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SOME PECULIARITIES OF ESSENTIAL OIL OF DAMASK ROSE GROWING IN HIGH ALTITUDE ARMENIAN LANDSCAPES

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As with any essential oil (EO), the content and composition of *Rosa* × *damascena* Mill L. (EO) can be changed significantly, depending on the cultivation methods and terroir. The purpose of this research is to determine the chemical composition and biological activities of EO distilled from Damask rose flowers cultivated in Armenia at high elevation. The EO was obtained by hydro-distillation method, the chemical composition was analyzed by gas-chromatography analysis. The EO main compounds were citronellol (38.04%), nonadecane (4.9 %), nerol (7.12%), geraniol (26.32%) and linalool (6.15%). The antioxidant activity of EO was measured by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), TBARS and metal-chelating activity determination assays. Antimicrobial activity was determined by agar-diffusion method. According to our investigations the minimal inhibitory concentration values against tested gram-positive and gram-negative bacteria were varied between the 2.5 and 20 $mg \cdot mL^{-1}$. Obtained data revealed high (DPPH) scavenging, metal chelating, lipid peroxidation inhibiting activities of EO. Thus, the results revealed a good potential of *R. damascena* EO.

***Keywords*:** Damask rose, essential oil, citronellol, geraniol, antioxidant activity, antimicrobial activity.

Introduction. The *Rosa* genus (Rosaceae family) includes approximately 300 species and thousands of cultivars [1]. All rose cultivars come from the single Damask roses group [2]. Cultivation and consumption of Damask rose (*Rosa* × *damascena* Mill L.) has a very long history, and the essential oil (EO) obtained from rose flowers is one of the most famous products since ancient times. Besides being useful in cosmetics and perfumery, Damask rose EO is also applicable in medicine due to its antimicrobial, antioxidant, relaxant, antiviral properties [3, 4]. For a long time, Damask rose EO has also always been a subject of interest to many scientists. There are data stating that analytical work has been done since the late 19th century, according to which some hydrocarbons (*n*-paraffins (15.9%), including heptadecane, nonadecane, eicosane and heneicosane (0.8–3.0%)); eicosene (0.8%); sesquiterpenes (0.2%); alcohols (linalool (2.7%); geraniol (15.8%); nerol (8.8%); citronellol (3%), farnesol (1.2%); 2-phenylethanol (1.2%)); aldehydes (pentanal (0.005%); nonanal (0.04%); citral (0.05%); cinnamaldehyde, etc.)); phenols (eugenol (0.6%) are present

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in this EO, but according to the Kovats [5], alcohols prevail among the isolated 127 components. Kovats noted that "Neither of the components is the real vehicle of the typical odor of oil of rose", and only by mixing several components in a certain ratio it is possible to gain specific odor. Phenyl ethyl alcohol is among the minor components of Damask rose oil, but it is one of the dominant odors emanating from the rose [6].

Recent studies show deep differences between the compositions of EO distilled from roses growing in different conditions [4]. According to [3], the main components of Damask rose EO are nonadecane (39.73%), heneicosane (32.38%), docosane (7.34%), citronellol (6.14%) and 9-nonadecene (5.69%). Meanwhile, Mahboubi et al. revealed the β-citronellol (48.2%), geraniol (17.0%), β-phenylethyl benzoate (5.4%) and phenyl ethyl alcohol (5.1%) as the main components [7]. Other studies also show that the main compound of rose EO is citronellol (8.76–48.24%) [8].

The International Organization for Standardization (ISO 9842, 2003) [9] specifies the main components and their percentages, which are as follows: citronellol (20–34%), nerol (5–12%), geraniol (15–22%), C17 paraffins (1.0–2.5%), C19 paraffins (8.0–15.0%) and C21 paraffins (3.0–5.5%).

The plant chemotype depends on growing conditions. So, the aim of the present work was to identify the chemical composition and some peculiarities of biological activity of EO distilled from the Damask rose cultivated in high altitude Armenian landscape as the chemotype of each plant determines the scope of its practical application.

Materials and Methods.

Plant Material. *R. damascena* plants were cultivated in Kotayk Province, Aragyugh Village, 1600 *m* a. s. l., and flowers were collected for EO distillation.

EO extraction was performed from flowers of fresh plant material by hydro-distillation, using a Clevenger-type apparatus and lasted 3 *h*. The distilled EO was dehydrated with anhydrous sodium sulphate and stored at 4°*C* in dark airtight bottles until further analysis [10].

Determination of EO Chemical Composition. The EO composition was analyzed in the "Nairian" CJSC laboratory (Armenia). The gas chromatography (GC) analysis was carried out using Bruker gas chromatograph (Bruker 450-GC, USA) equipped with $60\,m \times 0.25\,mm \times 0.25\,\mu m$ OPTIMA-FFAP column ("MACHEREY-NAGEL", Germany). The oven temperature varied from 40 to 220°*C* with the scanning rate of 3°*C* min^{-1}, evaporator temperature was 220°*C*. 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 of 70 *eV* and ion source temperature of 250°*C*. The diluted samples of EO in an amount of 2 μL were injected manually. To avoid overloading the GC column, EO was diluted in methanol in 1:50 proportion (v/v). The identification of peaks was tentatively carried out based on library search using NIST-2013 [11].

Metal-chelating Activity Determination. Metal chelating activity can be assessed by the method described by Alam et al. [12]. 0.4 *mL* of EO diluted in DMSO was added to 1 *mL* of ferrous chloride (0.2 *mM*) and left for 10 *min*. The interaction

begins after adding 0.4 *mL* of ferrozine (5 *mM*). After 10 *min* of incubation at room temperature, the absorbance is measured at a wavelength of 562 *nm*.

Percent metal chelating activity of EO was calculated according the following formula:

$$\text{chelating activity (\%)} = (A_c - A_t) \cdot 100 / A_c ,$$

where A_c is the absorbance of control solution; A_t is the absorbance of test solution. EDTA was used as a positive control.

Radical Scavenging Activity Determination. Scavenging free radical potentials were determined in ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [13]. Catechin was used as a standard. The sample solution contained 125 μL (1 *mM*) of DPPH, 375 μL of ethanol and 500 μL of test-solution (EO and catechin with different concentrations: 2.5 to 50 $mg \cdot mL^{-1}$ and 1.95 to 125 $\mu g \cdot mL^{-1}$, respectively). The test-solution was replaced by ethanol in the control sample. The absorbance was measured at the wavelength of 517 *nm* using Genesys 10S UV-Vis spectrophotometer.

The radical scavenging activity was calculated using the following formula:

$$\text{radical scavenging activity (\%)} = (A_c - A_s) \cdot 100 / A_c ,$$

where A_c is the absorbance of control (DPPH without the addition of test solution); A_s is the sample absorbance.

Determination of Antioxidant Activity with Thiobarbituric Acid Reactive Species Assay. A modified thiobarbituric acid reactive species (TBARS) assay [14] was applied using male Wistar rat (140–150 *g*) brain homogenate (1% in phosphate buffer, pH 7.4). For this, the protein concentration of brain homogenate was determined by the method described in [15]. The control tubes contained: 1 *mL* brain suspension and 1 *mL* 250 *mM* HCl. The test tube contained: 1 *mL* brain suspension, 0.7 *mL* 250 *mM* HCl and 0.3 *mL* EO (1000 $\mu g \cdot mL^{-1}$). The control and test tubes were incubated at 37°*C* (15 *min*), and then 2 *mL* reagent, which contained 0.375% thiobarbituric acid and 15% trichloroacetic acid (in 250 *mM* HCl), was added to each tube and boiled in a water bath for 30 *min*. The tubes were cooled and centrifuged at 1200×g for 10 *min*. The absorbance of the organic upper layer was measured at 532 *nm*, using Genesys 10S UV-Vis spectrophotometer, and the quantity of malondialdehyde (MDA) was measured by the formula:

$$C = D \cdot \varepsilon / A \cdot T,$$

where C is the MDA quantity, $nM \cdot min \cdot mg^{-1}$; extinction coefficient (ε) is $1.56 \cdot 10^5 M^{-1} \cdot cm^{-1}$; D is the absorbance; A is the protein quantity, $mg \cdot mL^{-1}$; T is the incubation period, *min*.

Data were expressed as the values based on the percentage of lipid peroxidation inhibition – the antioxidant index (AI, %), using the formula:

$$\text{AI} = (C_c - C_t) \cdot 100 / C_c,$$

where C_c , C_t are the MDA concentration in control and test tubes, $nM \cdot min \cdot mg^{-1}$.

α-tocopherol (1000 $\mu g \cdot mL^{-1}$) was used as a positive control.

Investigation of EO Antimicrobial Activity. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of EO were determined using agar diffusion method [16]. For this, different gram-positive (*Staphylococcus aureus* WDCM 5233 (Microbial Depository Center, Armbiotechno-logy Scientific and Production Center, laboratory control strain) and *Bacillus subtilis* WT-A1 (isolated from metal polluted soils of Kajaran, Armenia)); gram-negative (*E. coli*

VKPM M-17 (Russian National Collection of Industrial Microorganisms at the Institute of Genetics and Selection of Industrial Microorganisms, laboratory control strain), *Pseudomonas aeruginosa* GRP3 (Soil and Water Research Institute, Iran)) bacteria and two yeasts (*Debaryomyceshansenii* WDCM and 10070, *Candida guillermondii* WDCM 1002, (Microbial Depository Center, Armbiotechnology Scientific and Production Center, laboratory control strain)) were used. Test-organisms were grown in the Müller-Hinton agar. Tests were carried out with $1–2 \cdot 10^6$ CFU mL^{-1} containing plates. The final concentrations of EO reached 2.5, 5, 25, 50, 100 and 150 $mg \cdot mL^{-1}$.

MIC was determined as the lowest concentration of the investigated EO that completely inhibits the visible growth of the test microorganism. The desired concentration of the test organism suspension was obtained by serial dilutions. Ampicillin was used as a standard antibacterial agent.

Data Processing, Chemicals and Reagents. Experimental data ($n = 4$) were expressed as mean ± standard deviation (SD). The standard error did not exceed 3% (if not indicated). The validity of differences between experimental and appropriate control data was evaluated by Student's *t*-test criteria ($p<0.05$, if not indicated) using Microsoft Excel 2010.

The main chemicals and reagents were purchased from "Sigma Chemical Co." (USA) and antibiotics – from Kyiv Plant of Medicinal Preparations (Ukraine).

Results and Discussion. The hydro-distillation method allowed to have essential oil yield of 0.024%. According to the GC-MS data, the main components of EO distilled from Damask rose growing in high altitude Armenian landscape were citronellol, geraniol, nerol, linalool and nonadecane (see Table).

The chemical composition of R. damascena essential oil

N	RT	Component	%	N	RT	Component	%
1	5.67	pinene	0.74	13	15.95	geranial	0.72
2	7.80	limonene	0.52	14	19.88	eugenol	1.31
3	8.47	ocimene	0.42	15	20.48	caryophylene	0.31
4	10.44	linalool	6.88	16	21.03	humulene	0.3
5	10.88	phenylethyl alc.	0.81	17	26.44	eudesmol	0.81
6	11.02	roseoxide	0.15	18	27.71	heptadecane	1.0
7	12.91	terpine-4-ol	0.59	19	30.20	nonadecene	0.91
8	13.36	terpinelol	2.17	20	30.57	nonadecane	4.90
9	14.65	citronellol	38.04	21	31.64	eicosane	0.41
10	14.68	nerol	7.12	22	32.66	heneicosane	2.31
11	15.02	neral	0.82	23	–	Other minor components	2.44
12	15.56	geraniol	26.32			Total	100

Citronellol is the dehydrogenated form of geraniol, and nerol is the trans-isomeric form of geraniol. All of these substances are monoterpenoids and exist in rose EO as the primary components [7]. Linalool is a terpene alcohol, which is generally found in many flowers and spice plants. According to the ISO 9842, 2003, it is not a mandatory component in the Damask rose EO, but its appearance provides some pleasant odor [9, 17].

The investigated EO also possesses valuable biological activity. According to our investigations, the EO distilled from Damask rose growing in Armenian high altitude landscape showed the rather high DPPH radical scavenging activity. The IC_{50} value of Damask rose was 24.76 $mg \cdot mL^{-1}$ the same data of positive control, catechin, was 12.66 $\mu g \cdot mL^{-1}$ (Fig. 1).

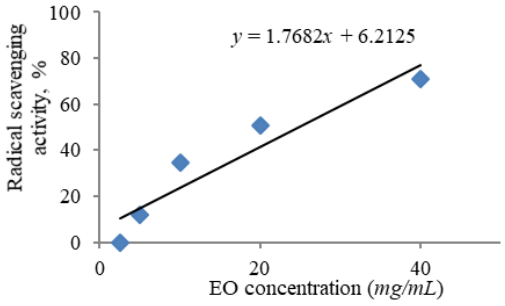

Fig. 1. The radical scavenging activity of *R. damascena* EO.

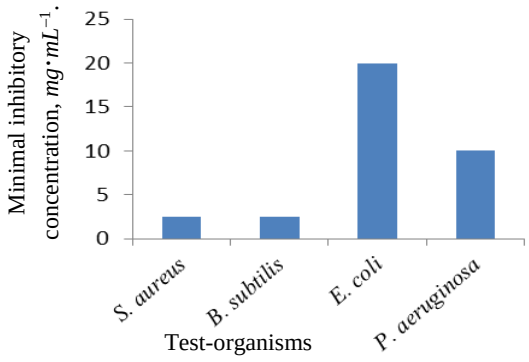

Fig. 2. *R. damascena* essential oil MIC against test-bacteria.

As a result of the Fenton and Haber–Weiss reactions in the presence of iron ions in living organisms, including humans, free radicals are formed, in particular hydroxyl radicals, resulting in an oxidation balance in the cell. This can lead to serious damages in different macromolecules. In this regard, it is important the presence of chelating factors in the organism that could diminish the excess metal in the cells, thereby preventing the formation of an excessive amount of free radicals. But, according to our results, the investigated concentration (see Materials and Methods) of Damask rose EO binds up to 3% of iron ions in the test solution, while the activity of the positive control was 94%. TBARs test also showed low activity of the investigated EO. According to the results of this test, AI was 7±0.4%, but in the case of the positive control (α-tocopherol) this parameter reached 91.1±1.9%. These data showed that Damask rose EO exhibited low or moderate antioxidant activity in chemical-based tests.

On the other hand, the EO distilled from Damask rose, growing in high altitude Armenian landscape, showed expressed antibacterial activity against various gram-positive and gram-negative bacteria. Our investigations showed that gram-positive bacteria were more sensitive to the chemical components of studied EO, and the value of MIC against *S. aureus* and *B. subtilis* were 2.5 $mg \cdot mL^{-1}$. Meanwhile, the same parameter in the case of *E. coli* and *P. aeruginosa* was 20 and 10 $mg \cdot mL^{-1}$, respectively. The tested yeasts were not sensitive against EO components (Fig. 2).

Our investigation has shown that EO distilled from *R. damascena* cultivated in high altitude Armenian landscape possess antibacterial and antioxidant activity and could be applied not only in cosmetics, but also in the pharmaceutical and food industry.

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ՀԱՅԱՍՏԱՆԻ ԲԱՐՁՐԱԴԻՐ ԳՈՏԻՆԵՐՈՒՄ ԱՃՈՂ ԴԱՄԱՍԿՈՍՅԱՆ ՄԱՍՐԵՆՈՒ ԵԹԵՐԱՅՈՒՂԻ ՈՐՈՇ ԱՌԱՆՁՆԱՀԱՏԿՈՒԹՅՈՒՆՆԵՐԸ

Ամփոփում

Ինչպես բոլոր յուղերի, *Rosa* × *damascena* Mill L. եթերայուղի պարունակությունն ու կազմը ևս կարող է զգալի փոփոխվել՝ կախված աճեցման

մեթոդներից և վայրից: Ներկայացված հետազոտության նպատակն է եղել որոշել Հայաստանի բարձրադիր գոտիներում աճող դամասկոսյան մասրենու ծաղիներից թորված եթերայուղի քիմիական կազմը և կենսաբանական ակտիվությունը: Եթերայուղը ստացվել է հիդրոթորմման մեթոդով և քիմիական կազմը որոշվել է գազ–քրոմատա-գրմամբ: Եթերայուղի հիմնական բաղադրիչ-ներն են՝ ցիտրոնելոլը (38.04%), նոնադեկանը (4.9%), ներոլը (7.12%), գերանիոլը (26.32%) և լինալոոլը (6.15%). Եթերայուղի հակաօքսիդանտային ակտիվությունը գնահատվել է 2,2-դիֆենիլ-1-պիկրիլ հիդրազիլ (ԴՖՊՀ), ճարպերի գերօքսիդացման և մետաղ-խելատացնող ակտիվության որոշման թեստերով: Հակաբակտերիական ակտիվությունը որոշվել է ագարում դիֆուզման մեթոդով: Համաձայն մեր հետազոտությունների արդյունքների, թեստավորված գրամ-դրական և գրամ-բացասական բակտերիաների նկատմամբ նվազագույն արգելակող կոնցենտրացիայի (ՆԱԿ) արժեքը 2.5 և 20 *մգ·մլ*$^{-1}$ սահմաններում է: Ըստ ստացված տվյալների եթերայուղն ունի բարձր հակառադիկալային (ԴՖՊՀ), մետաղ խելատացնող, լիպիդների գերօքսիդացումը արգելակող ակտիվություն: Արդյունքները վկայում են *R. damascena* եթերայուղի բարձր ներուժի մասին:

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НЕКОТОРЫЕ ОСОБЕННОСТИ ЭФИРНОГО МАСЛА ДАМАССКОЙ РОЗЫ, РАСТУЩЕЙ НА ВЫСОТНЫХ ПОЯСАХ АРМЯНСКОГО НАГОРЬЯ

Резюме

Как и в случае любого эфирного масла (ЭМ), химический состав (ЭМ) *Rosa* × *damascena* Mill L. может значительно меняться в зависимости от методов культивирования и зоны роста. В работе исследовался химический состав и биологическая активность ЭМ, выделенного из цветов дамасской розы, выращенной на высотных поясах Армении. ЭМ было получено гидродистиллированием, химический состав определялся газхроматографическим методом. Основными компонентами ЭМ были цитронеллол (38.04%), нонадекан (4.9%), нерол (7.12%), гераниол (26.32%) и линалоол (6.15%). Антиоксидантная активность ЭМ определялась ДФПГ-тестом по способности ингибировать перекисное окисление липидов и выявлением метал-хелатирующей способности. Антимикробная активность выявлялась методом диффузии в агар. Согласно нашим исследованиям, значения минимальной ингибирующей концентрации в отношении тестированных грам-положительных и грам-отрицательных микроорганизмов варьировались между 2.5 и 20 *мг·мл*$^{-1}$. Полученные данные выявили наличие антирадикальной (ДФПГ), метал-хелатирующей активности и способности к ингибированию перекисного окисления липидов. Таким образом, результаты выявили высокий потенциал ЭМ *R. damascena.*