

Research Article

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Composition and biological activity of essential oil from aboveground parts of *Artemisia messerschmidtiana*

The study of the chemical composition of biologically active compounds from plants of the natural flora is a promising direction for investigating their biological activity and potential use as sources for the medicinal, pharmaceutical, and cosmetic industries. This article presents the results of an evaluation of the composition and biological activity of essential oil isolated from *Artemisia messerschmidtiana* from the Far East. The essential oil was extracted by hydrodistillation. The obtained essential oil was tested for cytotoxic, fungicidal, antimicrobial, antimalarial, tuberculostatic, and antileishmanial activities. The composition of the extracted essential oil was analyzed by GC–MS methods. The total composition of the essential oil included 43 components, with the highest content observed for 1,8-cineole, camphor, and α -campholenal. Testing of the essential oil revealed weak antimicrobial, fungicidal, and antimalarial activities, and high cytotoxic activity. No antituberculosis or antileishmanial activity was detected in the essential oil. The results contribute to the understanding of the biological activity of components of the genus *Artemisia* L.

Keywords: *Artemisia messerschmidtiana*, essential oil, biological activity, chromatography-mass spectrometry

Introduction

The study of the composition of essential oils and the biological activity of medicinal plants is an important task for science. Numerous studies have shown that plants of the genus *Artemisia* L. differ within sections and species in terms of the content of many groups of biologically active substances, including the composition of essential oils. These differences are influenced by various factors, such as soil conditions, differences in moisture, solar insolation, temperature, altitude above sea level, and age characteristics. The composition and quantitative accumulation of individual components are influenced by the timing of raw material collection, fertility and fertilization, drying conditions, and the genetic characteristics of individual plant populations. Differences in chemical composition depend on the plant organ, and the extraction method. A review of the literature on the evaluation of essential oils of the genus *Artemisia* and compounds isolated from them shows that in recent years, numerous studies have been conducted on their antibacterial, antifungal, antiviral, and other anti-infective properties [1].

Despite extensive research on the genus *Artemisia*, not all species have been studied sufficiently, which creates potential for the search for new medicinal compounds in the natural flora.

Artemisia messerschmidtiana Besser (= *Artemisia gmelinii* var. *messerschmidtiana* Poljakov) is a semi-shrub 60–80 cm high of the *Asteraceae* family. It grows in Buryatia, Irkutsk and Chita regions, Krasnodar Krai, and Mongolia (Fig. 1) on slopes with shrubby meadow-steppe vegetation and forest edges [2].



Figure 1. Areas of *A. messerschmidtiana*
(from *Artemisia gmelinii* var. *messerschmidtiana* Poljakov | Plants of the World Online | Kew Science)

As a continuing our research on essential oils of wormwood from the Far East [3–6] and Kazakhstan [7–9], we investigated the chemical composition and some kinds of biological properties of *A. messerschmidtiana*'s essential oil from aboveground parts.

Previously, South Korean scientists isolated methyl esculin, daphnetin, 6-methyl esculetin, dimethyl daphnetin, esculin, and umbelliferone from *A. messerschmidtiana* raw materials [10].

The study of the aerial part of *A. messerschmidtiana* allowed the isolation of scopoletin, whose structure was established by spectral methods. The antibacterial and cytostatic activity of the dry extract and essential oil was studied. The content of 43 chemical elements in the plant was determined by atomic emission spectrometry. The anatomical structure of *A. messerschmidtiana* Bess. was also studied [11].

Experimental

Collection of raw materials. Raw materials of *A. messerschmidtiana* for research were collected in the second decade of September 2017, in an oak forest on the southern steep slope on the left bank of the Razdolnaya (Suifun) River, near the village of Chernyatino, Oktyabrsky District, Primorsky Krai (Russian Federation) (Fig. 2).



Figure 2. *A. messerschmidtiana*

The herbarium code for *A. messerschmidtiana* sample was 103564. Plant samples were collected into herbarium fund of G.B. Eljakov Pacific Institute of Bioorganic Chemistry, laboratory of chemotaxonomy (Far East Branch of RAS, Vladivostok, Russian federation).

Obtaining essential oil. Essential oil was obtained by hydro distillation methods from aboveground parts with a Clevenger apparatus, period of extraction was 2 hours [12], using hexane trap.

The essential oil of *A. messerschmidtiana* was tested on anti microbial and fungicidal activity, using the standard strains: *Aspergillus fumigates* ATCC 204305, *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Staphylococcus aureus* ATCC 29213, MRSATCC 33591, *Pseudomonas aeruginosa* ATCC 27853, *Candida glabrata* ATCC 90030, *Cryptococcus neoformans* ATCC 90113, *Escherichia coli* ATCC35218, and *Mycobacterium intracellulare* ATCC 23068 [13]. The reference preparations were amphotericin B and ciprofloxacin.

Primary screening was performed using a dose 50 µg/ml twice. Inhibition of bacterial and fungal growth (% Ing.) was taken into account in comparison with positive and negative controls. In secondary screening, samples were tested at concentrations ranging from 2 to 50 µg/ml of a suspension of 9 strains of microorganisms, and IC₅₀ was calculated based on the results.

The anti malarial activity of essential oil sample was tested as inhibition strains of *Plasmodium falciparum* (chloroquine-sensitive — D6, and chloroquine-resistant — W2) using negative and positive control. The preparation chloroquine was applied as a negative control. Also the essential oil was tested on the mammalian cellular line VERO. The selectivity index (SI) was assessed as the ratio IC₅₀ VERO to IC₅₀ D6 or W2.

Study of cytotoxic activity was conducted on nauplii of *Artemia salina* [14]. Mortality and survival rates of larvae after exposure to different concentrations of essential oil were recorded. Mortality (P) index was calculated by the formula:

$$P = (A - B - C) / N \times 100 \%$$

where, A is the number of dead larvae after 24 hours; B is the number of larvae that died before the start of the test; C is the average number of larvae that died in the negative control; N is the total number of larvae.

Mycobacteria testing was performed using the REMA method [15–18]. Working solutions of essential oil were diluted in Middle Brook 7H9 culture medium with the addition of OADC. 100 mL of Middle Brook 7H9 and essential oil were added to all test wells, with control wells containing no essential oil samples. Activity was assessed by color: blue color indicated no growth of mycobacteria, pink color indicated growth [19]. Isoniazid, amphotericin B, and chlorhexidine dihydrochloride were used as negative controls.

Testing for anti-leishmaniasis activity. The activity of essential oil was evaluated *in vitro* against *L. donovani* promastigotes (Pms) by flow cytometry (FACS). FACS analysis was performed to quantitatively assess fluorescence levels in treated and untreated groups. A decrease in fluorescence intensity indicated inhibition of parasite growth. J774 macrophages (5x10⁵ cells per well) in 12-well culture plates were infected with Pms at a ratio of 10:1. The infection level in infected macrophages before and after treatment with the preparation was measured using FACS.

All tests were performed in triplicate. The activity of the samples was also evaluated by Gimza staining [20].

Results and Discussion

Gas Chromatography-mass spectrometry (GC/MS) is a highly accurate analytical method that allows the qualitative and quantitative composition of essential oils to be determined. Using GC/MS, it is possible to characterize in detail all components present in essential oils, including major and minor components, which may also have biological activity and influence the aroma and properties of the oil.

GH/MS analysis of essential oils was conducted by literature data [4]. Retention indices were recalculated relative to normal hydrocarbons C₈-C₃₂.

In essential oil of *A. messerschmidtiana* was determined 43 components. The main components are 1,8-cineole (29.1 %), camphor (24.8 %), and α-campholenal (4.9 %) (Tab. 1).

Table 1

Component composition of the essential oil of *A. messerschmidtiana*

R _{calc}	Component	Area, %	R _{calc}	Component	Area, %
797	Hexanal	0,3	116	Borneol	2,3
843	2-Hexenal	0,2	1167	α-Santolin alcohol	1,9
916	Tricyclene	0,3	1168	Unidentified 2	0,8
926	α-Pinene	0,6	1169	α-Campholenal	4,9
941	Camphene	2,3	1174	Terpinene-4-ol	0,9

Continuation of Table 1

R _{calc}	Component	Area, %	R _{calc}	Component	Area, %
963	4(10)-Thujone	0.1	1182	<i>p</i> -Cymene-8-ol	0.9
985	1-Octen-3-ol	0.8	1188	α -Terpineol	0.4
1010	3,5,5-Tetramethyl-1,3-cyclohexadiene	0.3	1200	<i>trans</i> -Piperitol	0.2
1016	<i>o</i> -Diethylbenzene	0.2	1224	<i>trans</i> -Chrysoteny acetate	2.0
1020	<i>o</i> -Cymene	0.2	1278	Bornyl acetate	1.3
1033	1,8-Cineole	29.1	1288	Eucarvon	0.4
1052	γ -Terpinene	0.2	1307	5-Isopropenyl-2-methylcyclopent-1-ene-carboxaldehyde	0.1
1065	<i>cis</i> -Linalool oxide	0.1	1468	β -Eudesmene	0.2
1100	6-Methyl-3,5-heptadien-2-one	0.2	1562	Spatulenol	0.8
1100	Unidentified 1	8.1	1565	Caryophyllene oxide	1.2
1116	Chrysanthenone	0.4	1592	Gumulen-1,2-epoxide	0.1
1130	<i>cis</i> -2- <i>p</i> -Menten-1-ol	0.3	1646	Neointermedium	0.3
1133	<i>cis</i> -Chrysanthenol	0.5	1651	5 β ,10 α -Eudesm-11-en-4-ol	0.6
1147	Camphor	24.8	1680	(1 <i>R</i> ,7 <i>S</i> , <i>E</i>)-7-isopropyl-4,10-dimethylenecyclohex-5-enol	0.2
1148	<i>p</i> -Mentha-1,5-dien-8-ol	0.2	1794	Methyl ester of isocostonic acid	0.5
1155	Pinacurvone	1.0	1968	<i>n</i> -Hexadecanoic acid	0.3
1161	Isogeranial	0.2		Total	90.7

Testing on the anti microbial and fungicidal activity showed weak activity (Tab. 2).

Table 2

Anti microbial and fungicidal activity of *A. messerschmidtiana* essential oil

Essential oils / comparison preparation	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>	<i>A. fumigatus</i>	<i>C. neoformans</i>	<i>S. aureus</i>	MRS	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>M. intracellulare</i>	Test concentration (μ g/ml)
Amphotericin B	10	99	100	99	100	-	-	-	-	-	5
Ciprofloxacin	-	-	-	-	-	89	96	98	97	85	1
Essential oil	0	5	3	0	6	0	0	2	11	5	0

The essential oil from this plant has also been studied for its antimalarial activity and showed low activity with 40 % inhibition against *Plasmodium falciparum* D6 compared to the reference drug chloroquine (Tab. 3).

Table 3

Antimalarial activity of *A. messerschmidtiana* essential oil

Essential oils/comparison drug	<i>P. falciparum</i> D6 % Inh.
Chloroquine	9
Essential oil of <i>A. messerschmidtiana</i>	4

The essential oil of the plant was also studied for cytotoxic activity. All concentrations of essential oil demonstrated acute lethal toxicity, so, all larvae died.

The testing on anti tuberculosis activity (Tab. 4, 5) showed no significant effect.

Table 4

Activity of *A. messerschmidtiana* essential oil against mycobacteria (anti-tuberculosis activity) and yeast

Essential oils/comparison preparation	<i>M. tuberculosis</i> H37Rv ATCC 27294	<i>M. avium</i> ATCC 25291	<i>C. krusei</i> ATCC 6258	<i>C. parapsilosis</i> ATCC 22019
Essential oil <i>A. messerschmidtiana</i>	2000	>2000	>3000	>3000
Positive control	0.06 Isoniazid	>1.0 Isoniazid	1.0 Amphotericin B	0.5 Amphotericin B

Test concentration against *Mycobacterium* sp from 31.25 µg/ml to 2000 µg/ml. Concentration of reference preparations against *Mycobacterium* sp from 0.015 µg/ml to 1.0 µg/ml

Table 5

Activity of *A. messerschmidtiana* essential oil against bacteria

Essential oils/comparison preparation	<i>E. faecalis</i> ATCC 4082	<i>S. salivarius</i> ATCC 25975	<i>S. mitis</i> ATCC 49456	<i>S. mutans</i> ATCC 25175	<i>S. sanguinis</i> ATCC 10556	<i>S. sobrinus</i> ATCC 33478	<i>L. casei</i> ATCC 11578
Essential oil of <i>A. messerschmidtiana</i>	400	400	400	400	>400	400	400
Chlorhexidine dihydrochloride	7.375	1,844	7.375	0.922	3.688	1,844	3.688

Test concentration against yeast: 1.46 µg/ml to 3000 µg/ml. Concentration of reference products against yeast: 0.031 µg/ml to 16.0 µg/ml. Control: *C. parapsilosis* ATCC 22019—MIC value: 0.25–1.0 µg/ml. *C. krusei* ATCC 6258—MIC value: 0.25–2.0 µg/ml

The latest series of tests on *A. messerschmidtiana* essential oil showed no anti-leishmaniasis activity compared to existing drugs.

Conclusion

Thus, the studies conducted show that 43 components were found in the essential oil of *A. messerschmidtiana*. The major components were camphor, 1,8-cineole, and α -campholenal. Analysis of biological activities showed that the tested essential oil exhibited weak fungicidal, antimicrobial, and antimalarial effects, but high cytotoxic activity. No antituberculosis or antileishmanial activity was detected in the essential oil.

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Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Suleimen Ye.M.** — investigation, methodology, funding, writing-review & editing; **Mamytbekova G.K.** — conceptualization, essential oil isolation, antimicrobial and antifungal activity, data curation; **Serikbai G.** — antimicrobial activity; **Birimzhanova D.A.** — data curation, GS/MS data interpretation; **Doudkin R.V.** — investigation, methodology, plant material

collection; **Gorovoy P.G.** — investigation, methodology, plant material collection; **Ross S.** — GC/MS analysis; **Martins C.H.G.** — investigation of antituberculosis activity.

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***Artemisia messerschmidtiana* эфир майының құрамы мен биологиялық белсенділігі**

Дәрілік өсімдіктердің жаңа көздерін іздеу аясында эфир майларының химиялық құрамын зерттеу және олардың биологиялық белсенділігін бағалау маңызды міндет. Мақалада Қиыр Шығыстың *Artemisia messerschmidtiana* флорасынан бөлінген эфир майының құрамы мен биологиялық белсенділігін бағалау нәтижелері талқыланған. Эфир майы гидродистилляция арқылы бөлініп алынды. Алынған эфир майының микробқа, зеңге, безгекке, цитотоксикалық, туберкулезге және лейшманиозға қарсы белсенділігі тексерілді. Эфир майының құрамын хроматография-масс-спектрометрия көмегімен талдау 43 компоненттің бар екенін көрсетті. Максималды құрам 1,8-цинеол, камфора және α -камфоленал үшін белгіленді. Эфир майын сынау әлсіз микробқа, фунгицидтік пен безгекке қарсы және жоғары цитотоксикалық белсенділікті анықтауға мүмкіндік берді. Эфир майының туберкулезге қарсы немесе лейшманиозға қарсы белсенділігі анықталған жоқ. Алынған нәтижелер *Artemisia L.* туысының компоненттерінің биологиялық белсенділігін білуге ықпал етеді.

Кілт сөздер: *Artemisia messerschmidtiana*, эфир майы, биологиялық белсенділік, хроматографиялық масс-спектрометрия

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Состав и биологическая активность эфирного масла *Artemisia messerschmidtiana*

Изучение химического состава эфирных масел и оценка их биологической активности является важной задачей в свете поиска новых источников лекарственных растений. В настоящей статье рассматриваются итоги оценки состава и биологической активности эфирного масла, выделенного из *Artemisia messerschmidtiana* флоры Дальнего Востока. Эфирное масло было выделено методом гидродистилляции. Полученное эфирное масло было исследовано на антимикробную, антифунгальную, противомаларийную, цитотоксическую, антитуберкулезную и антилейшманиозную активность. Анализ состава эфирного масла с помощью хромато-масс-спектрометрии показал присутствие 43 компонентов. Максимальное содержание отмечено для 1,8-цинеола, камфоры и α -камфоленала. Тестирование эфирного масла позволило установить слабую антимикробную, фунгицидную и противомаларийную активность, а также высокую цитотоксическую. Противотуберкулезная и противолейшманиозная активность эфирного масла не выявлена. Полученные результаты вносят вклад в знание биологической активности компонентов рода *Artemisia L.*

Ключевые слова: *Artemisia messerschmidtiana*, эфирное масло, биологическая активность, хромато-масс-спектрометрия

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