

Chemical composition and antibacterial activity of essential oil of *Juniperus virginiana* L.

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A topical issue in modern pharmacy is the study of new types of medicinal plant raw materials and the development of pharmaceuticals based on them. Considerable interest is aroused by promising species of the genus *Juniperus*, which are cultivated in Ukraine and are widely used in folk medicine, homeopathy, and cosmetology, but remain insufficiently studied.

Aim: to investigate the component composition and determine the antimicrobial activity of the essential oil of *Juniperus virginiana* L. as an additional source for new antimicrobial herbal medicines.

Materials and methods. The object of the study was the essential oil of *Juniperus virginiana* cultivated at the educational and experimental plot of medicinal plants of Zaporizhzhia State Medical and Pharmaceutical University. The essential oil was obtained by hydrodistillation. The qualitative and quantitative composition of volatile compounds was determined using gas chromatography – mass spectrometry (GC-MS) on an Agilent 7890B chromatograph. The study of antimicrobial activity of *Juniperus virginiana* essential oil (experimental sample) and the reference preparation (essential oil of *Juniperus communis*) was carried out by the disc diffusion method *in vitro* using standard test strains of microorganisms from various groups: *Staphylococcus aureus* ATCC 29213/NCTC12973 (Gram-positive cocci), *Bacillus subtilis* ATCC 6633 (Gram-positive spore-forming rods), *Escherichia coli* ATCC 25922 (Gram-negative enterobacteria), *Pseudomonas aeruginosa* ATCC 27853 (non-fermenting Gram-negative microorganisms), and *Candida albicans* ATCC 885-653 (yeast-like fungi of the genus *Candida*).

Results. As a result of GC-MS, 57 compounds belonging to 6 different chemical classes were identified. The dominant components were Limonene (14.83 %), Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7 (12.65 %), and Safrole (12.42 %). The results of the *in vitro* disc diffusion method demonstrated a pronounced antibacterial activity of *Juniperus virginiana* essential oil against the reference strains of Gram-positive microorganisms: *Staphylococcus aureus* ATCC 29213/NCTC12973 (Gram-positive cocci) and *Bacillus subtilis* ATCC 6633 (Gram-positive spore-forming rods). High antifungal activity was also established against the reference strain of *Candida albicans* ATCC 885-653. The tested sample exhibited moderate antibacterial activity against the Gram-negative test strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Conclusions. The qualitative composition and quantitative content of volatile compounds in the essential oil of *Juniperus virginiana* were studied using GC-MS. A total of 57 components were identified in the essential oil, the major ones being Limonene (14.83 %), Naphthalene 1,2,3,4,4a,5,6,8a-octahydro-7 (12.65 %), and Safrole (12.42 %). Experimental testing of *Juniperus virginiana* essential oil for microbiological purity showed no microbial growth on the surface or within the nutrient media, allowing further investigation of its antimicrobial activity.

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Хімічний склад та антибактеріальна активність ефірної олії *Juniperus virginiana* L.

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Актуальним питанням сучасної фармації є дослідження нових видів лікарської рослинної сировини та створення на їхній основі лікарських засобів. Значну зацікавленість викликають перспективні види роду ялівець (*Juniperus*), які культивують на території України та широко застосовують у народній медицині, гомеопатії і косметології, але досліджені вони недостатньо.

Мета роботи – дослідження компонентного складу та визначення антимікробної активності ефірної олії ялівцю віргінського (*Juniperus virginiana* L.) як додаткового джерела нових фітопрепаратів антимікробної дії.

Матеріали і методи. Об'єкт дослідження – ефірна олія ялівцю віргінського, вирощеного на навчально-дослідній ділянці лікарських рослин Запорізького державного медико-фармацевтичного університету. Ефірну олію отримано методом гідродистиляції. Якісний склад і кількісний вміст летких речовин визначено методом газової хроматографії – мас-спектрометрії (ГХ-МС) за допомогою хроматографа Agilent 7890B. Протимікробну активність ефірної олії ялівцю віргінського (експериментальний зразок) та референс-препарату

(ефірна олія ялівцю звичайного) дослідили диско-дифузійним методом *in vitro* з використанням еталонних тест-штамів мікроорганізмів, що належали до різних груп: *Staphylococcus aureus* ATCC 29213/NCTC12973 (грампозитивні коки), *Bacillus subtilis* ATCC 6633 (грампозитивні спороутворювальні палички), *Escherichia coli* ATCC 25922 (грамнегативні ентеробактерії), *Pseudomonas aeruginosa* ATCC 27853 (неферментуючі грамнегативні мікроорганізми), *Candida albicans* ATCC 885-653 (дріжджоподібні гриби роду *Candida*).

Результати. За результатами ГХ-МС дослідження ідентифіковано 57 сполук, які належать до 6 різних класів хімічних речовин, серед них домінували Limonene (14,83 %), Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7 (12,65 %), Safrole (12,42 %). Результати вивчення антимікробної активності ефірної олії ялівцю віргінського диско-дифузійним методом *in vitro* дали підстави зробити висновок про його виражену антибактеріальну дію щодо еталонних штамів грампозитивних мікроорганізмів *Staphylococcus aureus* ATCC 29213/NCTC12973 (грампозитивні коки) та *Bacillus subtilis* ATCC 6633 (грампозитивні спороутворювальні палички). Виявлено високу протигрибкову активність ефірної олії ялівцю віргінського щодо еталонного штаму *Candida albicans* ATCC 885-653. Досліджений зразок мав помірну антибактеріальну активність щодо тест-штамів грамнегативних мікроорганізмів *Escherichia coli* ATCC і *Pseudomonas aeruginosa* ATCC 27853.

Висновки. За допомогою ГХ-МС методу досліджено якісний склад і кількісний вміст летких речовин ефірної олії *Juniperus virginiana*. В ефірній олії ялівцю віргінського визначено 57 компонентів, серед них найбільша частка Limonene (14,83 %), Naphthalene 1,2,3,4,4a,5,6,8a-octahydro-7 (12,65 %), Safrole (12,42 %). У результаті експериментального дослідження ефірної олії ялівцю віргінського щодо мікробіологічної чистоти не виявлено ріст мікроорганізмів на поверхні та в товщі живильних середовищ. Це дало змогу здійснити надалі дослідження протимікробної активності зразка.

Сучасні медичні технології. 2026. Т. 18, № 1(68). С. 46-54

Modern pharmacy is increasingly focused on the search for and study of new sources of biologically active substances of natural origin, particularly those derived from medicinal plant raw materials, which can serve as the basis for the development of effective and safe medicinal products. In this context, essential oils are of particular interest as complex multicomponent systems capable of exhibiting a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and others.

One of the promising objects of study is *Juniperus virginiana* L. (*Virginia juniper*), which belongs to the genus *Juniperus* (family *Cupressaceae*). Species of this genus are widely cultivated in various regions of Ukraine, distinguished by high adaptability, and are actively used in folk medicine, homeopathy, and the cosmetic industry. Despite their pharmacological potential, the biochemical composition and therapeutic properties of the essential oil of *Juniperus virginiana* L. remain insufficiently studied, especially regarding its antibacterial activity against clinically relevant infectious agents.

Considering the growing resistance of microorganisms to conventional antibiotics, the investigation of natural compounds with potential antimicrobial activity has become increasingly important. This necessitates a detailed analysis of the chemical composition of *Juniperus virginiana* L. essential oil, using modern analytical methods, along with the study of its biological activity to assess its suitability for pharmaceutical applications.

Thus, the study of *Juniperus virginiana* L. essential oil is both timely and scientifically justified, as it may contribute to expanding the raw material base for the development of new antimicrobial herbal medicines that meet the needs of contemporary medicine and pharmacy.

The genus *Juniperus* includes more than 70 species of evergreen coniferous trees and shrubs naturally found in the mountainous regions of the Carpathians and Crimea. These are evergreen trees or low-growing shrubs. Some species are cultivated as ornamental and essential oil-bearing plants. Juni-

pers have needle-like or scale-like leaves. Female cones are nearly spherical and become fleshy upon ripening, bluish-black or reddish-brown with a bluish waxy coating (galbuli), typically maturing in 2–3 years. Plants of the genus *Juniperus* are long-lived, drought- and frost-resistant, and grow extremely slowly [1].

They contain essential oils, sugars, anthocyanidins, organic acids, resins, wax, macroelements (potassium salts), microelements (salts of manganese, iron, copper, aluminum), tannins, flavonoids, steroids, diterpenoids, and sesquiterpenoids [2].

Pharmacological studies of *Juniperus virginiana* essential oil have shown anticancer, cytotoxic, antioxidant, antimalarial, and anti-inflammatory activities [3].

In folk medicine, juniper fruits are used as diuretic, disinfectant, expectorant, analgesic, and rubefacient remedies. Positive therapeutic effects have also been noted in the treatment of neuralgia, rheumatism, and gout [4].

Juniper fruits are included in the formulation of Zdenko's mixture, which is used for the treatment of urinary bladder papillomatosis, antacid gastritis, and peptic ulcer disease, as well as for edema, malaria, cystitis, polyarthritis, otitis, and skin diseases [5].

Juniper essential oil is used as a diuretic, antiseptic, tonic, and wound-healing agent [6]. It has a positive effect on emotional state, enhances lymphatic and blood circulation, facilitates the excretion of uric acid and excess fluid from the body, contributing to weight loss in obesity and the elimination of edema and musculoskeletal disorders.

Juniper-based preparations increase urine output and disinfect the urinary tract, stimulate gastric juice and bile secretion, enhance intestinal peristalsis, soften phlegm, and have anti-inflammatory and analgesic effects [7].

The use of juniper preparations is recommended for edema associated with renal failure and circulatory disorders, chronic pyelitis and cystitis, urolithiasis, gastroenteritis, diseases involving bile stasis and gallstone formation, and chronic respiratory tract diseases (tracheitis, laryngitis, bronchitis). In addition, galenic

preparations from juniper fruits enhance bile formation and secretion, increase gastric juice secretion, mildly stimulate intestinal peristalsis, and exhibit bactericidal activity [3,8].

Juniper essential oil is used in combination therapy for skin cancer along with radiation therapy and is applied externally for rheumatism in the form of alcoholic tinctures or ointments [3,9].

The essential oil of juniper has pronounced antimicrobial properties, which are attributed to its chemical composition. The main components – α - and β -pinene, myrcene, limonene, sabinene, terpinene-4-ol, and borneol – ensure a broad spectrum of activity against various microorganisms [7,10].

Aim

The aim of the study was to investigate the component composition and determine the antimicrobial activity of *Juniperus virginiana* L. essential oil as an additional source for the development of new antimicrobial herbal medicines.

Materials and methods

The object of the study was the essential oil extracted from the shoots and leaves of *Juniperus virginiana* harvested during the fruiting phase from the educational and experimental medicinal plant plot of Zaporizhzhia State Medical and Pharmaceutical University. The essential oil was obtained by hydrodistillation in accordance with the State Pharmacopoeia of Ukraine [11].

The phytochemical analysis was carried out in the phytochemical laboratory of the Educational and Scientific Medical Laboratory Center with vivarium at Zaporizhzhia State Medical and Pharmaceutical University.

The qualitative and quantitative determination of active compounds was performed at the Department of Toxicological and Inorganic Chemistry (Head of the Department: Prof. O. I. Panasenko, DSc, PhD), Zaporizhzhia State Medical and Pharmaceutical University [12,13].

The completeness of reactions and the individuality of the resulting compounds were monitored using an Agilent 7890B gas chromatograph equipped with a 5977B mass spectrometry detector. The chromatographic separation was performed using a DB-5ms column (30 m \times 250 μ m \times 0.25 μ m). The carrier gas was helium at a flow rate of 1.6 mL/min. The injection volume was 0.5 μ L, with a split ratio of 1:50. The injector temperature was programmed at 230 $^{\circ}$ C \rightarrow 12 $^{\circ}$ C/s \rightarrow 275 $^{\circ}$ C.

Oven temperature program: 240 $^{\circ}$ C (hold for 1 min) \rightarrow 5 $^{\circ}$ C/min \rightarrow 280 $^{\circ}$ C (hold for 1 min).

Total run time: 10 minutes. The interface temperature of the gas chromatography – mass spectrometry was 280 $^{\circ}$ C; ion source temperature – 230 $^{\circ}$ C; quadrupole mass analyzer – 150 $^{\circ}$ C. Ionization type: Electron Impact (EI), 70 eV. The scanning range was m/z 30–500.

The essential oil components were identified by comparing the obtained mass spectra of the separated compounds with the NIST 14 mass spectral library, which contains over 470,000 reference spectra [3,14].

The microbiological purity and antimicrobial activity of *J. virginiana* essential oil were studied at the microbiological laboratory

of the Educational and Scientific Medical Laboratory Center at Zaporizhzhia State Medical and Pharmaceutical University. The in vitro experiments were conducted in accordance with the requirements of the State Pharmacopoeia of Ukraine, 2nd edition, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards [15]. The essential oil of *Juniperus communis* L. served as the reference preparation.

To avoid false-positive results during antimicrobial testing, the microbiological purity (MP) of the experimental sample was preliminarily examined. This included the determination of total aerobic microbial count (TAMC), total combined yeast and mold count (TYMC), and the presence/absence of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and anaerobic microorganisms. TAMC and TYMC were determined by both pour plate and double-layer methods. For pour plate culture, dilutions of 1:100 and 1:1000 were prepared, and 1 mL of each dilution was inoculated into sterile Petri dishes, followed by 20 mL of melted, cooled (to 45 $^{\circ}$ C) soy-casein agar for bacteria, and Sabouraud dextrose agar for yeast and molds. For the double-layer method, 1 mL of each dilution was added to test tubes with 4 mL of molten, cooled Sabouraud agar, thoroughly mixed, and poured into Petri dishes. After solidification, the plates were incubated at 35 $^{\circ}$ C for 5 days (for bacteria) and at 25 $^{\circ}$ C for 7 days (for fungi). To test specific pathogens, 0.1 mL of each dilution was inoculated onto selective media: egg-yolk salt agar (for *S. aureus*), Endo medium (for *E. coli* and *P. aeruginosa*), and thioglycolate medium (for anaerobes). *S. aureus* and anaerobes were incubated at 35 $^{\circ}$ C for 48 hours, while *E. coli* and *P. aeruginosa* were incubated at 35 $^{\circ}$ C for 24 hours.

Antimicrobial activity was assessed using the disc diffusion method [16,17] in vitro against standard test strains from different microbial groups: *Staphylococcus aureus* ATCC 29213/NCTC12973 (Gram-positive cocci), *Bacillus subtilis* ATCC 6633 (Gram-positive spore-forming rods), *Escherichia coli* ATCC 25922 (Gram-negative enterobacteria), *Pseudomonas aeruginosa* ATCC 27853 (non-fermenting Gram-negative bacilli), *Candida albicans* ATCC 885-653 (yeast-like fungi).

Bacterial and fungal suspensions were prepared from 24-hour cultures using physiological saline, adjusted to a turbidity of 0.5 McFarland units (equivalent to 1.5×10^8 CFU/mL for bacteria and 5×10^6 CFU/mL for *Candida* species) using a DEN-1B densitometer (SIA "Biosan", Latvia). The suspensions were evenly inoculated on the surface of Mueller-Hinton agar (HiMedia, India) using sterile swabs. After drying for 5 minutes, sterile 6 mm paper discs (HiMedia, India) impregnated with *J. virginiana* essential oil were placed on the agar surface. Petri dishes were immediately incubated at 35 $^{\circ}$ C: for 18 hours for *S. aureus*, *E. coli*, and *P. aeruginosa*; and for 48 hours for *C. albicans*. The antimicrobial effect was determined by measuring the diameter of the inhibition zones around each disc in millimeters. The interpretation criteria were as follows: 0–2 mm: no activity, 3–10 mm – weak activity, 10–20 mm – moderate activity, 21 mm – strong activity. All experiments were performed in triplicate.

To assess the antimicrobial activity of the tested samples, the mean diameter of the inhibition zone and standard deviation were used. Since the data were obtained from a normally distributed population, an independent Student's t-test was applied to

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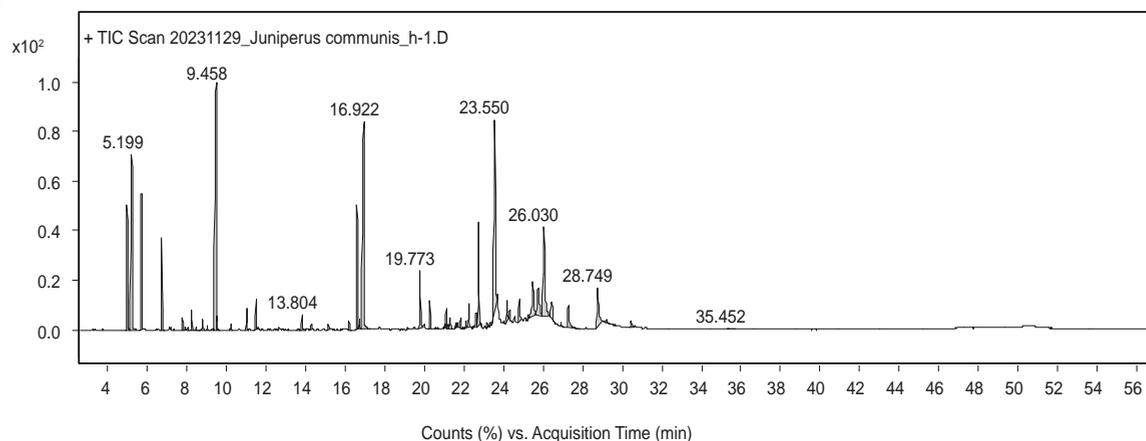


Fig. 1. Chromatogram essential oil components of *Juniperus virginiana* L.

evaluate the hypothesis of no statistically significant differences between the means of two independent groups. The level of statistical significance was set at $p < 0.05$.

Statistical analysis of the results was conducted using Statistica software (license No. JPZ804I382130ARCN10-J) and Microsoft Excel 7.0 (Microsoft Corp., USA).

Results

The essential oil obtained from the shoots and fruits of *Juniperus virginiana* is light yellow in color, transparent, and possesses a camphoraceous odor. The content of essential oil in the fruits of *J. virginiana*, calculated on an absolutely dry basis, was 1.74 ± 0.02 %.

The results of the component composition analysis of *J. virginiana* essential oil are presented in Fig. 1 and Table 1. As shown, 57 different compounds belonging to six groups were identified in the essential oil of *J. virginiana*, including: – 18 oxygenated sesquiterpenes (30.56 %), 4 oxygenated monoterpenes (2.03 %), 16 sesquiterpene hydrocarbons (8.05 %), 9 monoterpene hydrocarbons (23.34 %), 5 aromatic hydrocarbons (19.66 %), 3 phenylpropanoids (14.88 %), 5 other components (1.48 %).

The results of the microbiological study (Table 2) indicate that the essential oil of *Juniperus virginiana* exhibits pronounced antimicrobial properties, which are associated with its chemical composition [18,19,20,21].

The results of the study of the component composition of *Juniperus virginiana* essential oil are presented in Table 1. The analysis revealed 57 different compounds belonging to 6 chemical groups: 18 oxygenated sesquiterpenes (30.56 %), with the major constituents being elemol (12.65 %), eudesmol (6.04 %), isospathulenol (2.74 %), and 10- α -cadinol (2.54 %); 4 oxygenated monoterpenes (2.03 %), including linalool (1.00 %), eucalyptol (0.26 %), 4-terpineol (0.54 %); 16 sesquiterpene hydrocarbons (8.05 %), including δ -cadinene (3.71 %), α -muurolene (0.93 %), and caryophyllene (0.82 %); 9 monoterpene hydrocarbons (23.34 %), among which D-limonene (14.83 %),

1-(3-methylcyclopent-2-enyl)-cyclohexene (4.33 %), and 3-carene (2.31 %) were dominant; 5 aromatic hydrocarbons (19.66 %), including 3,5-dimethoxytoluene (0.26 %); and 3 phenylpropanoids (14.88 %), with the main ones being safrole (12.42 %), and methyl eugenol (2.24 %); 5 other components (1.48 %).

Experimental testing of the essential oil for microbial purity showed no microbial growth on the surface or in the depth of the nutrient media, which made it possible to proceed with the evaluation of the antimicrobial activity of the sample.

The obtained results of antimicrobial activity assessment of *J. virginiana* essential oil by the in vitro disc diffusion method indicate a pronounced antibacterial effect against reference strains of Gram-positive microorganisms – *Staphylococcus aureus* ATCC 29213/NCTC12973 (Gram-positive cocci) and *Bacillus subtilis* ATCC 6633 (Gram-positive spore-forming rods). A high antifungal activity was also observed against the reference strain *Candida albicans* ATCC 885-653. The test sample demonstrated moderate antibacterial activity against the test strains of Gram-negative microorganisms – *Escherichia coli* ATCC and *Pseudomonas aeruginosa* ATCC 27853 (Table 2). *J. virginiana* essential oil exhibits pronounced antimicrobial properties, which are associated with its chemical composition. Its major components – elemol, eudesmol, methyl eugenol, limonene, naphthalene, isospathulenol, and safrole – provide a broad-spectrum activity against various microorganisms.

As a reference substance in the microbiological study, the essential oil of common juniper (*Juniperus communis* L.) was used. The obtained results showed that *J. virginiana* essential oil also demonstrated antimicrobial activity against reference microbial strains (Table 3).

Analysis of the obtained results of the microbiological study of the experimental sample and the reference preparation (Table 4) indicates that the essential oil of *Juniperus virginiana* exhibits a higher mean diameter of the growth inhibition zone compared to the essential oil of *Juniperus communis* (statistically significant difference, $p < 0.05$), which demonstrates its more pronounced antimicrobial activity.

Table 1. Qualitative and quantitative composition of *Juniperus virginiana* essential oil

No.	Essential oil component	DB formula	RT	Area sum, %
1	D-Limonene	C10H16	9.458	14.83
2	Elemol	C15H26O	23.55	12.65
3	Safrole	C10H10O2	16.922	12.42
4	Eudesmol	C15H26O	26,03	6.04
5	1-(3-Methylcyclopent-2-enyl)cyclohexene	C12H18	16.582	4.33
6	δ -Cadinene	C15H24	22.732	3.71
7	Isospathulenol	C15H24O	28.749	2.74
8	10-epi- α -Cadinol	C15H26O	25.739	2.54
9	γ -Eudesmol	C15H26O	25.474	2.40
10	3-Carene	C10H16	6.713	2.31
11	Methyleugenol	C11H14O2	19.773	2.24
12	4,7(11)-Valeradien-12-al	C15H22O	27.269	1.18
13	Linalool	C10H18O	11.473	1.00
14	α -Murolene	C15H24	22.241	0.93
15	Opopanone Acetate	C17H28O3	24.78	0.90
16	Eudesma-7(11)-en-4-ol;	C15H26O	26.412	0.86
17	Caryophyllene	C15H24	20.272	0.82
18	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzene-3-ol	C15H26O	24.169	0.79
19	4-Carene	C10H16	11.001	0.54
20	(-)-4-Terpineol	C10H18O	13.804	0.54
21	(-)- α -Gurjunene	C15H24	21.091	0.51
22	β -Myrcene	C10H16	8.234	0.47
23	(+)- γ -Cadinene	C15H24	22,60	0.47
24	α -Elemene	C15H24	21.279	0.45
25	Caryophyllene Oxide	C15H24O	24.286	0.35
26	Sabinene	C10H16	7,77	0.29
27	3-Carene	C10H16	8.773	0.29
28	(-)-Bornyl Acetate	C12H20O2	16.697	0.29
29	Bicyclosesquifelandrene	C15H24	21.81	0.28
30	Kessanyl Acetate	C17H28O3	30.436	0.27
31	Eucalyptol; 1,8-Cineole	C10H18O	9.512	0.26
32	3,5-Dimethoxytoluene	C9H12O2	16.177	0.26
33	Citronellol	C10H20O	15.132	0.23
34	Estragole	C10H12O	14.277	0.22
35	cis-Muurolo-4(15),5-diene	C15H24	22.082	0.19
36	epi- γ -Eudesmol	C15H26O	25.23	0.18
37	Kessyl Acetate	C17H28O3	29.196	0.18
38	γ -Terpinene	C10H16	10.214	0.17

Cont. of Table 1.

No.	Essential oil component	DB formula	RT	Area sum, %
39	Epizonarene	C15H24	21.58	0.13
40	(+)- γ -Cadinene	C15H24	21.656	0.13
41	4-Carene	C10H16	9.018	0.11
42	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, acetate, [1R-(1 α ,2 α ,3 β ,6 α)]	C15H26O	26.888	0.11
43	cis-3-Hexenyl Benzoate	C13H16O2	24.008	0.10
44	(-)-Abietadiene	C20H32	35.452	0.10
45	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	C15H24	21.176	0.09
46	α -Copaene	C15H24	19.13	0.08
47	Cadina-3,5-diene	C15H24	21.011	0.07
48	Guaiol Acetate	C17H28O2	22.837	0.07
49	trans-Valerenyl Acetate	C17H26O2	29.61	0.07
50	1-Octen-3-ol	C8H16O	8.015	0.06
51	Methylenanthate	C8H16O2	8.446	0.06
52	o-Cymene	C10H14	9.254	0.06
53	(+)-Borneol	C10H18O	13.578	0.06
54	Caralene	C15H24	20.54	0.06
55	Eudesma-4(14),7(11)-diene	C15H24	20.658	0.06
56	β -Bisabolene	C15H24	22.469	0.06
57	trans-Valerenyl Acetate	C17H26O2	29.061	0.06

Table 2. Results of the microbiological study of *Juniperus virginiana* essential oil

Sample name	Test strain	Growth inhibition zone diameter, mm			Mean value, mm
		1 st test	2 nd test	3 rd test	
Essential oil of <i>Juniperus virginiana</i> (<i>Juniperus virginiana</i> L.)	<i>Staphylococcus aureus</i> ATCC 29213/NCTC12973	26	29	27	27.33 \pm 1.53
	<i>Bacillus subtilis</i> ATCC 6633	37	36	37	36.67 \pm 0.58
	<i>Escherichia coli</i> ATCC 25922	11	15	13	13.00 \pm 2.00
	<i>Pseudomonas aeruginosa</i> ATCC 27853	16	16	17	16.33 \pm 0.58
	<i>Candida albicans</i> ATCC 885-653	36	37	39	37.33 \pm 1.53

Table 3. Results of the microbiological study of common juniper essential oil (reference sample)

Sample name	Test strain	Growth inhibition zone diameter, mm			Mean value, mm
		1 st test	2 nd test	3 rd test	
Essential oil of Common juniper – <i>Juniperus communis</i> L.	<i>Staphylococcus aureus</i> ATCC 29213/NCTC12973	19	19	20	19.33 \pm 0.58
	<i>Bacillus subtilis</i> ATCC 6633	19	20	21	20.00 \pm 1.00
	<i>Escherichia coli</i> ATCC 25922	9	9	10	9.33 \pm 0.58
	<i>Pseudomonas aeruginosa</i> ATCC 27853	11	12	12	11.67 \pm 0.58
	<i>Candida albicans</i> ATCC 885-653	16	16	17	16.33 \pm 0.58

Table 4. Results of the microbiological study of the essential oils of *Juniperus virginiana* and *Juniperus communis*

Sample name	Microorganism cultures				
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
	Growth inhibition zone diameter, mm				
Essential oil of <i>Juniperus virginiana</i>	27.33 ± 1.53*	36.67 ± 0.58*	13.00 ± 2.00*	16.33 ± 0.58*	37.33 ± 1.53*
	t ₁ = 8.52	t ₂ = 28.58	t ₃ = 3.53	t ₄ = 13.11	t ₅ = 21.06
	p < 0.05	p < 0.001	p < 0.05	p < 0.001	p < 0.001
Essential oil of <i>Juniperus communis</i> (reference)	19.33 ± 0.58	20.00 ± 1.00	9.33 ± 0.58	11.67 ± 0.58	16.33 ± 0.58

*: Student's t-test, p < 0.05.

Discussion

Jha V. et al. identified 22 different compounds in the essential oil of *Juniperus virginiana*, represented mainly by sesquiterpenes, as well as terpenes, diesters, and organic compounds. The predominant constituents were α -cubebene (19.5 %), α -trans-atlantone (14.32 %), α -himachalene (13.62 %), γ -E-atlantone (9.6 %), diethyl phthalate (9 %), γ -himachalene (5.82 %), allo-himachalol (4.67 %), β -himachalene oxide (4.22 %), α -Z-atlantone (3.66 %), limona ketone (1.92 %), and calarene epoxide (1.74 %) [3].

Lafraxo S. et al. identified 31 compounds in the essential oil of *Juniperus thurifera* L. – a plant traditionally used in phytomedicine – by means of gas chromatography coupled with mass spectrometry (GC-MS). The dominant constituents were α -thujene (25 %), elemol (12 %), and muurolol (12 %) [22].

Jahanshiri Z. et al. demonstrated that the essential oil of *J. virginiana* inhibits the growth of *Candida albicans* and reduces biofilm formation, which is important for preventing recurrences and enhancing the efficacy of antifungal agents [18].

According to Lafraxo S., using the disk diffusion and microdilution methods, a pronounced antifungal activity of *J. thurifera* L. essential oil was detected against *C. albicans* and *F. oxysporum*, with activity values of 21.0 ± 2.1 mm and 32.0 ± 2.3 %, and a minimum inhibitory concentration (MIC) of 9.5 × 10⁻² ± 0.001 [22].

Of particular note is the high antifungal activity against *Candida albicans* (37.33 ± 1.53 mm), which significantly exceeds the corresponding values of the reference preparation – *Juniperus communis* L. essential oil (16.33 ± 0.58 mm). This result may be attributed to the presence of a considerable amount of phenylpropanoids (safrole and methyl eugenol) in the essential oil of *J. virginiana*, which, according to literature data, possess pronounced fungicidal properties [23].

As a result of our study of the essential oil obtained from the fruits of *Juniperus virginiana* L., a complex multicomponent chemical composition was established, including 57 individual compounds belonging to six main groups of terpenes and aromatic compounds. The qualitative and quantitative analyses revealed the predominance of monoterpene hydrocarbons and phenylpropanoids. A significant proportion of oxygenated sesquiterpenes, particularly elemol (12.65 %), eudesmol (6.04 %), and isospathulenol (2.74 %), indicates the presence of potentially biologically active substances in the essential oil composition.

Microbiological testing revealed that *J. virginiana* essential oil possesses pronounced antimicrobial properties. The highest susceptibility was observed in Gram-positive strains: *Staphylococcus aureus* (27.33 ± 1.53 mm) and *Bacillus subtilis* (36.67 ± 0.58 mm). These results are consistent with previous findings indicating that Gram-positive bacteria are more vulnerable to the effects of essential oils due to the structural simplicity of their cell walls.

A particularly noteworthy finding is the strong antifungal activity against *Candida albicans* (37.33 ± 1.53 mm), which significantly exceeded the reference sample – *Juniperus communis* L. essential oil (16.33 ± 0.58 mm). This may be attributed to the high content of phenylpropanoids, such as safrole and methyl eugenol, which are known for their antifungal properties.

Compared to the reference preparation, *J. virginiana* oil demonstrated statistically significantly higher antimicrobial activity against all tested microbial strains (Student's t-test, p < 0.05–0.001). The greatest differences were observed against *B. subtilis* and *C. albicans*, confirming the superior efficacy of *J. virginiana* essential oil as a potential antimicrobial agent.

While activity against Gram-negative bacteria (*E. coli*, *P. aeruginosa*) was moderate, it was still superior to that of *J. communis* oil. This relatively lower sensitivity is likely due to the outer membrane of Gram-negative bacteria, which impedes the penetration of hydrophobic essential oil constituents.

Overall, the antimicrobial activity of *J. virginiana* essential oil can be attributed to the synergistic effects of its major components, including monoterpenes, sesquiterpenes, and phenylpropanoids. The findings suggest promising applications for *J. virginiana* oil as a natural antiseptic agent in pharmaceutical, cosmetic, and food preservation industries.

Conclusions

1. The essential oil of *Juniperus virginiana* was analyzed by GC-MS, revealing 57 volatile components. The predominant compounds were Limonene (14.83 %), Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7 (12.65 %), and Safrole (12.42 %).

2. Microbiological testing confirmed the sterility of the *J. virginiana* essential oil sample, allowing reliable evaluation of its antimicrobial properties.

3. The essential oil demonstrated pronounced antimicrobial and antifungal activity, showing the largest inhibition zones against *Staphylococcus aureus* (27.3 mm), *Bacillus subtilis* (36.7 mm), and *Candida albicans* (37.3 mm), and moderate activity against *Escherichia coli* (13.0 mm) and *Pseudomonas aeruginosa* (16.3 mm).

4. Compared to *Juniperus communis* essential oil, *J. virginiana* oil exhibited significantly higher antimicrobial efficacy, with inhibition zones increasing by 1.4–2.3 times depending on the test strain ($p < 0.05$ – 0.001), indicating its potential as a broad-spectrum antimicrobial agent.

Prospects for further research. The findings demonstrate that *Juniperus virginiana* essential oil exhibits pronounced antimicrobial properties and holds promise for further research aimed at the development of new pharmaceutical products and herbal remedies. Additional clinical studies are required to evaluate its effectiveness as a potential medicinal agent.

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