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Component composition of *Achillea micrantha* essential oil and its biological activity

Data on study of the component composition and biological activity of the essential oil of *Achillea micrantha* Willd. (Asteraceae family) are presented in this article. The raw materials for the researching were collected at the territory of the Republic of Kazakhstan, Karaganda region during the flowering period. Essential oil of plant by hydrodistillation was obtained, the yield is 0.22 %. The component composition of essential oil was studied using GC/MS Clarus-SQ 8 (PerkinElmer). Antimicrobial, antifungal, antimalarial, cytotoxic, antiradical and anti-tuberculosis activities of essential oil were studied. Antibacterial and antifungal activity were identified using the strain of 5 human-pathogenic bacteria (*Staphylococcus aureus*, *St. aureus* (MRSa), *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium intracellulare*) and 5 fungi (*Candida albicans*, *C. glabrata*, *C. krusei*, *Aspergillus fumigates*, *Cryptococcus neoformans*). Antimalarial activity of the sample was tested for its ability to inhibit Plasmodium falciparum of protozoa. Cytotoxic activity carried out using test on larvae of *Artemia salina*. Determination of antiradical activity of essential oil was performed against 2,2-diphenyl-1-picrylhydrazyl (DPPH), as a reagent for comparison, gallic acid (GA) and butylhydroxyanisole (BHA). The tuberculosis activity of essential oil was determined on *Mycobacterium tuberculosis* H37Rv sensitive to all five first-line antituberculosis drugs (streptomycin, isoniazid, rifampicin, ethambutol and pirazinamide). As a result of research it was established that *A. micrantha* Willd essential oil doesn't possess or shows low degree of the above types of activity, shows lethal toxicity concerning *Artemia salina larvae* of crustaceans in all tested concentration (1–10 mg/ml).

Key words: *Achillea micrantha,* essential oil, GC/MS, antimicrobial, antifungal, antimalarial, cytotoxic, antiradical and anti-tuberculosis activities, *Artemia salina,* DPPH.

Introduction

The development of modern industry and rapid population growth in the coming years, puts the problem of finding new renewable resources: sources of power, biofuels, agricultural and medical products, cosmetics and personal care products; which will undoubtedly lead to increased interest in plant resources, on which you can obtain high-purity (quality) and biologically safe products.

Essential oils (EOs) are homogeneous mixtures of organic chemical compounds from the same chemical family; they are composed by terpenoids, especially monoterpenes and sesquiterpenes. Nevertheless, low molecular weight aliphatic compounds, acyclic esters or lactones may be present.

EOs of plants has been used traditionally for numerous applications in health-related areas, and in foods and commercial uses [1, 2]. In most medical applications the oils were applied directly to the skin, although the potential cytotoxicity of EOs precluded internal consumption [3]. This problem could, at least in theory, be avoided by inhalation of the vapors of EOs, as practiced in aromatherapy. Furthermore in many traditional remedies for colds and respiratory disorders, formulations often included plant EOs to provide relief through inhalation of the vapors [3].

Studies on the composition of the essential oils, isolation components from them, determination of biological activity and chemical modification carried out in scientific centers of United States, Russia, Turkey, Kazakhstan and others [4–7].

The Flora of Kazakhstan is rich one and about 5500 species was reported, and there are many aromatic plants among them. It is well known that, the composition of essential oils (EOs) can reach up to 200 components of different classes of chemical compounds. The EOs chemical composition is affected by some factors as species and subspecies, geographical location, harvest time, the part of the plant used and the extraction methods used to obtain the EO.

Achillea micrantha Willd. (Fam. *Asteraceae*) is a perennial grassy plant, 20–40 cm of high (Fig. 1). The plant is grayish-pubescent, timed to life on sands and sandy soils, at least — on the steppe and meadow communities. In Kazakhstan dwelling species mentioned in the following floristic regions: Tobol-Ishim, Caspian, Aktobe, Mugodzhar, Emba, Turgai, low hills west [4].

Figure 1. *Achillea micrantha* Willd.

In traditional medicine many people of the world widely apply different species of yarrow. In particular, grass of *A. micrantha* is used at gonorrhea, chronic diarrhea, acute respiratory diseases, the bleeding wounds, burns, anemia, for increase in lactation at nursing mothers [5].

Earlier the composition and antioxidant activity of *A. micrantha* essential oil from Iran [6] were studied, sesquiterpene lactones [7, 8] and flavonoids have been isolated [9].

The main physical properties of essential oil of *A. micrantha* were determined by authors of works [10]. In the [11] shows the data for the study of the biological activity of hydro-alcoholic extracts of *A. micrantha*.

Thus, *A. micrantha* and its essential oil are of great interest to researchers. In this report, we present the essential oil compositions for *A. micrantha* from Kazakhstan and data on it's biological activity.

Experimental part

Plant material for the study was collected in July 17, 2013 in Ulytau district of the Karaganda region in 90 km north of the city of Zhezkazgan in the phase of flowering. The plant is deposited in the herbarium of plants at the Zhezkazgan botanical garden. The number of a voucher specimen is 2011.06.15.04.01.

Essential oil was received from the dried crushed elevated mass of plants (stalks, leaves, flower baskets) by of steam distillation method on the «Alpha Midi» apparatus (Kaliningrad, «New Technologies» Ltd.) within 3 hours. The «Alpha Midi» apparatus consists from steam generator, tank for raw material, condenser and Florentine flask. The Apparatus can be used in two regimes: in stationary using electric power and can operate in the field on solid fuel (wood, coal). The «Alpha Midi» apparatus are given in Figure 2. The yield is 0.22 %.

Determination of component composition of *A. micrantha* essential oil was carried out on the Clarus-SQ 8 (PerkinElmer) Gas Chromatograph equipped with Mass-spectrometer (GC/MS apparatus).

Preparation of sample of essential oil: about 25 mg (exact weight) of essential oil *A. micrantha* placed into a 25 ml volumetric flask, dissolved in 15 ml of hexane, adjusted to volume and stirred until complete mixing of the oil.

Chromatographic conditions: capillary column — RestekRxi®-1 ms 0.25 mm × 30 m × 0.25 μm, sample volume: 1.0 μl, carrier gas — He, carrier gas speed: 1 ml/min, split ratio 1:25, t of column: 40 ºC, rise of 2 ºC/min to 280 ºC, t of evaporator — 280 ºC, mass spectrometric detection: t — 240 ºC, EI + = 70 eV, the scanning time from 4 to 120 minutes, the scan mode ion 39–500 m/z. The percentages of components are automatically calculated based on the total peak areas of the chromatogram of ions. Components were identified by mass spectra and the retention times, with use of NIST library.

Figure 2. The «Alpha Midi» apparatus

As shown in Table 1 the volatile composition of *A. micrantha* contains 1,8-cineole — 24.4 %, camphor — 12.4 %, camphene — 7,0 %, α-pinene — 5.7 %, sabinene — 5,5 %, o-cymol — 3.9 % and 4-terpineol — 2.8 % as main components.

Table 1

Component composition of essential oil of *A. micrantha*

RT	Compound	Content, %	RT	Compound	Content, %
4,55	Hexanal	0,3	23,117	Limona ketone	0,2
9,312	Santolina triene	0,7	23,305	cis-2-Menthenol	0,2
9,95	Cyclene	0,4	**23,998**	**Camphor**	**12,4**
10,314	β-Thujene	1,4	24,255	cis-Pinocarveol	0,3
10,647	**α-Pinene**	**5,7**	25,443	Pinocarvone	0,3
11,308	**Camphene**	**7,0**	26,478	endo-Borneol	2,3
12,823	**Sabinene**	**5,5**	26,786	cis-Chrysanthenol	0,3
12,944	β-Pinene	2,3	27,659	**4-Terpineol**	**2,8**
13,634	2,3-Dehydro-1,8-cineole	0,2	28,631	Terpineol	1,2
14,191	β-Myrcene	0,5	29,156	Myrtenol	0,2
14,797	α-Phellandrene	0,2	32,939	Piperitone	0,3
15,718	α-Terpinene	1,9	36,868	Bornyl acetate	0,3
15,963	**o-Cymol**	**3,9**	44,319	Cyclosativene	0,7
16,514	**1.8-Cineole**	**24,4**	45,298	Copaene	1,6
16,642	Limonene	0,9	45,746	β-Bourbonene	0,2
18,245	cis-β- Ocimene	0,5	48,307	Caryophyllene	0,3
18,81	γ-Terpinene	2,7	53,032	D-Germacrene	1,1
18,964	cis-Sabinene hydrate	0,7	54,21	γ-Gurjunene	0,2
21,059	α-Terpinolene	0,6	55,582	γ-Cadinene	0,2
21,228	trans-Sabinene hydrate	0,5	56,521	δ-Cadinene	0,6
22,292	α-Thujone	0,2	61,665	Copaborneol	0,9
22,472	2-Methylbutanoic acid 2-methylbutyl ester	0,2	64,655	β-Eudesmol	0,6
TOTAL					**87,6**

Data on studying of anti-malarial activity of essential oil (Table 2) was obtained for the first time. Antimalarial activity of the sample was tested for its ability to inhibit chloroquine-susceptible (D6) and/or

chloroquine-resistant (W2) *Plasmodium falciparum* of protozoa. The sample was tested twice in the first strains of *P. falciparum* D6. Percent inhibition (% Inh.) was counted in relation to the negative and positive control. Samples that showed ≥ 50 % inhibition sent to a secondary screening.

In the secondary screening, samples were dissolved in 20 mg/ml and tested for 47600, 15867 and 5289 ng/ml, and IC50s (test concentration in ng/ml, which gives 50 % inhibition of relatively simple negative and positive controls) and were performed in comparison with both D6 and W2 of strains. Samples were dissolved in 2 mg/ml and tested for 4760, 1587 and 529 ng/ml, and IC50s against both D6 and W2 strains. In addition to a strain of *P. falciparum*, the samples are tested in a mammalian cell line VERO, as an indicator of general overall cytotoxicity. Selectivity index (SI) — the ratio of VERO IC50 for D6 or W2 IC50 — was calculated. As a positive control were used antimalarial chloroquine and artemisinin.

Table 2

Data on the antimalarial activity of essential oil (primary screening)

The name of a plant — a source of essential oil	*P. falciparum* D6 % Inh.
A. micrantha	20

Primary screening showed that *A. micrantha* essential oil doesn't possess anti-malarial activity. Data on studying antimicrobial and anti-fungal activity of essential oil was obtained. The antimicrobial activity of essential oil was tested samples by their ability to inhibit growth a strain from 5 bacteria and 5 fungi which are pathogenic for the humans (Table 3).

Table 3

Strains from 5 bacteria and 5 fungi which are pathogenic for the humans

Bacteria	Fungus
Staphylococcus aureus	*Candida albicans*
Methicillin-resistant *Staphylococcus aureus* (MRSA)	*Candida glabrata*
Escherichia coli	*Candida krusei*
Pseudomonas aeruginosa	*Aspergillus fumigatus*
Mycobacterium intracellulare	*Cryptococcus neoformans*

In the beginning tested in primary screening for 50 μg/ml twice and growth inhibition percent (% Ing.) was calculated in relation to negative and positive control. Essential oil showing ≥50 % of inhibition directed to secondary screening.

In secondary screening samples dissolved in 20 mg/ml and checked at 50, 10 and 2 μg/ml and IC50s against all 10 strains of microorganisms. Samples dissolved in 2 mg/ml and carried out tests for 20, 4, 0.8 μg/ml and IC50s against all 10 strains of microorganisms. 7 μg/ml on secondary screening directed pure connections which have IC50 ≤ on tertiary screening.

The secondary screening samples were dissolved in 20 mg/ml and tested at 50, 10 and 2 μg/ml and IC50s against all 10 strains of microorganisms. Samples were dissolved in 2 mg/ml, and the test was conducted by 20, 4, 0.8 ug/ml and IC50s against all 10 strains of microorganisms. The antifungal agent — amphotericin B was used as a control, and as an antibacterial — ciprofloxacin. The results of primary screening showed low antimicrobial and antifungal activity of essential oil of *A. micrantha* (Table 4).

Table 4

Data on antimicrobial and anti-fungal activity of essential oil of A. micrantha (primary screening)

The name of a plant — a source of essential oil and a comparison preparation/ strains	*C. albicans* % Inh.	*C. glabrata* % Inh.	*C. krusei* % Inh.	*A. fumigatus* % Inh.	*C. neoformans* % Inh.	*S. aureus* % Inh.	*MRSA* % Inh.	*E. coli* % Inh.	*P. aeruginosa* % Inh.	*M.Intracellulare* % Inh.
Amphotericin B	98	98	97	99	98	ND	ND	ND	ND	ND
Ciprofloxacin	ND	ND	ND	ND	ND	93	99	100	95	76
A. *micrantha*	2	2	0	0	10	0	2	1	3	1

Determination of antiradical activity of essential oil. Studies antiradical activity of essential oil was performed with respect 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Absorbance analytes dependent on the concentration measured on a spectrophotometer Cary 60 UV-Vis at 520 nm wavelength. Antiradical activity of essential oil, we compared with gallic acid (GC) and butylhydroxyanisole (BHA). The values of antiradical activity (ARA) were calculated using the formula shown below:

$$\text{ARA } (\%) = (A_0 - A_t)/A_0 \times 100\ \%.$$

Here A_0 — optical density control; A_t — the optical density of the working sample [12].

DPPH molecule forms a free radical that is stable in the different environments and wide temperature range, due to the maximum freedom of the electron delocalization over the entire molecule and spatial shielding atoms bearing the greatest spin density as well as the lack of hydrogen atoms in the positions where the isomerization may occur or disproportionation. In addition, delocalization is causing intense violet color of this radical in the aqueous-alcoholic media, the interaction with the antioxidant, capable of donating a proton, there is a restoration of the radical, resulting in the violet color turns into yellow.

The experimental data show that the essential oil of *A. micrantha* showed middle antiradical activity (Tables 5, 6).

T a b l e 5

Change of optical density from concentration

No	Sample	Values of optical density depending on concentration (mg/ml)				
		0,1	0,25	0,5	0,75	1,0
1	BHA	0,1278	0,1240	0,1260	0,1250	0,1240
2	*A. micrantha* Willd.	0,2686	0,3038	0,3194	0,3021	0,4537

T a b l e 6

Antiradical activity of essential oil in different concentrations (%)

No	Sample	The concentrations of essential oil (mg/ml)				
		0,1	0,25	0,5	0,75	1,0
1	BHA	80,0	80,7	80,3	80,5	80,7
2	*A. micrantha* Willd.	49,41	42,78	39,83	43,09	14,55

Determination of the cytotoxic activity of essential oil was carried out for the first time.

Separating funnel filled with 55 ml of artificial sea water and 200 mg of eggs *Artemia salina*. Allowed standing for 3 days at the air supply until soft crustaceans gave the egg. One side of the tube covered with aluminum foil and 5 minutes later, the larvae that are going on the bright side of the funnel, removed Pasteur pipette.

20–40 larvae were placed in 990 μl of seawater into each of the 24 micro titer plates. Dead larvae were counted under a microscope. Added 10 μl of dimethylsulfoxide solution of 10 mg/ml sample. As a comparison, the drug actinomycin D or staurosporine. For a negative control 10 μl was added only DMSO. After 24 h of incubation and further maintaining micro titer plates for 24 hours (to ensure immobility) counts the dead larvae under the microscope.

Mortality P determined by the following formula:

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

Here A — amount of dead larvae after 24 h; N — amount of larvae died before the test; B — the average amount of larvae died in a negative control; Z — the total amount of larvae [13].

Results of the study the cytotoxic activity of essential oils are shown in Table 7.

Based on this experiment it can be assumed that the essential oil of *A. micrantha* in all concentrations tested exhibit acute lethal toxicity — all larvae are died.

Table 7

The cytotoxic activity of essential oils of *A. micrantha*

Parallel	The amount of larvae in the control		The amount of larvae in a sample			The amount of surviving larvae in the control, %	The amount of surviving larvae in sample, %	Mortality, *P*, %	The percentage of neurotoxicity, %
	survivors	died	survivors	died	paralyzed				
10 mg/ml									
1	22	0	0	23	0	96	0	96	0
2	25	1	0	26	0				
3	24	2	0	26	0				
Medium	24	1	0	25	0				
5 mg/ml									
1	22	0	0	29	0	96	0	96	0
2	25	1	0	23	0				
3	24	2	0	25	0				
Medium	24	1	0	26	0				
1 mg/ml									
1	22	0	0	25	0	96	0	96	0
2	25	1	0	22	0				
3	24	2	0	20	0				
Medium	24	1	0	22	0				

Also essential oil *A. micrantha* was tested for activity against aerobic microorganisms, mycobacterium, and yeast according to [14–18] for the first time. It is found that the essential oil of *A. micrantha* has no activity against aerobic microorganisms, mycobacterium (anti-tuberculosis), yeast and mold.

Conclusions

Thus, during the researches the chemical composition of *A. micrantha* Willd. essential oil was determined. It is found that the composition of the essential oil is dominated by the following substances: 1,8-cineole — 24.4 %, camphor — 12.4 %, camphene — 7.0 %, α-pinene — 5.7 %, sabinene — 5.5 %, o-cymol — 3.9 % and 4-terpineol — 2.8 %. Flowers essential oil of *A. micrantha* from Iran [6] were characterized by higher amounts of binapacryle — 83.6 %, 1,8-cineol — 3.8 % and α-selinene — 4.5 %.

It is experimentally proved that the essential oil of *A. micrantha* Willd. does not possess antimicrobial, anti-tuberculosis, antifungal, antimalarial activity. The experimental data show that the essential oil of *A. micrantha* showed middle antiradical activity. The essential oil of *A. micrantha* from Iran [6] had more antioxidant activity with (IC50 0.184±0.0475 μg/ml in dry weight in same method). It was determined that the essential oil *A. micrantha* Willd. had a high cytotoxic activity.

Investigations of anti-tuberculosis, antifungal, antimalarial and cytotoxic activity were carried out for the first time.

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Achillea micrantha эфир майының компоненттік құрамы және оның биологиялық белсенділігі

Мақалада *Achillea micrantha* Willd. (күрделігүлділер тұқымдастығы) өсімдігі эфир майының компоненттік құрамы және биологиялық белсенділігі бойынша мәліметтер келтірілген. Зерттеуге алынған өсімдік шикізаты Қазақстан Республикасының Қарағанды аймағында гүлдеу кезеңінде жиналған. Өсімдіктің эфир майы сулы дистилляция әдісімен алынды, шығымы 0,22 %-ды құрады. Эфир майының компоненттік құрамы Clarus-SQ 8 (PerkinElmer) масс-спектрометриялық детекторлы газдық хроматограф аспабы көмегімен анықталды. Эфир майының микробтарға, зеңге, безгекке қарсы, цитоуыттылық, радикалдарға және туберкулезге қарсы белсенділік түрлері зерттелді. Микробқа және зеңге қарсы белсенділіктер адам үшін қауіпті саналатын 5 бактерия (*Staphylococcus aureus*, *St. aureus* (MRSa), *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium intracellulare*) және 5 зең (*Candida albicans*, *C. glabrata*, *C. krusei*, *Aspergillus fumigates*, *Cryptococcus neoformans*) штаммдарын пайдалану арқылы анықталды. Безгекке қарсы белсенділік *Plasmodium falciparum* D6 қарапайымдарын басу қабілеті бойынша зерттелді. Цитотоксикалық белсенділік *Artemia salina* дернәсілдеріне қатысты сынақ бойынша анықталды. Эфир майының радикалға қарсы белсенділігі 2,2-дифенил-1-пикрилгидразил затына қатысты зерттелді, салыстыру реагенті ретінде галл қышқылы және бутилгидроксианизол қолданылды. Эфир майының туберкулезге қарсы белсенділігі бірінші қатардағы барлық бес препарат (стрептомицин, изониазид, рифампицин, этамбутол және пиразинамид) бойынша сезімтал саналатын H37Rv туберкулез микобактериясына қатысты зерттелді. Зерттеу нәтижесінде *A. micrantha* Willd эфир майының жоғарыда келтірілген белсенділік түрлерін көрсетпейтіндігі немесе төмен дәрежеде көрсететіндігі анықталды. Эфир майы *Artemia salina* дернәсілдеріне қатысты барлық сыналған концентрация мәндерінде (1–10 мг/мл) жоғары улылық көрсетеді.

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Компонентный состав эфирного масла *Achillea micrantha* и его биологическая активность

В статье приведены данные по исследованию компонентного состава и биологической активности эфирного масла *Achillea micrantha* Willd. (семейство сложноцветных). Растительное сырье для исследования было собрано в Карагандинской области в период цветения. Эфирное масло растения было получено с помощью метода гидродистилляции, выход составил 0,22 %. Компонентный состав эфирного масла изучен с помощью газового хроматографа с масс-спектрометрическим детектором Clarus-SQ 8 (PerkinElmer). Изучены антимикробная, противогрибковая, противомалярийная, цитотоксическая, антирадикальная и противотуберкулезная активности эфирного масла. Антимикробная и антигрибковая активность была определена с использованием штаммов патогенных для человека 5 бактерий (*Staphylococcus aureus*, *St. aureus* (MRSa), *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium intracellulare*) и 5 грибов (*Candida albicans*, *C. glabrata*, *C. krusei*, *Aspergillus fumigates*, *Cryptococcus neoformans*). Антималярийная активность образцов была определена по их активности к ингибированию простейших *Plasmodium falciparum* D6. Цитотоксическая активность проведена с использованием теста на личинках рачков *Artemia salina.* Определение антирадикальной активности эфирного масла проводили по отношению к 2,2-дифенил-1-пикрилгидразилу, в качестве реагента для сравнения использовали галловую кислоту и бутилгидроксианизол. Туберкулезная активность эфирного масла была определена на микобактерии туберкулеза H37Rv, который чувствителен для всех пяти туберкулезных препаратов первого ряда (стрептомицин, изониазид, рифампицин, этамбутол и пиразинамид). В результате исследования установлено, что эфирное масло *A. micrantha* Willd. не обладает или обладает низкой степенью приведенных выше видов активности, проявляет летальную токсичность в отношении личинок *Artemia salina* во всех испытанных концентрациях (1–10 мг/мл).