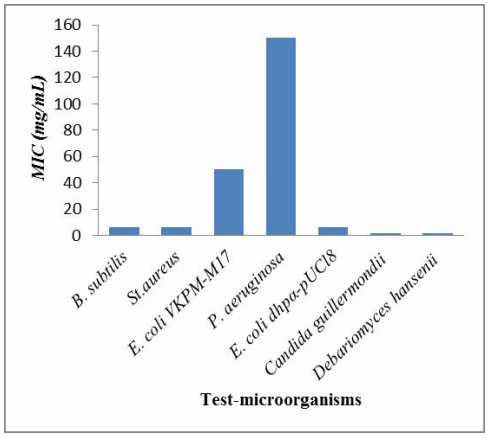
[a] – for HP-5 capillary column.

Besides estragol, *A. dracunculus* EO contained also a rather high amount of monoterpenes (ocimenes, limonene, 3-caren), which were responsible for specific sweet, pungent odor and expressed biological activity. According to some recent investigations 3-caren (bicyclic monoterpene containing a carbon-carbon double bond

and a gem-dimethylcyclopropane ring), is a constituent of many EO and turpentine oils. It was reported that the isomeric mixture of 3-caren showed a wide spectrum of activities, such as antimicrobial, anticancer, antioxidant and fumigant activities [15].

To understand the role of different chemical components of EO it should be noted that another monoterpene is limonene commonly used in perfumes, household cleaners, food, and medicines. Limonene has numerous medicinal benefits demonstrated in human and animal studies. Limonene is one of the components of different plant essential oils that has been identified as having antioxidant and anticancer properties. It demonstrates also anxiolytic effects in a mouse maze model where (comparable to diazepam, but not antagonized by flumazenil, implying a nonbenzodiazepine mechanism) it demonstrates antidepressant activity via 5-HT_{1A} receptor pathway. Limonene has anti-inflammatory effects in models of osteoarthritis and asthma. Limonene is metabolized into perillyl alcohol, which is also a subject of numerous cancer-related studies [16].

The ocimens are a group of monoterpenes (isomeric hydrocarbons) found within a variety of plants and fruits. Ocimene has anti-inflammatory effects in white blood cell through a variety of pathways. Antifungal effects are also observed against the human specific Candida species and antiviral effect against SARS virus [17, 18]. According to our investigations the amount of ocimens in *A. dragunculus* EO was approximately 7%.



A. dragunculus essential oil MIC values against test-microorganisms.

Linalool is a volatile natural plant product with antifungal, antimicrobial and insecticidal properties with a pleasant aroma associated with the fragrance of lavender and laurel. It is the major bioactive compound in basil oil active against tephritid fruit flies, *Ceratitis capitata* (Wiedemann) and *Bactrocer*, *Musca domestica* L. Linalool repels mosquitoes indoors by 93%. Linalool strongly suppresses oxidant-induced genotoxicity, which is predominately mediated by radical scavenging activity; it is a reversible inhibitor of acetylcholinesterase. Linalool-containing plants have been used in folk medicine as anticonvulsant, sedative and antinociceptive remedy [19].

Antibacterial Activity. Nowadays the increased multidrug resistance of bacterial strains leads 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 [20]. In addition, the 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 [21].

The present investigation revealed that gram-positive bacteria were more sensitive to *A. dragunculus* EO. The MIC of *A. dracunculus* EO was 6.25 *mg*/*mL* against *St. aureus* and *B. subtilis* (see Figure).

MIC values of oil under investigation against *E. coli* were 50 *mg*/*mL*, but *P. aeruginosa* was less susceptible to EO components and MIC value reached 150 *mg*/*mL*. The antibiotic resistant *E. coli* was the most sensitive gram-negative microorganism against the investigated oil with 6.25 *mg*/*mL* MIC value. Tested fungi were more susceptible against oil components: MIC = 1.56 *mg*/*mL* against both tested yeasts.

Thus, the MIC values determined are acceptable, effective, and the action of essential oils in this study was evaluated to be bactericidal.

Conclusion. The qualitative and quantitative composition of EO *A. dragunculus* revealed that most of the components are monoterpenes, the main component was estragol. These oils had expressed antibacterial effect against gram-positive bacteria and yeasts and less against gram-negative ones. Being the very common spice, this plant could be important in preventing and treating of bacterial infections, including caused by the antibiotic-resistant bacteria. But taking into account the dose-depended carcinogenetic and mutagenic effect of *A. dragunculus* EO main components the use of this oil requires a lot of caution and further thorough investigations.

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