

Antimicrobial activity of essential oil of *Monarda citriodora* Cerv. ex Lag.

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Abstract. The results of studying the antimicrobial activity of an essential oil obtained by steam distillation from dry raw materials of lemon monarda (*Monarda citriodora* Cerv. ex Lag.), grown in the conditions of the Steppe Crimea (Prisivashye). The major components of the studied essential oil are thymol (61.52%), p-cimene (7.55%), carvacrol (6.54%), γ -terpin (5.56%). The high antimicrobial activity of the essential oil against reference cultures and clinical isolates of Gram-positive and Gram-negative microorganisms has been established, which indicates the prospects of its use as a potential antibacterial drug of natural origin.

1 Introduction

Genus *Monarda* L. (the Lamiaceae family) includes 22 species that naturally grow in North America, are grown as ornamental, essential oil and medicinal crops in Asia and Europe [1]. The high antibacterial activity of essential oils accumulating in the aboveground part of monarda plants is mainly due to the presence of biologically active isomeric monoterpenoid components in them, in particular carvacrol and thymol [2].

Monarda citriodora Cerv. ex Lag. (lemon monarda) is an annual herbaceous plant, cultivated as a medicinal plant in the Crimea, Siberia, and some regions of the Caucasus [3]. According to the literature, the aboveground mass of lemon monarda contains valuable biologically active compounds, vitamins C and B₁, B₂, tannins, organic acids, mineral elements [4], as well as up to 0.85% essential oil (EO) with a high content of phenols in the oil (about 70%), which has a floral-spicy scent with thyme and citrus tones [5]. The entire aboveground part of the plants serves as the raw material for the production of essential oil, the maximum accumulation is observed during the flowering phase [6].

The high mass fraction of phenolic compounds such as thymol, carvacrol, *p*-cymene and γ -terpinene in the EO of lemon monarda determines its high antibiotic, disinfecting and antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Aspergillus brasiliensis*, *Klebsiella pneumoniae*, *Streptococcus*

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pyogenes [7]. There is also evidence of the antitumor properties of lemon monarda essential oil [8]. Due to its antimicrobial and anti-inflammatory properties, lemon monarda is used for infectious diseases of the upper respiratory tract and lungs, diseases of the mucous membrane of the oral cavity and nasopharynx, secondary immunodeficiency, atherosclerosis, anemia. Monarda essential oil, when added to tea, helps well with colds [9, 10]. The lemon monarda EO is widely used in medicine as an antiseptic, in the perfumery and cosmetics industry for perfuming cosmetics, and is also used to flavor food and as a fragrant and useful spice that improves digestion [11].

The high concentration of thymol in essential oil causes a high lauricidal effect, which has a detrimental effect on the larval stage of insects, mainly mosquitoes, flies, gnats [12].

The purpose of this study is to study the antimicrobial activity of lemon monarda essential oil in relation to reference cultures and clinical isolates of gram-positive and gram-negative microorganisms.

2 Materials and methods

The object of the study is an essential oil extracted from dry raw materials (the leafy part of the aboveground mass, cut at a height of 10-15 cm from the soil surface during the mass flowering phase) of the lemon monarda, grown in the conditions of the Crimean Prisivashye in the Dry Steppe zone in 2023. In the conditions of Prisivashye, the plants reach a height of 90 cm, form a branched bush. The leaves are narrow, 5.5 ± 0.64 cm long, dentate, lanceolate. The inflorescences consist of 2-7 whorls with tubular pink-purple flowers. The duration of the growing season is 152-163 days; budding is observed in the second half of June, mass flowering – in the second half of July; the yield of the aboveground mass is 0.80 ± 0.11 kg/m².

The mass fraction of essential oil was determined by steam distillation using the Dalmatov method from dried raw materials (GOST 34213-2017). The component composition of EO was studied on a chromatograph Chromatek-Crystal 5000.2 with a mass spectrometric detector. The EO components were identified by comparison with data from the NIST14 MS Search mass spectrum library [13, 14].

The antimicrobial effect of EO on the growth of reference strains and clinical isolates of opportunistic microorganisms was determined by the disc diffusion method in accordance with the recommendations of the European Committee for the Determination of Sensitivity to Antimicrobial Drugs [15]. To do this, a suspension of a daily culture with a density of 1.0 units according to McFarland was introduced into Petri dishes with beef-extract agar for bacteria and Sabouraud agar for fungi. 0.005 ml of oil was applied to a sterile paper disc with a diameter of 6.5 mm (the Saint-Petersburg Pasteur Institute, St. Petersburg), which was placed in the center of a cup with culture. The culture was incubated in a thermostat for 24-48 hours at a temperature of 37 °C. The effect of the oil was assessed by the diameter of the zone of absence of culture growth around the disc. Growth suppression of less than 15 mm was assessed as stability, a diameter of 16-20 mm as moderate sensitivity, a diameter of more than 20 mm as sensitivity, and more than 35 mm as high sensitivity.

3 Results and discussion

By steam distillation, 1.6% of warm straw-colored essential oil with a spicy bitter aroma was obtained from dry raw materials of lemon monarda. 37 compounds were identified, among which thymol (61.52%), *p*-cymene (7.55%), carvacrol (6.54%) predominate (Fig. 1, Table 1).

Table 1. The component composition of essential oil of *Monarda citriodora* Cerv. ex Lag.

No.	Name of the component	RI	Specific Index	Square, %	Compounds
1	α -thujene	932	4.39	0.80	bicyclic monoterpene
2	α -pinene	942	4.57	0.34	bicyclic monoterpene
3	1-octen-3-ol	977	5.27	1.36	monatomic unsaturated alcohol
4	3-octane	981	5.37	0.38	monatomic ketone
5	β -pinene	985	5.46	0.11	bicyclic monoterpene
6	β -myrcene	989	5.55	1.48	acyclic monoterpene
7	3-octanol	993	5.64	0.19	monatomic secondary alcohol
8	octanal	998	5.76	0.21	monoaldehyde
9	α -phellandrene	1009	6.01	0.17	monocyclic monoterpene
10	3-carene	1015	6.13	0.08	bicyclic monoterpene
11	α-terpinene	1021	6.28	2.32	monocyclic monoterpene
12	<i>p</i>-cymene	1029	6.44	7.55	monocyclic monoterpene
13	limonene	1035	6.58	0.50	monocyclic monoterpene
14	eucalyptol + β -phellandrene	1037	6.64	0.32	bicyclic + monocyclic monoterpenes
15	γ-terpinene	1064	7.31	5.56	monocyclic monoterpene
16	1-octanol	1070	7.47	0.05	monatomic primary alcohol
17	cis-sabinene hydrate	1073	7.57	1.12	monoterpene alcohol
18	1-nonen-3-ol	1079	7.74	0.06	monatomic unsaturated alcohol
19	α -terpinolene	1091	8.07	0.19	monoterpene
20	linalool	1098	8.29	0.35	aliphatic monoterpene alcohol
21	nonanal	1100	8.37	0,25	monaldehyde
22	trans-sabinene hydrate	1102	8.42	0.32	bicyclic monoterpene alcohol
23	trans-p-2-Menten-1-ol	1128	9.10	0.15	monocyclic monoterpene alcohol
24	endo borneol	1175	10.52	0.67	bicyclic monoterpene alcohol
25	terpinene-4-ol	1183	10.80	2.97	monocyclic monoterpene alcohol
26	α -terpineol	1194	11.18	0.20	monocyclic monoterpene alcohol
27	methyl carvacrol	1241	12.57	1.61	monoterpene phenol
28	thymol	1295	14.41	61.52	monoterpene phenol
29	carvacrol	1303	14.57	6.54	monoterpene phenol
30	thymol acetate	1346	15.84	0.29	monoterpene phenol ester
31	β -caryophyllene	1427	18.39	1.21	bicyclic sesquiterpene
32	γ -murolene	1481	20.01	0.11	bicyclic sesquiterpene
33	germacrene D	1487	20.20	0.17	monocyclic sesquiterpene
34	γ -cadinene	1519	21.14	0.09	bicyclic sesquiterpene
35	δ -cadinene	1525	21,30	0.19	bicyclic sesquiterpene
36	caryophyllene oxide	1587	23.12	0.43	bicyclic sesquiterpene oxide

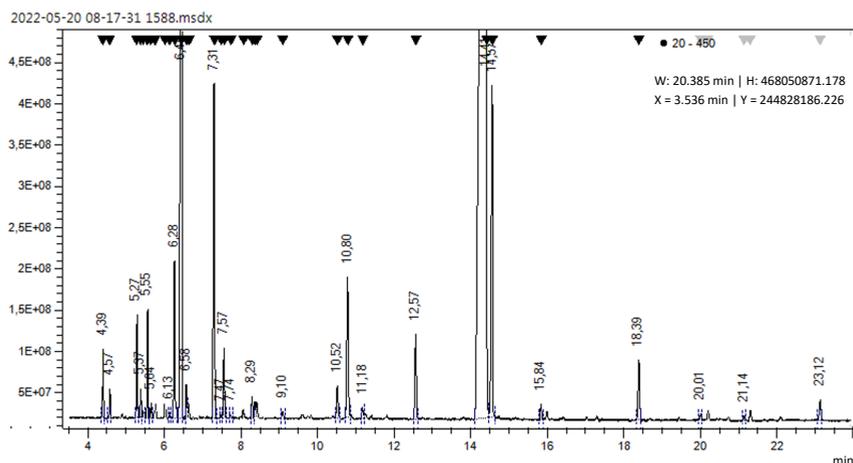


Fig. 1. Chromatogram of essential oil of *Monarda citriodora* Cerv. ex Lag.

The ratio of the main groups of components of lemon monarda essential oil is as follows: phenols – 70%, monoterpenes - 19%, alcohols – 8%, sesquiterpenes – 2%, ketones – 0.38% (Fig. 2).

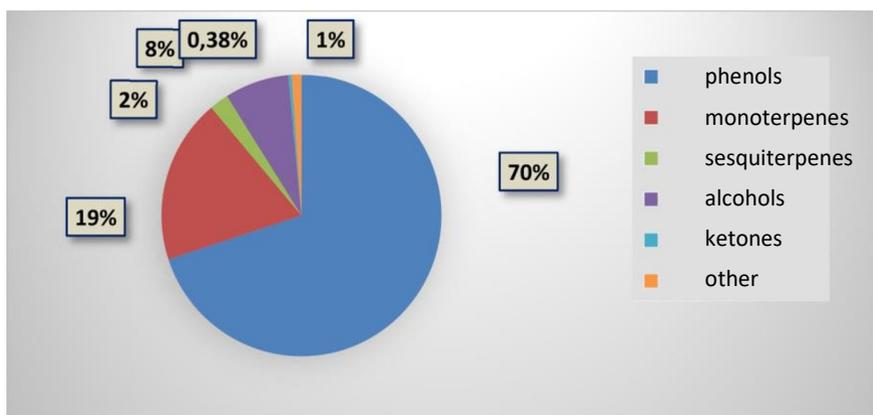


Fig. 2. The ratio of the main components of essential oil of *Monarda citriodora* Cerv. ex Lag.

The predominance of monoterpene phenols in EO, which is typical for the studied oil, determines its pronounced antimicrobial properties and prospects for use in pharmacology and cosmetology. Thus, a number of authors have proven that thymol significantly reduces adhesion, which is an essential prerequisite for the pathogenesis of bacterial and fungal infections [16]. Also, there is data from Mattarelli P., Epifano F. (2017), that EO of *Monarda fistulosa* and *Monarda didyma*, with a thymol fraction above 60%, inhibits the growth of both gram-negative and gram-positive bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pyogenes*, *MRSA*, *Streptococcus pneumoniae*, *Haemophilus influenzae*), while showing a more pronounced activity against pathogenic rather than beneficial microorganisms (*Bifidobacterium animalis* and *Lactobacillus casei*), i.e. it acts selectively [17].

Studies of the antimicrobial activity of lemon monarda EO by the paper disk method have shown a pronounced inhibitory effect on all test cultures of both museum strains and clinical opportunistic microorganisms, with the exception of some clinical strains of *Pseudomonas aeruginosa* (Table 2).

Table 2. Inhibition of the growth of pathogenic test cultures with essential oil of *Monarda citriodora* Cerv. ex lag.

Test cultures of bacteria	Inhibition area, mm	Degree of sensitivity of the strain
<i>Reference strain (museum)</i>		
<i>Staphylococcus aureus</i> ATCC 23923	32	S
<i>Escherichia coli</i> ATCC 25922	42	V
<i>Clinical strain</i>		
<i>Escherichia coli</i> (uropathogia)	35	V
<i>Escherichia coli</i> (hemolytics)	40	V
<i>Escherichia coli</i> (pharynx, ENT)	32	S
<i>Reference strain (museum)</i>		
<i>Candida albicans</i> CCM 885	54	V
<i>Clinical strain</i>		
<i>Candida krusei</i>	57	V
<i>Candida tropicalis</i>	55	V
<i>Pseudomonas aeruginosa</i> No. 1	19	I
<i>Pseudomonas aeruginosa</i> No. 2	10	R
<i>Pseudomonas aeruginosa</i> No. 3	30	S

*R – low sensitivity, growth suppression less than 15 mm;

*I – moderate sensitivity, growth suppression from 16 to 20 mm;

*S – sensitivity, growth suppression from 20 mm;

*V – high sensitivity, growth suppression of 35 mm or more.

According to the data obtained, the studied essential oil is characterized by high antimicrobial activity, as evidenced by an inhibition area of more than 30 mm (with the exception of two strains of *Pseudomonas aeruginosa*). Lemon monarda essential oil had the strongest inhibitory effect on reference and clinical strains of *Escherichia coli* and *Candida* strains. It should also be noted that there is a fairly strong inhibition of clinical strains of *Pseudomonas aeruginosa* isolated from patients who underwent an unsuccessful course of antibiotic therapy, which indicates the prospects of using the studied essential oil of lemon monarda to obtain an antibacterial drug of natural origin.

4 Conclusions

The high antimicrobial activity of *Monarda citriodora* essential oil, grown in the conditions of the Steppe Crimea (Prisivashye), and accumulating in the aboveground mass (1.6% of air-dry raw materials) essential oil with a high mass fraction of thymol (61.52%), with respect to reference cultures and clinical isolates of gram-positive and gram-negative microorganisms.

The studied oil had the most pronounced suppressive effect on reference and clinical strains of *Escherichia coli* and *Candida* strains, and the suppression of the growth of clinical strains of *Pseudomonas aeruginosa* indicates the prospect of its use in the development of multifunctional phytobiotics.

The study was carried out within the framework of the research project "Creation of varieties of essential oils and medicinal plants containing biologically active substances significant for human health, development on their basis and testing of means to improve the quality of human life (FNNS-2022-0006)" using the equipment of the Common Use Center "Physiological and Biochemical studies of plant objects" of the Nikitsky Botanical Garden – National Scientific Center of the Russian Academy of Sciences (Yalta, Russia) and with the financial support of the RSF grant No. 23-15-20015.

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