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Development of composition and technology for obtaining antimicrobial composition based on mono- and sesquiterpenoids

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The article reflects the results of the essential oils selection for the compositions with antibacterial and antifungal properties. The technology of their production is represented.

The aim of the work was the development of technology and the study structure of new essential oils compositions with antibacterial and antifungal activities.

Material and methods. The following plants have been used in the work: the herb of *Hyssopus ambiguus* (Trautv.) Iljin, the herb of *Thymus crebrifolius* Klokov, the herb of *Thymus marschallianus* Willd, the herb of *Thymus serpyllum* L., and the essential oils obtained from them. The composition of the essential oils was determined by Gas Chromatography Mass Spectrometry. The main physical and chemical parameters of the compositions were evaluated in accordance with the requirements of the Russian State Pharmacopoeia, the XIVth edition. The tests for the antimicrobial activity were carried out using the strains of *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231.

Results. Compositions with an activity against microorganisms *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and fungi *Candida albicans* have been obtained and studied. The composition based on essential oils of *Hyssopus ambiguus* (Trautv.) Iljin and *Thymus marschallianus* Willd. contained 139 components, the main ones of which are eucalyptol (6.51%) and terpinen-4-ol (1.95%). The composition of essential oils of *Hyssopus ambiguus* (Trautv. Iljin) and *Thymus crebrifolius* Klokov contained 137 components (eucalyptol (20.37%), terpinen-4-ol (7.03%), Ţ-muurol (2.28%), γ-terpinene (2.23%), β-myrcene (2.09%), etc.). The composition of the essential oils of *Hyssopus ambiguus* (Trautv.) Iljin and *Thymus serpyllum* L. contained 149 components (the main ones are eucalyptol (7.33%) and α-terpineol (0.9%)).

Conclusion. The technology has been proposed and the structure of the essential oils compositions with antibacterial and antifungal activities has been established.

Keywords: essential oils; isoprenoids; essential oils composition; technology; antibacterial and antifungal activities

Abbreviations: SP – State Pharmacopoeia; RK – Republic of Kazakhstan; RF – Russian Federation; BAS – biologically active substances; MPRMs – medicinal plant raw materials; EOs – essential oils; IgA – immunoglobulin A; PhM – pharmacopoeial monograph; GPhM – general pharmacopoeial monograph.

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Разработка состава и технологии получения антимикробной композиции на основе моно- и сесквитерпеноидов

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В статье отражены результаты подбора эфирных масел для композиций с антибактериальными и противогрибковыми свойствами. Приведена технология их получения.

Цель. Разработка технологии и изучение состава новых композиций эфирных масел, обладающих антибактериальной и противогрибковой активностью.

Материал и методы. В работе использованы: трава иссопа сомнительного (*Hyssopus ambiguus* (Trautv.) Iljin), трава тимьяна частолистого (*Thymus crebrifolius* Klokov), трава тимьяна Маршалла (*Thymus marschallianus* Willd.), трава тимьяна ползучего (*Thymus serpyllum* L.), и полученные из них эфирные масла. Состав эфирных масел определяли методом газовой хроматографии с масс-спектральной детекцией. Основные физико-химические показатели композиций оценивали согласно требованиям Государственной фармакопеи Российской Федерации XIV изд. Испытания на антимикробную активность проводили с применением штаммов *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231.

Результаты. Получены и исследованы композиции, обладающие активностью в отношении микроорганизмов *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* и грибков *Candida albicans*. Композиция на основе эфирных масел иссопа сомнительного и тимьяна Маршалла содержала 139 компонентов, из которых основные – эвкалиптол (6,51%) и терпинен-4-ол (1,95%). Композиция из эфирных масел иссопа сомнительного и тимьяна частолистого содержала 137 компонентов (эвкалиптол (20,37%), терпинен-4-ол (7,03%), Ţ-муурол (2,28%), γ-терпинен (2,23%), β-мирцен (2,09%) и др.). Композиция из эфирных масел иссопа сомнительного и тимьяна ползучего содержала 149 компонентов (основные – эвкалиптол (7,33%) и α-терпинеол (0,9%)).

Заключение. Предложена технология и установлен состав эфирномасличных композиций, обладающих антибактериальной и противогрибковой активностью.

Ключевые слова: эфирные масла; изопреноиды; эфирномасличная композиция; технология; антибактериальная и противогрибковая активность

Список сокращений: ГФ – Государственная фармакопея; РК – Республика Казахстан; РФ – Российская Федерация; БАВ – биологически активные вещества; ЛРС – лекарственное растительное сырье; ЛП – лекарственный препарат; ЭМ – эфирные масла; IgA – иммуноглобулин А; ФС – фармакопейная статья; ОФС – общая фармакопейная статья.

INTRODUCTION

To date, one of the actively developing scientific areas is the development of new medicinal drugs (MDs) intended for the treatment and prevention of the upper respiratory tract infections, using innovative technologies and based on natural biologically active substances (BASs) of the plant origin. The essential oils (EOs), complex multicomponent mixtures consisting mainly of isoprenoids, are known to have a wide range of pharmacological activities, in particular, they are able to effectively inhibit the development of pathogenic microflora, and therefore they are of great interest for the development of new drugs [1–4].

Mono- and sesquiterpenoids are the main part of EOs; oxygen-containing derivatives are the rest. Due to the content of phenols, terpenes, alcohols, aldehydes and flavonoids, EOs are able to have bactericidal and bacteriostatic effects, an antiviral effect, and exhibit an antifungal activity. EOs have an immunomodulatory effect, strengthen cell membranes, and increase the antioxidant activity of blood plasma [5–8].

Numerous studies by domestic and foreign scientists prove the effectiveness of a number of EOs in relation to viral, bacterial and fungal agents. Antiviral and antimicrobial effects, as well as a restorative effect on the protective barriers of the upper respiratory tract, have biologically active substances of the representatives of the following families: *Adoxaceae, Acoraceae*, *Amaryllidaceae, Asteraceae*, *Betulaceae*, *Fabaceae*, *Ericaceae*, *Hypericaceae, Salicaceae*, *Zingiberaceae*, *Onagraceae*, *Cupressaceae*, *Alliaceae*, *Myrtaceae*, *Parmeliaceae*, *Rosaceae*, *Polemoniaceae*, *Pinaceae*, *Lamiaceae,* etc. [9–12].

The components of the EOs that exhibit antiviral and antibacterial actions are phenolic compounds (thymol, carvacrol, eugenol, etc.), terpene alcohols (linalool, geraniol, and menthol), aldehydes (neral, geranial, and citronellal), ketones (thujone, fenchon, carvone, pinocamphone, camphor, menthon, etc.), esters (anethole, estragol and others) [13–16].

The use of EOs and the drugs based on them in respiratory infections has a great potential and requires further research due to the problem of their increasing antibiotic resistance, because they are able to exert an antimicrobial effect without causing the development of resistance in microorganisms, unlike antibiotics and antiviral chemotherapy drugs [17].

The EOs inhalation is one of the effective methods for preventing colds [18-21]. They do not cause any resistance; have expectorant and secretolytic effects; increase the production of immunoglobulin A (IgA), which helps to increase immunity; favorably affect the symptoms of SARS; increase the activity of the ciliary epithelium of the nasal cavity [22].

In order to develop a new medicinal product with antimicrobial properties, combinations of the EOs obtained from MPRMs of the *Lamiaceae* plants growing on the territory of the Republic of Kazakhstan (RK), i. e, *Hyssopus ambiguous* (Trautv.) Iljin, *Thymus marschallianus* Willd., *Thymus serpyllum L.* and *Thymus crebrifolius* Klok. were studied.

THE AIM of the work was the development of technology and the study structure of the new essential oils compositions with antibacterial and antifungal activities.

MATERIALS AND METHODS

Objects of study

The objects of the study were the EOs compositions, made up on the basis of the results of microbiological studies in compliance with the rules for compiling EOs compositions in aromatherapy. The samples of the MPRMs were collected during the flowering period. It is during this period that the EOs of these plants have the most suitable qualitative and quantitative components composition. In all the cases, the areal part was used.

The confirmation of the species affiliation of producing plants was carried out with the participation of Professor of the Department of Botany M.Yu. Ishmuratova; the plant samples were deposited in the herbarium of the Faculty of Biology and Geography of Karaganda University named after E.A. Buketov (Karaganda, RK). Previously, MPRMs passed a radiation control and a test for heavy metals. When studying these species, for the first time, a morphological and histochemical study of the herb of *Hyssopus ambiguus* (Trautv.) Iljin) was carried out [23].

Methods for obtaining EOs

EOs were obtained in a setup consisting of a reaction round-bottomed flask with a capacity of 1 dm^3, a bent steam pipe, a refrigerator, a receiver with a drain cock, and a drain pipe. About 15-20 g of raw materials were placed in a flask, 300 ml of water was added, the flask was connected through a thin section to a steam pipe and filled with water through a tap using a hose with a funnel. The contents of the flask were heated and boiled at the intensity at which the distillate flow rate was 60–65 drops per 1 min for 2 h. The EOs volume was measured 5 min after the end of the distillation. The percentage content was determined by the volumetric method. For each name of the medicinal plant, the production of EOs was carried out separately and until at least 10 ml of each studied object was obtained, that was sufficient to study the physical and chemical properties and the production of samples of the studied compositions. The output of EOs for each of the used types of plant materials is presented in Table 2.

The EOs samples were dissolved in n-hexane (50 ml); the solution was kept with anhydrous magnesium sulfate for 1 h, then filtered through filter paper and evaporated under vacuum on a Stegler XD-52AA rotary evaporator (Russia) until the organic solvent was completely removed.

Determination of the component composition of essential oils compositions

The following essential oils compositions were used in the work: composition 1 “hyssop dubious+Marshall’s thyme”, composition 2 “hyssop dubious+Mother-of-thyme” and composition 3 “hyssop dubious+creeping thyme”.

The component of the EOs compositions was determined by GC-MS according to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan

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(SP RK, Vol. I (2.2.28))[1]. For the analysis, an Agilent-7890A chromatograph (Agilent Technologies, USA) with a capillary column HP-5 ms 30 m×0.25 mm (film thickness 0.25 µm) and in combination with a selective mass spectral detector 5975C was used. The results obtained were processed using the Agilent ChemStation software and the EOs components were identified using the NIST-2017 mass spectrum library.

The EOs in the amount of 150 µl was dissolved in 800 µl of the solvent (chloroform for composition 1 and 70% ethyl alcohol for compositions 2 and 3) and stirred until the oil was completely dissolved. The analysis was carried out using a temperature program: starting from +70°C for 2 min, then raising the temperature at the rate of 20°C/min to +270°C (holding for 30 min). The carrier gas was helium. The injector temperature was +250°C; the detector temperature was +230°C. Mass spectra were recorded using an ionization energy of 70 eV and a separation temperature of +280°C; the acquisition mass range was M/z 10–650.

Study of antimicrobial activity of essential oils compositions

The analysis of the EOs antimicrobial activity, compositions and results was carried out at the Department of Biomedicine in the microbiological laboratory of Medical University of Karaganda (RK). The following materials were used in the work: Chistovich's nutrient media, meat-peptone agar, Endo's medium, Sabouraud's medium, blood agar: museum culture strains of *Staphylococcus aureus, Bacillus subtilis, Ecsherichia coli, Candida albicans;* the disks manufactured by Bioanalyse Limited (Turkey) impregnated with benzylpenicillin sodium salt (1 U/disk), ampicillin trihydrate (25 µg/disk), nystatin (100 µg/disk) and fluconazole (10 µg/disk) to determine the sensitivity of microorganisms to antibiotics; a ruler-template for measuring the zones size of the growth inhibition of microorganisms (PW096), size – 370×65 mm (HiMedia Laboratories Pvt. Limited, India); a densimeter to determine the concentration of culture in saline.

An antimicrobial activity was determined in accordance with the requirements of the SPh of the Republic of Kazakhstan according to the method for determining the sensitivity to antibiotics by the microbiological method, i.e., the agar diffusion method[2].

For the cultivation of *Staphylococcus aureus*, Chistovich's medium was used; for *Escherihia coli*, it was Endo's medium; for *Bacillus subtilis*, it was meat-peptone broth; for *Candida albicans* - Saburo's medium was used. According to the methods used, daily cultures diluted in saline were used. Filter paper discs impregnated with an undiluted essential oil composition were placed on one segment of the Petri dish; the discs with the control preparation (benzylpenicillin sodium salt, nystatin) were placed on the other. Taking into account that medicinal products containing the studied EOs in their composition are not represented on the market of the RK, it was decided to use a cosmetic product with antimicrobial properties as a comparison drug, i. e, the "Breathe"/"Dyshi" spray (ser. 061022, manufacturer JSC "Akvion", Russia).

Petri dishes with bacterial cultures were placed in a thermostat for 24 h and incubated; for bacteria, the temperature regime was +36(±1)°C, for fungi of the genus *Candida* – +28°C. The zones of the microorganism growth absence were determined with a special ruler-template. The diameters of the growth inhibition zones less than 10 mm and a continuous growth in the cup were evaluated as the absence of the antimicrobial activity, 10–15 mm – as a weak activity, 15–20 mm – as a moderately pronounced activity, more than 20 mm – a pronounced one.

Standardization of resulting essential oils compositions

The above essential oils compositions were tested in accordance with the requirements of the Russian State Pharmacopoeia XIV edition (hereinafter SP RF XIV ed.), GPhM.1.5.2.0001.15 Essential oils[3].

Description. The essential oils compositions were placed in a transparent glass cylinder with a diameter of 3 cm, the mobility of the liquid and their color were determined in the transmitted diffused daylight. The odor determination was as follows: about 0.1 ml (2 drops) of the EOs composition was applied to a strip of filter paper 12 cm long and 5 cm wide so that the oil did not wet the edges of the paper, then the odor was evaluated every 15 minutes.

Authenticity. Its determination was carried out by high-performance gas chromatography in accordance with the requirements of GPhM.1.2.1.2.0004.15 Gas chromatography (see "Determination of the component composition of essential oils compositions"). To establish the authenticity of essential oils compositions, relative retention times of the predominant and specific components were used: for composition 1, eucalyptol and terpinen-4-ol; for composition 2, eucalyptol, terpinen-4-ol, Ʈ-muurolol, ɣ-terpinene, β-myrcene; for composition 3, eucalyptol and α-terpineol.

[1] State Pharmacopoeia of the Republic of Kazakhstan. Vol. 1; Almaty: Zhibek Zholy Publishing House; 2008, 592 p. Available from: https://pharmacopoeia.ru/wp-content/uploads/2017/01/Gosudarstvennaya-farmakopeya-Respubliki-Kazahstan-PDF.pdf

[2] Ibid.

[3] Russian State Pharmacopoeia XIV edition. T. 1–4; Moscow: FEMB, 2018. Available from: https://femb.ru/record/pharmacopea14

Solubility. The essential oils compositions obtained are insoluble in water. The solubility of the essential oils compositions in hexane, chloroform and dimestyl sulfoxide were also tested.

To determine the solubility of an EOs composition in 70% ethyl alcohol, 1 ml of the essential oil was placed in a 25 ml test tube with a friction-fitted lid. The determination was carried out at the temperature of 20±2°C. Until the oil was completely dissolved, alcohol was added in portions of 0.1 ml with frequent vigorous stirring. The volume of the alcohol consumed to obtain a clear solution was recorded. Then the alcohol was added in portions of 0.5 ml with vigorous stirring until the total volume of alcohol added was 20 ml.

If the solution became cloudy or opalescent from the addition of 20 ml of the solvent, then its volume was recorded at the point of appearance of the first signs of opalescence, and the volume of alcohol at which the turbidity or opalescence disappeared. If a clear solution did not form after the addition of 20 ml of alcohol, the test was repeated using 90% ethyl alcohol.

Ethyl alcohol. 1) 2 drops of the EOs composition were added to a few drops of water placed on a watch glass. The composition passed the test if there was no noticeable haze around the EOs droplets on the black background.

2) 1 ml of the EOs composition was poured into a test tube, closed with a loose piece of cotton wool, in the middle of which a crystal of basic fuchsin was placed, then heated to a boil in a water bath. The composition passed the test if the cotton wool did not turn purple-pink.

Fatty and mineral oils. 1) 1 ml of the EOs composition was shaken in a 20 ml test tube with 10 ml of 96% ethyl alcohol. The composition passed the test if cloudiness and oily droplets were not observed.

2) 0.05 ml of the tested essential oils composition was placed on filter paper. The oil stain completely evaporated from the paper within 24 h without a trace.

Essential oil residue after evaporation. About 5 g of the EOs composition was placed in a pre-weighed cup 8 cm in diameter. The cup had been heated in a boiling water bath until the oil completely evaporated, then cooled in a desiccator over anhydrous calcium chloride and weighed. The water level in the bath throughout the experiment was 1–20 mm below the bottom of the evaporating dish.

Water. 0.5 ml of the EO was mixed with 10 ml of petroleum ether. The composition passed the test if no haze was observed.

Density. The test was carried out using an automatic viscometer brand SVM 3000 Stabinger (Austria) in accordance with GPhM.1.2.1.0014.15 Density.

Optical rotation. The test was carried out on an AP-300 polarimeter from ATAGO CO, LTD (Japan) in accordance with GPhM.1.2.1.0018.15 Polarimetry.

Refractive index. The test was carried out on an IRF-454 B2M refractometer manufactured by OAO KOMZ (Tatarastan) in accordance with GPhM.1.2.1.0017.15 Refractometry.

Acid number. The number of milligrams of potassium hydroxide, which is necessary to neutralize the free acids in 1 g of the essential oil, was determined in 2 g (accurately weighed) of the oil dissolved in 5 ml of 95% ethyl alcohol, previously neutralized with phenolphthalein.

Quantitation. The GC-MS method was used to determine the contents of the main components of essential oils compositions: for composition 1, eucalyptol and terpinen-4-ol; for composition 2, eucalyptol, terpinen-4-ol, Ţ-muurolol, ɣ-terpinene, β-myrcene; for composition 3, eucalyptol and α-terpineol.

Statistical processing. Statistical processing of the results was carried out using the STATISTICA 12.6 program (StatSoft, USA). Variation series were tested for a normal distribution using the Kolmogorov-Smirnov test, the significance level in this study was taken as p=0.05. For all the groups, the mean and standard error of the mean were calculated. Student's t-test was used to assess intergroup differences.

RESULTS AND DISCUSSION

EOs selected as components of essential oils compositions were tested for an antimicrobial activity.

Screening of the antimicrobial activity of EOs of *Hyssopus ambiguus* (Trautv.) Iljin, *Thymus marschallianus* Willd., *Thymus crebrifolius* Klokov and *Thymus serpyllum* L. made it possible to obtain the following data presented in Table 3. To confirm the presence or absence of growth of microorganisms, Petri dishes with crops were viewed in the transmitted light.

As can be seen from the data in Table 3, the most pronounced antibacterial activity was shown by *Hyssopus ambiguous* (Trautv.) Iljin EOs, while the analysis of *Thymus marschallianus* Willd EOs, *Thymus crebrifolius* Klokov and *Thymus serpyllum* L. showed a higher antifungal activity. Taking into account the data obtained, it was decided to combine the EOs of the investigated thyme species (in all compositions, the EOs of *Hyssopus ambiguous* (Trautv.) Iljin was present). Thus, three different essential oils compositions have been obtained: composition 1 "*Hyssopus ambiguous* (Trautv.) Iljin+*Thymus marschallianus* Willd", composition 2 "*Hyssopus ambiguous (*Trautv.*)* Iljin+*Thymus crebrifolius* Klokov" and composition 3 "*Hyssopus ambiguous (*Trautv.*)* Iljin+*Thymus serpyllum* L.)".

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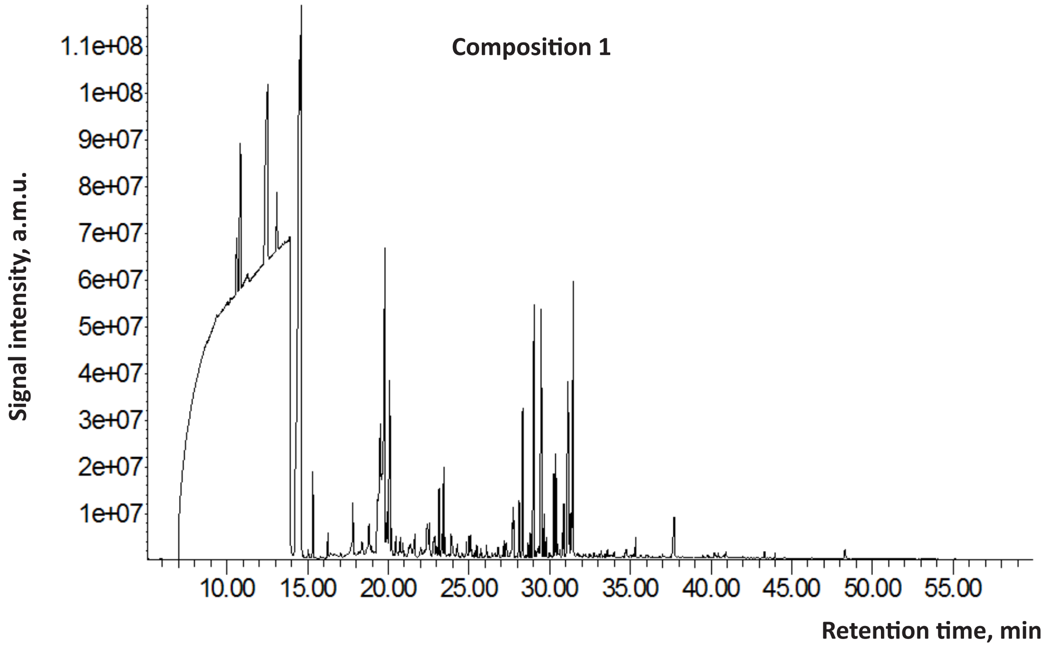

Figure 1 – Chromatogram of essential oils composition 1

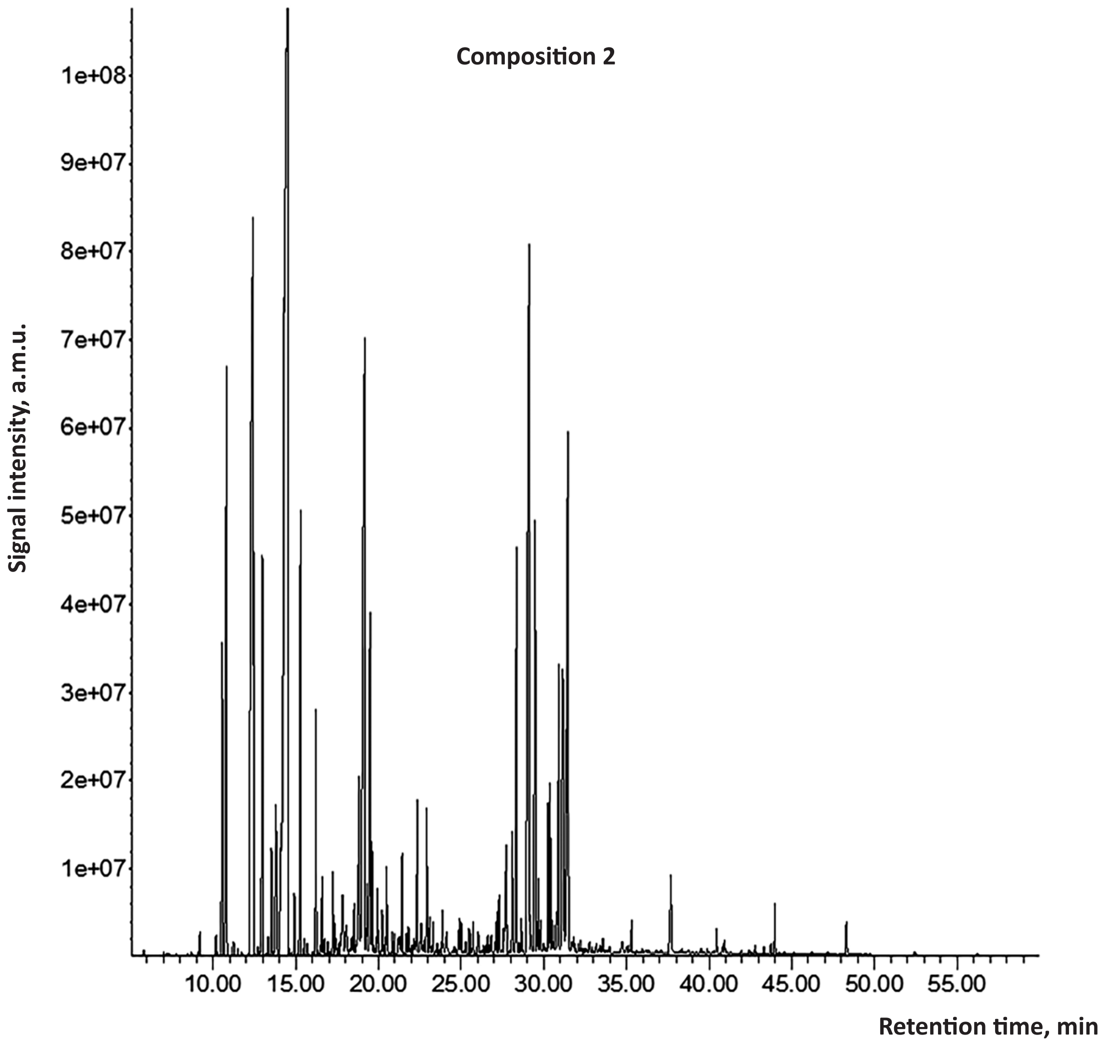

Figure 2 – Chromatogram of essential oils composition 2

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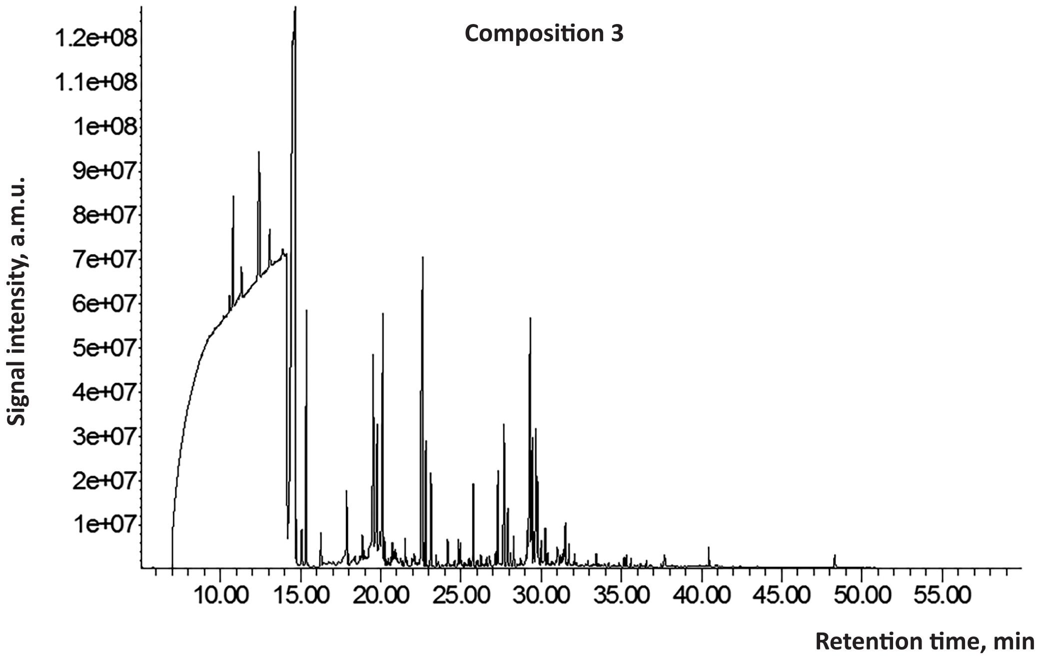

Figure 3 – Chromatogram of essential oils composition 3

Table 1 – Characteristics of MPRMs used to obtain EOs

Name of the producing plant (Russian and Latin)	Gathering place	Geographical coordinates	Collection time, development phase
Hyssopus ambiguous (Trautv.) Iljin	Sorting stlm., Karaganda region, RK	49.970554°N 73.2226789° E	September 2021, flowering phase
Hyssopus ambiguous (Trautv.) Iljin	Karkalinsk suburbs Karaganda region, RK	49.412951° N, 75.477275° E	August 2021, flowering phase
Thymus marschallianus Willd.		49.412951° N, 75.477275° E	June 2020
Thymus serpyllum L.		49.412951° N, 75.477275 E	Flowering phase
Thymus crebrifolius Klok.	Surroundings of the Ulytau mountains, Karaganda region, RK	48.652828° N, 67.025743° E	June 2020

Note: MPRMs – medicinal plant raw materials, EOs – essential oils, RK – Republic of Kazakhstan, stlm. – settlement, N – north latitude, E – east longitude.

Table 2 – Output of EMs in used MPRMs

Name of producing plant (Russian)	Amount (g) of MPRMs to obtain 1 ml of EO	EOs output, %
Hyssop dubious (Karkaralinsk)	65.5	0.40
Hyssop dubious (Sorting)	147	0.60
Marshall's thyme (Karkaralinsk)	197.5	0.50
Mother-of-thyme (Ulytau)	79.5	1.13
Creeping thyme (Karkaralinsk)	201.0	0.40

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Table 3 – Antimicrobial activity of EOs collected on territory of Karaganda region

Name of the producing plant (Russian)	Microorganisms growth inhibition zone, mm			
	Staphylococcus aureus ATCC 6538	*Bacillus subtilis* ATCC 6633	*Escherihia coli* ATCC 25922	*Candida albicans* ATCC 10231
Hyssopus ambiguous (Trautv.) Iljin (Karkaralinsk)	33.0±7.0	33.7±4.9	12.5±2.1	9.7±2.1
Hyssopus ambiguous (Trautv.) Iljin (Sorting)	39.0±3.0	25.5±2.6	10.0±3.0	7.5±0.6
Thymus marschallianus Willd (Karkaralinsk)	7.8±1.5	9.0±3.0	10.7±5.0	22.7±10.8
Thymus crebrifolius Klokov (Ulytau)	7.0±0.8	8.5±2.1	10.2±3.2	9.8±4.3
Thymus serpyllum L. (Karkaralinsk)	13.3±1.7	14.5±0.7	11.0±2.8	13.0±4.1

Table 4 – Main components in essential oils composition 1

Retention index	Retention time, min	Component	Content, %
1022±5 (394)	14.4452	Eucalyptol	6.51
1164±5 (512)	18.9980	Terpinen-4-ol	1.95
1175±5 (540)	19.4381	α-Terpineol	0.61
1086±3 (646)	16.5737	Linalool	0.29
1592±4 (15)	30.3548	β-Oplopenone	0.22
1050±4 (484)	17.2230	ɤ-terpinene	0.21
1472±6 (181)	27.1513	ɤ-Tsuurolen	0.13
1270±5 (28)	22.2448	ρ-Cymen-7-ol	0.12
1494±5 (178)	27.7718	α-Muurolen	0.12
1376±4 (377)	24.5755	Kopaen	0.13
1477±5 (364)	27.2812	Germacren D	0.08
1531±5 (59)	28.7747	α-Kalakorn	0.06
1382±5 (186)	24.8280	(-)-β-Bourbonene	0.05
1419±5 (656)	25.7227	caryophyllene	0.04
1372±4 (94)	25.2753	Methyleugenol	0.02
1451±5 (468)	26.5884	Humulen	0.02
1447±8 (2)	30.8455	Aromadendren	0.01

Table 5 – Main components of essential oils composition 2

Retention index	Retention time, min	Component	Content, %
1022±5 (394)	14.4452	Eucalyptol	20.34
1164±5 (512)	18.9980	Terpinen-4-ol	7.03
1632±5 (119)	31.1125	Ʈ-muurolol	2.28
1050±4 (484)	17.2230	ɤ-terpinene	2.23
983±3 (580)	12.9299	β-Myrcene	2.09
1592±4 (15)	30.3548	β-Oplopenone	0.70
1586±11 (64)	30.2250	Ledol	0.65
1025±7 (52)	14.0555	o-Cymen	0.63
1494±5 (178)	27.7718	α-Muurolen	0.41
1131±9 (76)	17.9879	trans-verbenol	0.33
1086±3 (646)	16.5737	Linalool	0.31
1477±5 (364)	27.2812	Germacren D	0.27
1419±5 (656)	25.7227	caryophyllene	0.22
1164±N/A (1)	18.5146	Pinocarvone	0.33
1472±6 (181)	27.1513	ɤ-Muurolen	0.16
1104±5 (66)	17.3818	α-Campholenal	0.14
1460±6 (178)	26.7760	Alloaromadendren	0.14
998±4 (356)	13.2979	α-Phelandrene	0.12
1241±4 (13)	21.6748	Citral	0.12
860±6 (210)	9.1924	ρ-Xylene	0.10
1270±5 (28)	22.2448	ρ-Cymen-7-ol	0.10
1382±5 (186)	24.8280	(-)-β-Bourbonene	0.10
1222±10 (18)	20.9317	D-Carvone	0.09
1454±6 (7)	27.5626	cis-Muurola-4(15),5-diene	0.08
1372±4 (94)	25.2753	Methyleugenol	0.07
946±5 (423)	11.2560	camphene	0.06
1185±7 (2)	19.7194	(-)-trans-Isopiperithenol	0.05
1376±4 (377)	24.5755	Kopaen	0.05
1038±3 (356)	14.5678	trans-β-ocimene	0.04
1531±5 (59)	28.7747	α-Kalakorn	0.04
1211±3 (146)	20.4917	Citronellol	0.035
1447±8 (2)	30.8455	Aromadendren	0.03
1089±5 (52)	17.0787	thuyon	0.02

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Table 6 – Main components of essential oils composition 3

Retention index	Retention time, min	Component	Content, %
1022±5 (394)	14.4452	Eucalyptol	7.33
1175±5 (540)	19.4381	α-Terpineol	0.90
1164±5 (512)	18.9980	Terpinen-4-ol	0.54
1086±3 (646)	16.5737	Linalool	0.35
1477±5 (364)	27.2812	Germacren D	0.26
1419±5 (656)	25.7227	caryophyllene	0.22
1500±5 (196)	27.9160	β-Bisabolene	0.17
1037±4 (15)	14.8997	β-ocimene	0.15
1038±3 (356)	14.5678	trans-β-ocimene	0.11
1401±8 (51)	29.7487	Longifolen-(V4)	0.09
1577±N/A (1)	30.9898	(-)-Spatulenol	0.07
1335±8 (207)	24.0486	Eugenol	0.06
1472±6 (181)	27.1513	ɤ-Muurolen	0.06
1632±5 (119)	31.1125	Ţ-Muurol	0.06
1270±5 (28)	22.2448	ρ-Cymen-7-ol	0.05
1222±10 (18)	20.9317	D-Carvone	0.04
1447±8 (2)	30.8455	Aromadendren	0.04
1376±4 (377)	24.5755	Kopaen	0.03
1460±6 (178)	26.7760	Alloaromadendren	0.03
1170±N/A (1)	20.5492	D-Verbenone	0.02
1370±6 (83)	24.4672	Ulangen	0.02
1372±4 (94)	25.2753	Methyleugenol	0.02
1401±8 (51)	29.7487	Longifolene	0.01

Table 7 – Antimicrobial activity of essential oils compositions

Name of the composition / comparison preparation	Zone of growth inhibition of microorganisms, mm			
	Staphylococcus aureus ATCC 6538	*Bacillus subtilis* ATCC 6633	*Escherihia coli* ATCC 25922	*Candida albicans* ATCC 10231
Composition 1	28.7±2.3	21.7±2.1	9.7±2.3	16.0±10.4
Composition 2	27.7±4.9	25.0±14.8	9.0±1.0	18.0±10.1
Composition 3	27.5±0.7	19.5±3.5	9.5±2.1	10.0±1.4
"Breathe" / "Dyshi" spray	7.4±0.9	8.5±3.0	16.25±2.2	7.7±0.6
Benzylpenicillin sodium salt	26.2±5.2	31.2±8.5	11.0±1.7	–
Ampicillin trihydrate	27.0±2.1	30.2±23.0	18.2±2.8	–
Nystatin	–	–	–	19.8±3.9

Note: "–" – There is no growth inhibition zone.

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Table 8 - Indicators of standardization of essential oils compositions

Quality indicator	GPhM.1.5.2.0001.15 Essential oils	Test results		
		Composition 1	Composition 2	Composition 3
Description	Colorless or tinted transparent movable liquid, often yellowish in color, with a characteristic odor; lighter than water.	Mobile liquid with a characteristic odor, light orange in color; lighter than water.	Mobile liquid with a characteristic odor, light orange in color; lighter than water.	Mobile liquid with a characteristic odor, light orange in color; lighter than water.
Authenticity	The chromatogram of the test solutions of the compositions should contain peaks of essential oil components to be determined.	The relative retention times of the peaks on the chromatogram of the test solution corresponded to those of eucalyptol and terpinen-4-ol.	The relative retention times of the peaks on the chromatogram of the test solution corresponded to those of eucalyptol, terpinen-4-ol, Ţ-muurolol, ɣ-terpinene and β-myrcene.	The relative retention times of the peaks in the chromatogram of the test solution corresponded to those of eucalyptol and α-terpineol.
Solubility	Slightly soluble, very little soluble or practically insoluble in water; easily soluble or soluble in alcohol of various concentrations, ether and other organic solvents.	Insoluble in water. With ethyl alcohol it formed a white precipitate, which disappeared when 18 ml of 70% ethyl alcohol was added. When the essential oil composition was dissolved in hexane, fatty drops formed at the bottom, it was completely dissolved in chloroform, and when dissolved in dimethyl sulfoxide, a gelatinous precipitate appeared.	Not soluble in water. Completely dissolved in 70% ethyl alcohol already with the addition of 1 ml of alcohol. When dissolved in hexane, a cloudy solution was formed; when dissolved in chloroform, the oily solution was formed; when dissolved in dimethyl sulfoxide, a white precipitate was formed.	Not soluble in water. Completely dissolved in 70% ethyl alcohol already with the addition of 1 ml of ethyl alcohol. When dissolved in hexane, oily droplets formed at the bottom; with chloroform, an oily solution; when dissolved in dimethyl sulfoxide – a white precipitate.
Ethyl alcohol	Method 1 – there should be no turbidity around the drops of the essential oil composition Method 2 – there should be no purple-pink staining of cotton wool.	It does not give haze by Method 1 and does not give purple-pink staining by Method 2.		
Fatty and mineral oils in essential oils	There should be no turbidity of the solution and the formation of greasy drops.	Turbidity was not observed, fatty drops were not formed.	Turbidity was not observed, fatty drops were not formed.	Turbidity was not observed, fatty drops were not formed.
Residue of the essential oil after evaporation.	Norms have not been established.	1.35%	1.01%	1.60%
Density	Norms have not been established.	0.900 g/cm³	0.901 g/cm³	0.910 g/cm³
Optical rotation	Standards have not been established.	+0.01°	+0.01°	+0.01°
Refractive index	No standards have been set.	1.3800	1.5400	1.4800
Acid number	It should not exceed 4	1.02	1.03	1.00
Quantification	Norms have not been established.	Eucalyptol content – 6.51%; terpinen-4-ol – 1.95%	Eucalyptol content – 20.34%; terpinen-4-ol – 7.03%, Ţ-muurolol – 2.28%, ɣ-terpinene – 2.23% and β-myrcene – 2.09%.	Eucalyptol content – 7.33%; α-terpineol – 0.90%.

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An important factor in the preparation of essential oils compositions was the synergism of the EOs included in the composition. The *Hyssopus ambiguous* (Trautv.) Iljin oil, which has a medium volatility, is combined with the thymes EOs, which have a high volatility. When developing the compositions, attention has also been paid to the smell of the final composition, because *Hyssopus ambiguous* (Trautv.) Iljin EOs in its pure form has a rather pungent odor due to a high content of eucalyptol, while the EOs of the thyme species collected had a less pungent odor, which in total could correct the final smell of the composition [24]. The odors of the obtained essential oils compositions were evaluated subjectively. All the presented EOs compositions were mobile liquids from light yellow to light orange color with a pleasant characteristic odor.

The developed compositions were tested for an antimicrobial activity. According to the results of the study, only the samples that showed a pronounced activity against at least one of the studied strains of microorganisms were selected.

After screening for antimicrobial and antifungal activities, which made it possible to make a choice in favor of the most promising composition, their component composition was studied by GC-MS.

Fig. 1 shows the chromatogram of composition 1.

Table 4 shows the main components found in essential oils composition 1.

The predominant components of composition 1 were eucalyptol, terpinen-4-ol and α-terpineol.

Fig. 2 shows the chromatogram of essential oils composition 2.

In composition 2, 137 components were found, among which eucalyptol, terpinen-4-ol, Ʈ-muurolol, ɤ-terpinene, and β-myrcene predominated.

Table 5 shows the main components of essential oils composition 2.

Figure 3 shows the chromatogram of essential oils composition 3.

In composition 3, 149 components were found, among which eucalyptol and α-terpineol predominated.

Table 6 shows the main components of essential oils composition 3.

The study results of the antimicrobial activity of the developed compositions are presented in Table 7.

As can be seen from the data presented in the table, the EOs compositions developed had pronounced antibacterial and significant antifungal activities.

The carried out analysis of the chemical composition made it possible to characterize the dominant components:

Eucalyptol (1,8-cineol), a monoterpenoid, which is a colorless liquid, insoluble in water. By its structure, it is a cyclic ether. It is part of the EOs of a number of plants, for example, many species of eucalyptus. Eucalyptol shows a pronounced activity against *Enterococcus faecalis* ATCC 29212; a moderate activity against *Pseudomonas aeruginosa* ATCC 27853. Eucalyptol is highly active against *Botrytis cinerea, Colletotrichum acutatum, Aspergillus flavus* ATCC 22546; moreover, the level of its antimicrobial activity significantly depends on the components of the EO which are in synergy with eucalyptol [25, 26].

Terpinen-4-ol[4] is a monoterpenoid, insoluble in water. It has a sharp citrus scent. Terpinen-4-ol has strong antibacterial and antifungal properties. The literature studies have shown that terpinen-4-ol induces antitumor effects by selectively causing the death of necrotic melanoma cells. Terpinen-4-ol is highly active against biofilms formed by *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis, and Fusobacterium nucleatum* [26].

Ʈ-Muurol is a sesquiterpenoid that is practically insoluble in water. It has a slight spicy smell and a herbal taste. It exhibits an antifungal activity against *Trametes versicolor* BCRC 35253, *Gloeophyllum trabeum* BCRC 31614, *Laetiporus sulphureus* BCRC 35305, *Lenzites betulinus* BCRC 35296 [26].

γ-Terpinene is a branched unsaturated hydrocarbon, it is an isoprenoid. It is present in all eukaryotic organisms. It has a "herbal" smell and a bitter taste. It has antioxidant, anti-inflammatory and antiproliferative properties [26].

β-Myrcene is an acyclic monoterpene, an important component of the EOs of a number of plants. It has a pleasant smell and taste. It has analgesic, anti-inflammatory, antibacterial and antimutagenic activities [26, 27].

α-Terpineol is a monoterpene alcohol that smells like lilac. It has anti-inflammatory, antitumor properties. It is active against the strains of *Serratia liquefaciens, Cordulephya divergens, Listeria innocua* and *Salmonella Typhimurium,* and has an antioxidant activity [26, 28].

As a result of the study, compositions have been determined and optimal technological methods for the manufacture of essential oils compositions from the medicinal plant raw materials, common in the RK, the plants with antimicrobial properties, have been proposed. In the course of testing the obtained compositions, it was found that the developed compositions correspond to the basic physicochemical and technological requirements for essential oils of the SP RF XIV ed. The results are presented in Table 8.

CONCLUSION

In the course of complex studies, the samples of the EOs obtained from the medicinal plant raw materials, representatives of the *Lamiaceae* family of the RK, screening of their antimicrobial activity was carried out. Taking into account the chemical composition and activity of the obtained samples, 3 essential oil compositions with pronounced

[4] Merck KGaA, Darmstadt. Available from: https://www.sigmaaldrich.com/KZ/en/product/aldrich/223190.

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antibacterial and significant antifungal effects, have been developed.

The developed essential oils compositions have a number of features, in particular:

1. A complex chemical composition, including isoprenoids of a plant origin; it prevents the development of resistance in microorganisms and fungi, and may be an advantage of the developed compositions over synthetic analogues of antibacterial and antifungal activities.

2. The revealed chemical structure of essential oils compositions provides a wide range and severity of antibacterial and antifungal actions compared to the reference drugs - benzylpenicillin sodium salt, ampicillin trihydrate, nystatin and essential oils composition – the “Breathe”/“Dyshi” (spray). The developed compositions, as simultaneously possessing antibacterial and antimicrobial activities, can be considered as promising objects for the creation of new drugs based on them, intended for the treatment and prevention of diseases of the upper respiratory tract. Essential oils compositions can be administered by passive inhalation, which is characterized by ease of use and safety.

3. The development and further study of compositions based on the EOs from the MPRMs growing in the territory of the Republic of Kazakhstan allows the development of localized production of high-quality and relatively inexpensive therapeutic and prophylactic agents that can effectively fight infectious diseases of the upper respiratory tract.

The conducted microbiological and technological studies proved that the obtained essential oils compositions met the quality standards (SP RF XIV ed., SP RK) and the generally accepted rules for compiling essential oils compositions in aromatherapy. The resulting compositions can be both an alternative and a supplement to currently used antimicrobial and antifungal drugs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTION

Ekaterina V. Lakomkina – collection of medicinal plant materials, production of essential oils, screening of essential oils for antimicrobial activity, structuring of essential oils compositions and their subsequent screening for antimicrobial activity, GC-MS of the obtained compositions, development of the concept of the article, article writing, literature sources collection; Gayane A. Atazhanova – management of the work on the technological part of the study, approval of the text for the publication, approval of the final version of the publication; Saule B. Akhmetova – management of the work on the microbiological part of the study, approval of the text for the publication, approval of the final version of the publication; Ifrat N. Zilfikarov – consulting, editing and approval of the text for the publication, approval of the final version of the publication.

All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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