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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *ROSA DAMASCENA MILL*. (VARIETY RAINBOW) FROM CLONAL MICROPROPAGATION

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Damask rose of the Veselka variety is an important industrial rose variety used to obtain essential oil. It is widely used in modern cosmetology, perfumery, and aromatherapy. In addition, the essential oil of Damask rose has a whole spectrum of pharmacological properties.

The scientific innovation of this research lies in its foundation on cultivating and acquiring planting materials of the Damask rose in vitro. This approach guarantees controlled conditions for plant growth, the production of robust seedlings, and an enhancement in the precision and credibility of the research outcomes.

Moreover, the study has scientific novelty in that it explores to assess both the quantitative and qualitative constituents of the essential oil in the acquired plant material of the Damask rose. This assessment takes place within the context of cultivating regenerative plants in an outdoor environment. Such an approach acknowledges the potential distinctions in the oil's component composition acquired from plants propagated through this method in comparison to traditional vegetative reproduction. Lastly, the research has scientific novelty in investigating the potential antimicrobial properties of Damask rose essential oil, which could have significant practical implications in the development of new drugs and combatting infectious diseases.

The purpose *of the study was to establish the component composition of the essential oil of Rosa damascena Mill., which was grown in vitro, and to determine its antimicrobial effect.*

Methods*. The object of the study was the essential oil of Damask rose of the Veselka variety, which was grown by the method of clonal micropropagation in vitro. The essential oil was extracted from fresh petals collected during dry weather conditions prior to sunrise by hydrodistillation. Determination of the qualitative composition and quantitative content of volatile substances was carried out by the GC-MS method using an Agilent 7890B chromatograph. Antimicrobial activity was studied in vitro using the disk diffusion method with reference test strains of microorganisms Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Candida albicans ATCC 885-653.*

Results*. According to the results of the chromato-mass spectrometric study, 41 compounds (6 of which were in the isomeric state) were identified, which was belong to 13 different classes of chemicals. Dominant compounds among terpenoid substances were shown: geraniol – 30.96 %, citronellol – 27.08 %, alkanes: nonadecane – 17.29 %, and heneicosene – 5.46 %.*

It was established that the essential oil of Damask rose had a significant antimicrobial effect against strains of C. albicans and E. coli, the diameters of which growth retardation zones ranged from 32–35 mm and 20–23 mm, respectively. In studies with P. aeruginosa and S. aureus, the essential oil showed moderate antibacterial activity: the diameters of the growth retardation zone of these microorganisms ranged from 13 to 15 mm and 11 to 12 mm, respectively.

Conclusion. *For the first time, the qualitative composition and quantitative content of volatile substances in the essential oil extracted from the petals of Rosa damascena Mill., Veselka variety, cultivated through the clonal micropropagation in vitro, were explored by chromatography-mass spectrometry techniques. The results of the study of antimicrobial activity showed that the studied essential oil exhibits significant fungicidal effects against Candida microorganisms, along with moderate bactericidal effects on gram-negative (E. coli, P. aeruginosa) and gram-positive (S. Aureus) bacteria. These results highlight the potential of this essential oil for further investigation in the realm of developing novel medicines and herbal preparations. Further clinical studies are needed to assess this potential.*

Keywords*: Rosa Damascena Mill., method of clonal micropropagation, GC-MS, antibacterial activity.*

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1. Introduction

There are several thousand garden varieties and hybrids of roses in the world. Most of them were formed because of selection, multiple repeated crossings, and selection, but there are also wild varieties [1]. It is believed that a significant number of rose varieties were obtained by crossing the Damask rose with modern varieties of hybrid tea roses and floribunda roses [2].

Damask rose *Rosa damascena Mill.* is a perennial branchy shrub up to 1.5 m tall and belongs to the Rosaceae family (Rosaceae). According to the classification of roses, *Rosa damascena* belongs to the old garden roses, and its homeland is the Middle East, the city of Damascus in Syria [3]. Rose essential oil has been widely used in aromatherapy for its soothing properties since ancient times [4].

Damask rose, as a source of biologically active substances, constantly attracts the attention of scientists [5, 6]. Thus, Iranian scientists have proven the positive effect of Damask rose extract as a special food additive and alternative means in the treatment of non-alcoholic fatty liver disease [7]. Further studies of the antioxidant effect confirmed the prospects of using this extract in Alzheimer's disease [8]. There are also data on the analgesic properties of rose oil in patients with migraine [9] and on reducing the level of pain and anxiety in the first stage of childbirth when using aromatherapy with rose essence [10].

It should be noted the presence of antimicrobial and anti-inflammatory action. For example, in an article by Turkish scientists [11], the effect of an alcohol extract against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). However, the antimicrobial effect was confirmed only against *E. coli.* Research by Iranian scientists established the dependence of the antimicrobial effect on the dosage form [12]. Antimicrobial activity was confirmed against *Xanthomonas axonopodis* spp. *Vesicatoria*, *Chromobacterium violaceum, Erwinia carotovora* strains, *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, and *Pseudomonas fluorescens*. In turn, Japanese researchers were able to argue the feasibility of using rose water in the treatment of inflammatory skin infections caused by *Candida albicans* and/or methicillin-resistant *Staphylococcus aureus* (MRSA) [13].

The works of scientists contain a wide array of data on the antifungal/antimicrobial action of geraniol and nerol against *C. albicans* [14, 15] and *E. coli* [16], antifungal action of citronellol against *C. albicans* [17], a wide spectrum of antimicrobial and antifungal action nonadecane and heneicosene [18].

It is the combination of antimicrobial and anti-inflammatory action that determines the fact that rose essential oil is part of many skin care products produced by such cosmetic companies as Melvita (France), Germaine de Capuccini (Spain), Milani (USA), Leganza (Bulgaria) and others.

The analysis of Scopus and Web of Science revealed information on the dependence of the chemical composition of the essential oil and, accordingly, its biological action [19] on climatic and growing conditions [20].

Panasenko O.I. at all investigated for the first time the chemical composition of the freon extract of *Rosa damascena Mill.*, grown in vitro using the chromato-mass-spectrometric method. The main components of rose petals: phenylethyl alcohol – 64.070 %, citronellol – 6.090 %, nonadecane – 4.636 %, heneicosane – 2.590 %, geraniol – 1.749 % [21].

Tanjga B.B. et al. Studied the volatile composition of the hydrosol R. hybrida. There were 44 volatile compounds detected in the hydrosol by using GC–MS, among which the dominant ones were phenylethyl alcohol (23.5 %), nerol (17.2 %), linalool (13.2 %), and geraniol (8.3 %). It was also established that the total phenolic content in R. hybrida hydrosol was 4.96 μg GAE/mL. Similar results (5.2 μg GAE/mL) were obtained from Turkish R. damascena hydrosol. Significantly higher values of TPC are noted in R. damascena, from 32.52 μg GAE/mL to 57.02 μg GAE/mL, while hydrosol of R. alba contains 72.72 μg GAE/mL [22].

Studies of the antimicrobial effect of Damask rose essential oil propagated *in vitro* are relevant.

2. Planning (methodology) of the research

Rosa damascena is classified as an old garden rose, the essential oil of which is widely used in medicine, cosmetics, perfumery, and aromatherapy. We obtained the essential oil from the fresh petals of *Rosa damascena*, a variety of Rainbow, the planting material of which was grown by the method of clonal micropropagation *in vitro*. The qualitative composition and quantitative content of volatile substances of the obtained essential oil were determined by GC-MS method, the antimicrobial activity was studied, and the prospect of its use for the creation of new medicinal and cosmetic products was shown (Fig. 1).

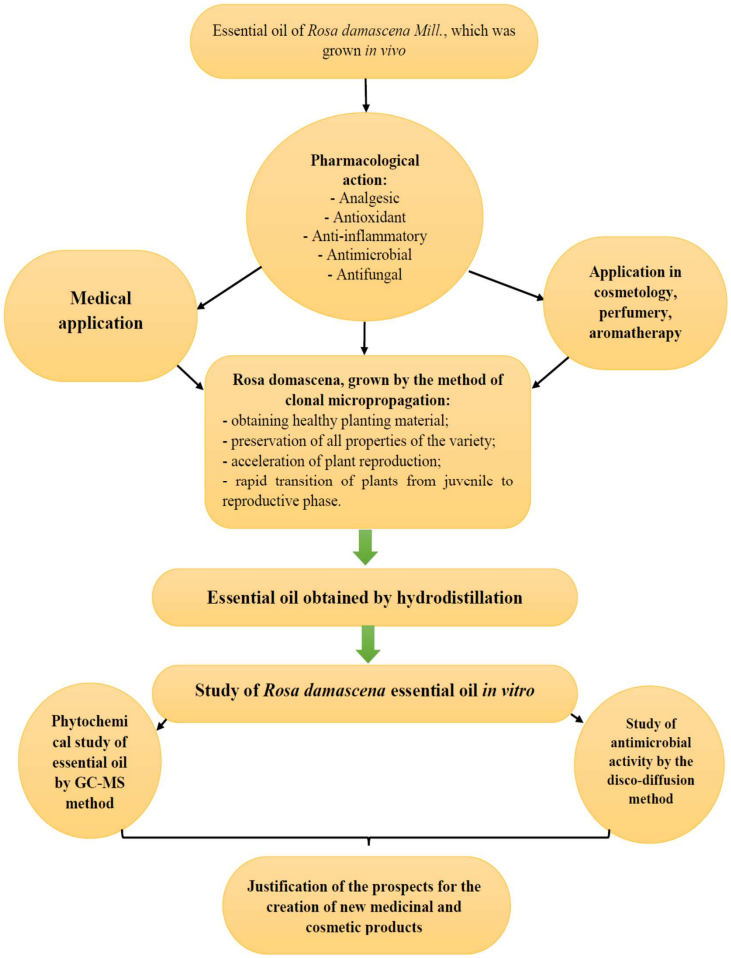

Fig. 1. Scheme of substantiation of the relevance and planning of the experiment on the study of *Rosa damascena* essential oil *in vitro*

3. Materials and Methods

3. 1. Ethical consideration

The study was carried out as part of the research work of the Department of Pharmacognosy, Pharmacology and Botany of Zaporizhzhia State Medical University «The searching and researching new sources of medical plant raw materials and creating the substances and medicines that based on them» No. 0120U102600. Biosafety ethics were observed by all scientists during the conducted research.

3. 2. Plant material

The object of the study was the essential oil of Damask rose (*Rosa damascena* Mill.), grown by the method of clonal micropropagation *in vitro.*

Cultivation of plants *in vitro* was carried out at the Educational and Scientific Medical and Laboratory Center with a vivarium of Zaporizhzhia State Medical University. The advantage of the method of clonal micropropagation *in vitro* is obtaining healthy planting material identical to the original one with the preservation of all the properties of the variety, rapid reproduction of plants, and acceleration of the transition of plants from the juvenile to the reproductive phase.

Parts of shoots with buds of the Rosa (Variety Rainbow) were used for injecting into *in vitro* culture. The entire process was carried out according to the methods generally accepted in biotechnology [23]. Explants were cultured *in vitro* from March to May; they were cultivated on a modified nutrient medium of Murashige and Skoog with growth regulators at an air temperature of 22–24 °C, relative air humidity of 65–70 %, and the illumination of 2500–3000 lux with a photoperiod of 16 hours. The nutrient medium was sterilized in an autoclave under a pressure of 0.11 MPa for 25 minutes. The duration of the passage is 28–30 days.

For injecting *in vitro*, Murashige and Skoog (MS) nutrient medium was used with the addition of 2.0 mg/l 6-benzylaminopurine (6-BAP), 0.2 mg/l indolyl-3-acetic acid (IOC), and 25 0 mg/l of ascorbic acid. Explants 0.8–1.2 sm in size with one node were planted.

Removal of apical dominance and induction of the development of axillary buds were used as the main method of propagation at the subcultivation stage. The best morphometric indicators were recorded on the MS medium with the addition of 2.0 mg/l BAP, 0.2 mg/l IUC and 0.5 mg/l adenine. Under such cultivation conditions, the reproduction ratio ranged from 1:7 to 1:12 for one passage (30 days), while the length of the shoots reached from 11 to 28 mm.

The essential oil was obtained by hydrodistillation, according to the State Pharmacopoeia of Ukraine [24].

Qualitative and quantitative determination of active compounds was carried out at the Department of Natural Sciences for Foreign Students and Toxicological Chemistry (Head of the Department – PhD, DSc, Professor O. I. Panasenko) of Zaporizhzhia State Medical University. Standard methods of determining chemical compounds were applied for this [25, 26].

The completeness of the reactions and the individuality of the resulting compounds were controlled by the gas chromatograph Agilent 7890B with a 5977B mass spectrometrydetector. The column is DB-5ms 30 m×250 μm×0.25 μm with length. The gas-carrier speed (helium) is 1.6 ml/min. Injection volume – 0.5 μl. Separation of the flow is 1:50. The temperature of the sampling unit is 230 °C→12 °C/s→275 °C.

Thermostat temperature: programmable, 240 °C (1-minute delay)→5 °C/min→280 °C (delay 1 min). The total time of examination is 10 min. Temperature of interface GS/MS – 280 °C; ion sources – 230 °C; quadrupole mass analyzer – 150 °C. Type of ionization: EI with an electron energy of 70 eV. The range of mass numbers that were scanned was 30–500 m/z.

3. 3. Antimicrobial activity

The study of antimicrobial activity was carried out in the microbiological laboratory of the Department of Microbiology, Virology, and Immunology of Zaporizhzhia State Medical University.

Antimicrobial activity was studied *in vitro* with the disk diffusion method [27, 28] using reference test strains of the American Collection of Type Cultures: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 885-653. Previously, strains of *E. coli*, *P. aeruginosa*, and *S. aureus* were grown on meat-peptone agar, and *C. albicans* – on Sabouraud's medium (HiMedia, India).

Sterile paper discs manufactured by HiMedia (India) were used to produce oil-impregnated discs. They were immersed in Damask rose essential oil for a few seconds and then dried.

During the experiments, 18-hour cultures of bacteria were used, from which suspensions with a McFarland density of 0.5 were prepared in an isotonic sodium chloride solution using a DEN-1B densitometer (SIA "Biosan", Latvia). Freshly prepared bacterial suspensions of Escherichia coli, pseudomonads, and staphylococcus were evenly inoculated using a sterile cotton swab on the surface of Mueller-Hinton agar (HiMedia, India), and the Candida suspension was inoculated on the surface of modified Mueller-Hinton agar (HiMedia, India). Culture seeds were dried for 5 min., and then oil-soaked discs were placed on the surface of the agar. The crops were incubated at a temperature of 35±1°. The study results were calculated after 20 hours of crop incubation. The sensitivity of test strains to Damask rose essential oil was determined by the presence/absence of zones of growth retardation around the disk. The diameter of growth retardation was measured in millimetres with an accuracy of 1 mm. The study was conducted three times.

4. Research results

In the chromato-mass spectrometric study of the essential oil of *Rosa damascena* Mill. 41 compounds were found in its composition (6 of them were in the isomeric state). It was established that they belong to 13 classes of chemical substances: monoterpene alcohols – 70.80 %, alkanes – 22.76 %, alkenes – 6.42 %, sesquiterpenes – 4.43 %, sesquiterpene alcohols – 1.74 %, monoterpenes – 1.72 %, aromatic alcohols – 1.59 %,

monoterpene esters – 1.36 %, aromatic esters – 1.30 %, simple alcohol – 1.22 %, fatty acid esters – 0.27 %, monoterpene aldehyde – 0.26 %, ketone – 0.08 % (Table 1).

The following components prevailed by percentage: geraniol – 30.96 %, citronellol – 27.08 %, nonadecane – 17.29 %, heneicosene – 5.46 %.

Table 1

Essential oil components of *Rosa damascena* Mill.

No.	RT	Compound	Chemical class	Percentage, %
1	13.835	ethanol	simple alcohol	1.221
2	13.943	α-pinene	monoterpene	0.100
3	16.134	sabinene	monoterpene	0.071
4	16.356	*cis-roseoxide	monoterpene	0.423
5	17.141	myrcene	monoterpene	0.380
6	19.609	limonene	monoterpene	0.053
7	21.613	γ-terpinene	monoterpene	0.560
8	24.348	β-linalool	monoterpenealcohol	0.106
9	25.134	*trans-roseoxide	monoterpene	0.135
10	25.271	phenyleethylalcohol	aromaticalcohol	0.770
11	33.396	citronellol	monoterpene	27.080
12	34.177	nerol	monoterpenealcohol	0.101
13	35.112	*geraniol	monoterpenealcohol	15.866
14	36.164	geranial	monoterpenealdehyde	0.264
15	39.735	methylgeranate	monoterpeneester	0.106
16	41.647	citronellylacetate	monoterpeneester	0.629
17	42.007	eugenol	aromaticalcohol	0.824
18	42.382	nerylacetate	monoterpeneester	0.087
19	43.640	geranylacetate	monoterpeneester	0.541
20	43.972	α-bourbonene	alkene	0.130
21	44.380	β-elemene	sesquiterpene	0.163
22	45.011	methyleugenol	aromaticester	0.245
23	46.217	caryophyllene	sesquiterpene	0.777
24	47.348	α-guaiene	sesquiterpene	0.546
25	48.355	α-humulene	sesquiterpene	0.585
26	50.066	germacrene D	sesquiterpene	1.105
27	50.904	**n*-tridecane	alkane	0.779
28	51.130	aciphyllene	sesquiterpene	0.194
29	51.550	α-bulnesene	sesquiterpene	0.653
30	54.790	trans-nerolidol	sesquiterpenealcohol	0.069
31	56.514	ethyleicosanoate	fattyacidester	0.046
32	56.807	**n*-tridecane	alkane	0.171
33	61.197	*docosene	alkene	0.459
34	62.483	*nonadecane	alkane	2.483
35	63.744	farnesol	sesquiterpenealcohol	1.667
36	64.845	*geraniol	monoterpenealcohol	15.097
37	66.124	bensylbenzoate	aromaticester	0.050
38	66.510	*docosene	alkene	0.068
39	67.770	*nonadecane	alkane	0.021
40	70.806	phenylethyltiglate	fattyacidester	0.083
41	71.265	oxacycloheptadecenone	ketone	0.076
42	71.681	heptadecane	alkane	1.480
43	72.284	*docosene	alkene	0.068
44	73.132	*nonadecane	alkane	12.179
45	77.470	ethyleicosanoate	fattyacidester	0.087
46	77.743	*nonadecane	alkane	2.602
47	81.137	*docosene	alkene	0.232
48	82.476	eicosane	alkane	1.247
49	85.471	methyllinolenate	fattyacidester	0.055
50	90.678	heneicosene	alkene	5.464
51	95.120	*n*-tricosane	alkane	1.802

*Note: * – these compounds are in the form of isomers*

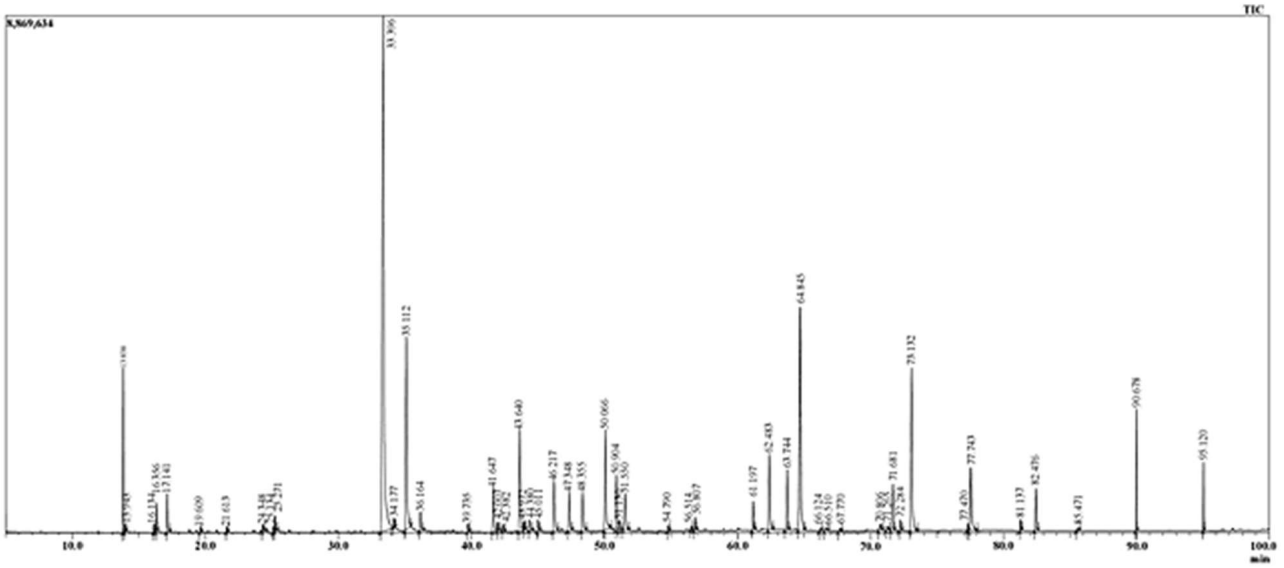

Fig. 2. Chromatogram essential oil components of *Rosa damascena Mill*.

On the chromatogram of the essential oil components of *Rosa damascena* Mill. (Fig. 2) was identified citronellol (*RT*=33.396) and geraniol (*RT*=35.112).

The obtained results of microbiological studies allowed us to conclude that Damask rose essential oil had significant antimicrobial activity. The highest activity of the oil was found against *C. albicans* and *E. coli* strains: the diameters of growth retardation around the discs ranged from 32 to 35 mm (mean 33.3 mm) with Candida and from 20 to 23 mm with Escherichia (mean 21.3 mm). The moderate antibacterial activity of the essential oil was determined in experiments with staphylococcus and pseudomonas. Thus, in cultures with *P. aeruginosa*, the diameters of growth retardation of the strain ranged from 13 to 15 mm (mean 14.0 mm), with *S. aureus* culture – from 11 to 12 mm (mean 11.3 mm) (Table 2).

Table 2

Antimicrobial activity of essential oil of *R osa damascena Mill*

Sample name	Tests train	The diameter of the zone of detention grows by mm			
		1 study	2 study	3 study	Mean
Essential oil of *Rosa damascena Mill.*	*E. coli*	21	23	20	21.3
	S. aureus	11	11	12	11.3
	P. aeruginosa	13	14	15	14.0
	C. albicans	35	32	33	33.3

According to this research, the *Rosa Damascena Mill.* (Variety Rainbow), grown *in vitro*, can be recommended for further research as a promising plant with antimicrobial activity.

5. Discussion of the results

External conditions inevitably affect the quality of plant raw materials, which is why there is potential for the use of *in vitro* plant cultures for the production of medicinal products. Plants propagated by *in vitro* microtonal propagation are genetically homogeneous with the donor plant, healthier, and have an optimal chemical composition, thus having a greater advantage compared to those grown *in vivo*. They can be acclimatized in a shorter period of time. Rapid propagation of selected material allows for high yields of *Rosa damascena* Mill. raw material throughout the year, regardless of the vegetation period.

The essential oil of Rosa Damascena Mill, Rainbow variety, was obtained by hydrodistillation, and we investigated its chemical composition, quantified the volatile compounds, and studied its antimicrobial activity.

These results indicated the antibacterial effectiveness of the studied Damask rose essential oil against enterobacteria, non-glucose-fermenting gram-negative bacteria, gram-positive cocci, and Candida. The high antibacterial activity of Rosa Damascena essential oil is probably due to the significant content of such compounds as geraniol, citronellol, nonadecane, heneicosene.

Turkish scientists have proven the antimicrobial activity of the alcohol extract of *Rosa damascena* Mill. using disk diffusion and well diffusion methods against Escherichia coli (ATCC 25922) bacteria, as well as inhibitory activity against tyrosinase according to TLC-bioautography data [11].

Japanese scientists have proven that rose water suppressed neutrophil activation induced by stimulants at 3–15 %, inhibited mycelial and yeast growth of C. albicans at ca. 2.2 and 50 %, respectively, and >50 % rose water killed MRSA within a short time, these results suggest that its cutaneous application may inhibit the growth of microbes on the skin surface.

The concentrations of citronellol, geraniol, and phenethyl alcohol in 2.2 % rose water (IC50) were calculated to be 0.00038, 0.00031, and 0.00091 %, re-

spectively. The IC50 value of geraniol was ca. 0.00045, and 0.0006 % citronellol contributed to the activity of rose water. The research was focused on microorganisms as the cause and neutrophils, which played an important role in the inflammatory process, and found that rose water has antimicrobial and anti-inflammatory effects [13].

Study limitations. During the study of the essential oil of Damask Rose for its antimicrobial activity, only archival strains of microorganisms were used in the research, although it would have been interesting to test its activity against clinical strains obtained from real patients.

The prospects for further research. Further studies will be conducted to compare the volatile compound content and antimicrobial activity of the essential oil of Damask Rose grown through *in vitro* clonal micropropagation with that of the raw material grown under *in vivo* conditions.

6. Conclusion

For the first time, the qualitative composition and quantitative content of volatile substances in the essential oil extracted from the petals of *Rosa damascena Mill.*, Rainbow variety, cultivated through the clonal micropropagation in vitro, were explored by chromatography-mass spectrometry techniques.

1. According to the results of research, the *Rosa damascena* Mill. is a valuable source of such compounds as geraniol, citronellol, nonadecane, and heneicosene.

2. The obtained results of microbiological research remained to show that the studied essential oil exhibits significant fungicidal effects against *Candida* microorganisms, along with moderate bactericidal effects on gram-negative (*E. coli, P. aeruginosa*) and gram-positive (*S. Aureus*) bacteria.

3. Further clinical studies are needed to assess the effectiveness of the research object as a potentially medicinal product.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

The manuscript does not have any associated data.

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