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A comparative microbiological study of the essential oils from the genus *Rosa* L.

The medicinal plant raw material containing essential oils always attract the attention of scientists from various countries worldwide. The search for new sources of the plant raw material rich in biologically active substances, particularly volatile compounds, remains a relevant task in modern pharmaceutical science. Our focus has been drawn to *Rosa damascena* Mill. of the “Veselka” (“Rainbow”) variety grown in the natural environment of Zaporizhzhia; its essential oil exhibits anti-inflammatory, antimicrobial, and antiseptic effects. To obtain a valuable medicinal plant raw material from *Rosa damascena* with a high content of essential oil, it was introduced into *in vitro* culture to produce aseptic regenerants, followed by their reintroduction into the natural environment.

Aim. To conduct a comparative microbiological study of essential oils from *Rosa damascena* of the “Veselka” variety grown via clonal micropropagation in *in vitro* culture and in the natural environment of Zaporizhzhia.

Materials and methods. The study objects were essential oils obtained by water distillation from rose petals cultivated using the method of clonal micropropagation in *in vitro* culture (at the premises of the Educational and Scientific Medical Laboratory Center with a vivarium) and the natural environment of Zaporizhzhia. The study of the antimicrobial activity was conducted in the microbiological laboratory of the Department of Microbiology, Virology, and Immunology at Zaporizhzhia State Medical and Pharmaceutical University.

Results and discussion. The essential oils from *Rosa damascena* grown via clonal micropropagation in *in vitro* culture and under natural conditions in Zaporizhzhia effectively inhibited the growth of *E. coli* (21.3 mm and 12.0 mm, respectively) and *S. aureus* (11.3 mm and 10.2 mm, respectively). The results of the study on *C. albicans* indicate a high antifungal activity in both essential oils; the mean inhibition zone diameter in the experiments using the oil from *Rosa damascena* cultivated in *in vitro* culture is less: 33.3 mm compared to 40 mm in the experiments with the oil from roses grown in the natural environment of Zaporizhzhia.

Conclusions. The method of clonal micropropagation of *Rosa damascena* of the “Veselka” variety is effective for obtaining a large amount of planting material in a short time with the subsequent reintroduction into the natural environment to expand the raw material base of valuable medicinal plants with a high content of essential oils. The results of the study of the antimicrobial activity of the essential oil of *Rosa damascena* of the “Veselka” variety cultivated through clonal micropropagation in *in vitro* culture have exhibited a high antimicrobial activity and moderate antifungal activity compared to the essential oil from *Rosa damascena* grown in the natural environment of Zaporizhzhia. Therefore, *Rosa damascena* Mill. of the “Veselka” variety cultivated under *in vitro* conditions is a promising source of essential oil with a high antibacterial effect in order to create new herbal formulations.

Keywords: *Rosa damascena* Mill. “Veselka” variety; clonal micropropagation in *in vitro* culture; essential oil; antimicrobial activity

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Порівняльне мікробіологічне дослідження ефірної олії представників роду *Rosa* L.

Лікарська рослинна сировина, яка містить ефірні олії, завжди привертає увагу науковців з різних країн світу. Пошук нових джерел рослинної сировини з високим вмістом біологічно активних речовин, зокрема летких сполук, є актуальним завданням сучасної фармацевтичної науки. Нашу увагу привернула *Rosa damascena* Mill. сорту Веселка, вирощена в природному середовищі м. Запоріжжя, ефірна олія якої володіє протизапальною, антимікробною та антисептичною дією. Задля одержання цінної лікарської рослинної сировини *Rosa damascena* з високим вмістом ефірної олії було введено її в культуру *in vitro* для отримання асептичних регенерантів із подальшою реінтродукцією в природне середовище.

Метою роботи було порівняльне мікробіологічне дослідження ефірних олій троянди дамаської сорту Веселка, вирощеної методом клонального мікророзмноження в культурі *in vitro* та в природному середовищі в умовах м. Запоріжжя.

Матеріали та методи. Об'єктами дослідження були ефірні олії, отримані методом гідродистиляції з пелюсток троянд, вирощених методом клонального мікророзмноження в культурі *in vitro* (на базі Навчально-наукового медико-лабораторного центру з віварієм) та в природному середовищі в умовах м. Запоріжжя. Дослідження протимікробної активності проведено на базі мікробіологічної лабораторії кафедри мікробіології, вірусології та імунології Запорізького державного медико-фармацевтичного університету.

Результати та їх обговорення. Ефірні олії троянди дамаської, вирощеної методом клонального мікророзмноження в культурі *in vitro* та в природному середовищі в умовах м. Запоріжжя, досить ефективно пригнічували зростання *E. coli* (відповідно 19,7 та 12,0 мм) та *S. aureus* (відповідно 11,3 та 10,2 мм). Результати дослідження з *C. albicans* свідчать про високу протигрибкову активність обох ефірних олій, середнє значення діаметра зони затримки зростання в експериментах з олією троянди дамаської, вирощеної в культурі *in vitro*, є меншим: 33,3 мм проти 40 мм в експериментах з ефірною олією троянди, вирощеної в природному середовищі в умовах м. Запоріжжя.

Висновок. Метод клонального мікророзмноження троянди дамаської сорту Веселка ефективний для одержання великої кількості посадкового матеріалу в короткі строки з подальшою реінтродукцією в природне середовище для розширення сировинної бази цінних лікарських рослин з високим вмістом ефірних олій. Результати дослідження протимікробної активності ефірної олії троянди сорту Веселка, вирощеної методом клонального мікророзмноження в культурі *in vitro*, проти ефірної олії троянди дамаської, вирощеної в природному середовищі в умовах м. Запоріжжя, засвідчили високу антибактеріальну та помірну протигрибкову активність. *Rosa damascena* Mill. сорту Веселка, вирощена в умовах *in vitro*, є перспективним джерелом отримання ефірної олії з високою антибактеріальною властивістю з метою створення нових фітопрепаратів.

Ключові слова: *Rosa damascena* Mill. сорту Веселка; клональне мікророзмноження в культурі *in vitro*; ефірна олія; протимікробна активність

Introduction. In modern medical practice, significant attention is paid to identifying additional sources of the medicinal plant material with antimicrobial and anti-inflammatory properties.

The Damask rose (*Rosa damascena* Mill.) from the *Rosaceae* family widely used in medicine and cosmetology is of particular interest.

There are approximately several thousand garden varieties and hybrids of roses globally [2].

The Damask rose, *Rosa damascena* Mill., is a perennial branched shrub up to 1.5 meters high, with its raw material being petals (*Rosae petales*), which contain the essential oil in exogenous formations – oil glands [3].

A significant portion of the chemical composition of the Damask rose is represented by components of the essential oil, which is one of the primary biologically active compounds of this plant. The essential oil of *Rosa damascena* has a complex chemical nature and contains several dozen different components. According to studies by French and Bulgarian scientists, the main components of the essential oil from the Damask rose petals are citronellol (35-46 %), geraniol (10-26 %), nerol (9-18 %), and their acetates, which give it a characteristic fragrance [4-8].

Rose petals also contain such flavonoids as kaempferol, quercetin, apigenin, myricetin, rutin, roxyloside (kaempferol-3-O- β -D-glucopyranosyl (1-4)- β -D-xylopyranoside), afzelin, isoquercitrin, quercetin 3-O-galactoside, and quercetin 3-O-xyloside, cyanidin-3-O-beta-glucoside, quercetin gentiobioside; hydroxycinnamic acids – gallic, caffeic, chlorogenic; carotenoids – beta-carotene, rubixanthin; triterpenoids – ursolic acid, oleanolic acid, and quinic acids; vitamins (A, C, E, K), and minerals (potassium, calcium, sodium, phosphorus, magnesium) [1, 4, 9-11].

The antibacterial activity of the rose methanolic extract against *Pseudomonas aeruginosa*, *Mycobacterium bovis*, and *Salmonella typhimurium*, as well as its antifungal activity, are associated with phenolic compounds and terpenes [12-15].

The literature sources suggest that acetone extracts have more pronounced antibacterial activity compared to methanolic extracts [14]. *Rosa damascena* has long

been used in traditional medicine as an anti-inflammatory, antispasmodic, antipyretic, tonic, and sedative agent for bronchitis, ulcers, depression, and skin damage [1, 7, 13, 14, 16], as well as for kidney, liver, and biliary tract diseases [17].

Inhalations with the rose oil are used for allergies, headaches, and migraines [7, 18], and the effectiveness of the oil in treating periodontitis and pulpitis has also been proven [19].

The results of the previous studies on the essential oil of *Rosa damascena* grown in *in vitro* conditions indicate its antibacterial effectiveness against enterobacteria (*Escherichia coli* ATCC 25922), gram-negative bacteria that do not ferment glucose (*Pseudomonas aeruginosa* ATCC 27853), gram-positive cocci (*Staphylococcus aureus* ATCC 25923), and *Candida* (*Candida albicans* ATCC 885-653) [20].

The antimicrobial action of the alcoholic extract of rose petals against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) has been demonstrated [21, 22].

The state of war presents a complex and dangerous situation, requiring the search for new approaches to ensure public safety and health. As part of this research, we focused on the comparative assessment of the microbiological properties (microbiological purity, antimicrobial activity) of two essential oils from the genus *Rosa L.*, one of which was produced from the raw material grown in the natural environment of Zaporizhzhia, while the other was obtained through water distillation of rose petals grown in *in vitro* culture via clonal micropropagation.

These oils can also be used to produce medicinal products and be applied in the treatment and prevention of diseases, particularly in conditions of limited resources and insufficient access to medical care. Through clonal micropropagation, it is possible to rapidly obtain a significant amount of the raw material base from the medicinal plant, which will enable the production of sufficient quantities of an essential oil in a short time.

Therefore, a comparative microbiological study of essential oils from representatives of the *Rosa L.* genus obtained using different methods is highly relevant in the context of the potential development of medicinal products based on them.

The **aim** of this work was to conduct a comparative microbiological study of the essential oils from *Rosa damascena* of the “Veselka” (“Rainbow”) variety grown via clonal micropropagation in *in vitro* culture and in the natural environment of Zaporizhzhia, to further substantiate their potential use in medical practice and develop new medicinal products based on them.

Materials and methods. The study was carried out as part of the research project No. 0120U102600 “The search and study of new sources of the medical plant raw material, and creation of substances and medicines based on them” of the Department of Pharmacognosy, Pharmacology, and Botany of Zaporizhzhia State Medical University.

The study objects were essential oils obtained by water distillation [2] from rose petals grown using the method of clonal micropropagation in *in vitro* culture (hereafter referred to as Sample 1) at the Educational and Scientific Medical Laboratory Center with a vivarium, and under natural conditions in Zaporizhzhia (hereafter referred to as Sample 2).

For the introduction into *in vitro* culture, stem parts with buds of the “Veselka” rose variety were used. Explants were introduced into *in vitro* culture from March to May, and they were cultivated on a modified Murashige and Skoog nutrient medium with the addition of 2.0 mg/L 6-benzylaminopurine (6-BAP), 0.2 mg/L indole-3-acetic acid (IAA), and 25.0 mg/L ascorbic acid at an air temperature of 22-24 °C, relative humidity of 65-70 %, and lighting of 2500-3000 lx with a photoperiod of 16 hours. The nutrient medium was sterilized in an autoclave under 0.11 MPa pressure for 25 minutes. The passage duration was 28-30 days. Explants, 0.8-1.2 cm in size with one node, were planted. The clonal micropropagation method for the *Rosa damascena* “Veselka” variety in *in vitro* conditions and its advantages were described in previous works [20].

The study of the antimicrobial activity was conducted at the Microbiological Laboratory of the Department of Microbiology, Virology, and Immunology at Zaporizhzhia State Medical and Pharmaceutical University. The study of the microbiological purity and antimicrobial activity of the rose essential oils (Sample 1 and Sample 2) was carried out *in vitro* in accordance with the existing standards of the 2nd edition of the State Pharmacopoeia of Ukraine and taking into account the existing requirements of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [23]. The microbiological purity (MP) was studied based on such indicators as the total viable aerobic microbial count (TAMC), the total viable yeast and mold count (TYMC), the presence/absence of *S. aureus*, *P. aeruginosa*, *E. coli*, and anaerobic microorganisms. The TAMC and TYMC were determined using the pour plate and double-layer methods. For the pour plate method, dilutions of samples 1:100 and 1:1000 were prepared, and 1 ml was placed into sterile Petri dishes, followed by the addition of 20 ml of molten and cooled (to 45 °C) soy-casein agar (to detect bacteria) or Sabouraud-dextrose agar (to detect yeasts and molds). In the double-layer

method, 1 ml of samples from 1:100 and 1:1000 dilutions were added to tubes containing 4 ml of molten and cooled (to 45 °C) soy-casein agar or Sabouraud-dextrose agar. The contents of each tube were quickly mixed and poured over the surface of the corresponding agar in Petri dishes. After the agar solidified, the plates were incubated at 35 °C for 5 days to detect bacteria and at 25 °C for 7 days to detect fungi. The presence/absence of *S. aureus*, *P. aeruginosa*, and *E. coli* was determined by inoculating 0.1 ml from each dilution of the samples onto the dried surface of the respective nutrient media (egg-yolk salt agar for staphylococcus, Endo medium for *E. coli* and pseudomonads). The thioglycolate medium was used to detect anaerobes and facultative anaerobes, in which 0.1 ml from each sample dilution was inoculated into the medium. The cultures for the detection of staphylococci and anaerobic microorganisms were incubated at 36 °C for 48 hours, while the cultures for enterobacteria and pseudomonads were incubated for 24 hours.

The study of the antimicrobial activity of the oils was conducted using the disk diffusion method. Paper disks (6 mm in diameter) were soaked separately with the essential oil from Sample 1 and Sample 2. The tests used 24-hour cultures of reference test strains from different groups of microorganisms: *E. coli* ATCC 25922 (a gram-negative rod, representative of the Enterobacteriaceae family), *P. aeruginosa* ATCC 27853 (a gram-negative rod that does not ferment glucose), *S. aureus* ATCC 25923 (a gram-positive coccus), and *C. albicans* ATCC 885-653 (a yeast-like fungus from the Candida genus). A bacterial suspension with a density of 0.5 McFarland units (using a DEN 1B densitometer, SIA “Biosan”, Latvia) was prepared from each culture in physiological saline and evenly inoculated over the Mueller-Hinton agar surface using a sterile cotton swab. The surface of the nutrient medium was dried for 5 minutes, after which the soaked disks were placed on the agar, and the cultures were immediately transferred to an incubator. The cultures were incubated at 35±1 °C with *E. coli*, *P. aeruginosa*, and *S. aureus* for 18 hours, and with *C. albicans* for 48 hours. The sensitivity/resistance to the essential oils was determined by the presence/absence of the growth inhibition zones around the disk with the oil. The activity was assessed based on the diameter of the growth inhibition zones around the disk: 0-2 mm – no antimicrobial effect, 3-10 mm – weak antimicrobial effect, 10-20 mm – moderate antimicrobial effect, more than 21 mm – high antimicrobial effect. The experiments were conducted in triplicate. The HiMedia (India) nutrient media were used in the study.

Results and discussion. Testing of the microbiological purity of both essential oil samples showed no microbial growth on the surface or within the nutrient media, indicating the absence of any bacteria (*S. aureus*, *P. aeruginosa*, *E. coli*, and anaerobic microorganisms), as well as yeasts and molds in the samples studied.

The results of the comparative study of the antimicrobial activity demonstrated that the oils tested exhibited the antibacterial and antifungal activity although the

Table

The study of the antimicrobial activity of essential oils

The sample name	The name of the test strain	The diameter of the growth retardation zone, mm			The average value, mm
		Study 1	Study 2	Study 3	
Sample 1	<i>E. coli</i>	21	23	20	21,7
	<i>S. aureus</i>	11	11	12	11,3
	<i>P. aeruginosa</i>	13	14	15	14
	<i>C. albicans</i>	35	32	33	33,3
Sample 2	<i>E. coli</i>	11	12	13	12
	<i>S. aureus</i>	9,5	10	11	10,2
	<i>P. aeruginosa</i>	0	0	0	0
	<i>C. albicans</i>	50	35	35	40

level and spectrum of this activity varied in different experiments. For example, the analysis of the mean diameters of the growth inhibition zones showed that the essential oil of rose (Sample 1) more effectively inhibited the growth of *E. coli* compared to the essential oil from Sample 2 (21.3 mm vs. 12.0 mm, respectively). In experiments with *S. aureus*, both samples exhibited nearly identical bactericidal activity: the mean diameter of the growth inhibition zones in experiments with Sample 1 was 11.3 mm, and with Sample 2, it was 10.2 mm. It was also found that the second sample of the essential oil, unlike the first one obtained by clonal micropropagation, did not inhibit the growth of *P. aeruginosa* (the diameters of the growth inhibition zones were 0 mm and 14 mm, respectively). In the studies with *C. albicans*, the results indicated a high antifungal activity for both essential oils although the mean diameter of the growth inhibition zone in experiments with the first sample of damask rose oil (Sample 1) was less – 33.3 mm compared to 40 mm in experiments with Sample 2 of the essential oil.

According to the results of the study of essential oils obtained from petals of *Rosa damascena* “Veselka” variety (Sample 1 and Sample 2), *Rosa damascena* (Sample 1) can be recommended for further research as a promising plant with the antimicrobial activity.

The analysis of scientific literature shows that recent studies have focused on investigating the antimicrobial and wound-healing properties of plants, particularly *Rosa damascena*, and their potential in treating bacterial infections and accelerating wound healing.

For example, researchers from Ghana have actively studied the antimicrobial activity of essential oils and extracts from several plants cultivated in that country, including *Rosa damascena* [24].

The literature provides data on the antimicrobial properties of an ethanol extract from rose petals against food-borne bacteria [25].

A randomized controlled study demonstrated the effectiveness of the *Rosa damascena* extract in accelerating wound healing [26].

The studies using rat models have shown that the essential oil and extracts of *Rosa damascena* have wound-healing and rapid skin recovery properties, confirming their potential utility in clinical practice [27-30].

Similar results were obtained in experiments on wound treatment using the aqueous extract of *Rosa damascena* in rats [31].

These studies highlight the potential efficacy of rose extracts and oils in combating pathogens of purulent-inflammatory wound infections, which could be significant for the development of new treatments and methods of care.

The results of our study have revealed that the essential oils of *Rosa damascena* “Veselka” variety obtained through clonal micropropagation from the raw material cultivated in natural conditions in Zaporizhzhia possess antimicrobial properties. Both samples have demonstrated a high antifungal activity, which justifies their potential use in treating wounds in patients with candidal infections. Additionally, the samples showed a moderate antistaphylococcal activity. Furthermore, the essential oil obtained through clonal micropropagation exhibited a high antibacterial activity against *Escherichia coli* and a moderate activity against *Pseudomonas aeruginosa*. In contrast, the essential oil obtained from the raw material grown in natural conditions showed a moderate activity against *E. coli*, but lacked bactericidal effects against *P. aeruginosa*. Therefore, we can confidently conclude that the essential oil of *Rosa damascena* obtained through clonal micropropagation possesses a broader spectrum of the antimicrobial activity than the oil from the raw material naturally cultivated. The high content of citronellol, geraniol, as well as the presence of nerol and eugenol [20], contribute to the antimicrobial activity of the essential oil from rose petals grown through clonal micropropagation *in vitro* (Sample 1), particularly against *P. aeruginosa*.

The results of the microbiological purity studies of both samples showed that the essential oils of *Rosa damascena* “Veselka” variety obtained through clonal micropropagation and from the raw material grown in natural conditions in Zaporizhzhia fully complied with the existing standards of the State Pharmacopoeia of Ukraine, 2nd edition.

The results of the study indicate the potential of using the essential oil of *Rosa damascena* “Veselka” variety grown through clonal micropropagation *in vitro* in medicine and pharmacy for developing new drugs against various infectious diseases, particularly for treating purulent-inflammatory wound infections.

Conclusions

1. The essential oil obtained by water distillation of petals from the *Rosa damascena* "Veselka" variety grown through clonal micropropagation *in vitro* exhibits a high antimicrobial activity against *E. coli* and *C. albicans*, and a moderate activity against *S. aureus* and *P. aeruginosa*.

2. The essential oil of *Rosa damascena* from the raw material grown in the natural environment in Zaporizhzhia shows a high antimicrobial activity against *C. albicans*, while a moderate activity against *E. coli* and *S. aureus*. *P. aeruginosa* has been found to be completely resistant to the action of this oil.

3. *Rosa damascena* Mill. "Veselka" variety grown *in vitro* is a promising source of essential oil with the

antimicrobial activity, it is suitable for creating new herbal formulations.

The prospects for further research. The results of this study can serve as a scientific basis for developing medicinal formulations based on the essential oil of *Rosa damascena* Mill. "Veselka" variety grown through clonal micropropagation *in vitro*.

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