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Component composition of *Achillea salicifolia* Besser essential oil and its biological activity

In the present article the component composition and biological activity of the essential oil of *Achillea salicifolia* Besser (*Asteraceae* Family) were studied. The raw material of plant was collected during the flowering period in the Akmola region of the Republic of Kazakhstan. The plant materials were dried at the shade and their essential oils were obtained by hydrodistillation using Clevenger-type device, the yield was 0.34 %. The component composition of essential oil was analyzed using GC/MS Clarus-SQ 8 (Perkin Elmer). Anti-radical activity of essential oil was evaluated according to 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (GA) and butylhydroxyanisole (BHA) were used as comparison reagents. Cytotoxic activity was carried out using test on larvae of *Artemia salina*. Antibacterial effect of EO was evaluated *in vitro* against 3 pathogenic bacteria species, namely gram-positive — *Staphylococcus aureus* 6532, *Bacillus cereus*, gram-negative — *Salmonella enteridis* and microscope fungi *Candida albicans* SC5314. Forty seven components representing 91.2 % composition of the essential oil were characterized. The main components of the oil were α-thujone (43.0 %), 1,8-cineole (11.0 %), terpinen-4-ol (5.3 %), camphor (5.3 %) and sabinene (3.1 %). According to the results of DPPH assay, *A. salicifolia* showed low antiradical activity comparing with BHA and lethal toxicity concerning crustaceans of *Artemia salina* larvae in all tested concentrations (1–10 mg·ml^{-1}).

Keywords: Achillea salicifolia Besser, essential oil, gas chromatography–mass spectrometry, antimicrobial, cytotoxic and anti-radical activities, *Artemia salina,* 2,2-diphenyl-1-picrylhydrazyl.

Introduction

Achillea L. is one of the most important genera of the *Asteraceae* (*Compositae*) family, which includes over 120 species. This genus is widely distributed in Europe, Asia and Northern Africa, and is naturalized in other parts of the world [1]. *Achillea* species have been previously reported with pharmaceutical useful properties, such as antioxidant, antimicrobial [2], spasmolytic, antidiabetic, antiulcer, anti-tumor, choleretic, hepatoprotective activity and cytotoxic effects [3–9].

The *Achillea* genus has a wide distributional range, and the differences in oil composition may be affected by different environmental factors such as plant genetic type, seasonality, and developmental stage, because it is a chemically polymorphic and perennial plant. Terpenoids (1,8-cineole, camphor, borneol, pinenes, artemisia ketone, santolina alcohol, farnesane, caryophyllene and its oxides, cubebene, germacrenes, eudesmol, α-bisabolol and oxides, farnesene, γ-gurjunene, γ-muurolene and chamazulene) are the main components of *Achillea*'s essential oils [10].

The component composition and antimicrobial activity of essential oil of *A. salicifolia* Besser (collected from Ardahan between Gole, Turkey) were studied by Turkish researchers. The study showed that the main components of essential oil were camphor (55.3 %), 1,8-cineole (22.8 %), 2,5,5-trimethyl-3,6-heptadien-2-ol (4.4 %), camphene (3.2 %), artemisia alcohol (3.2 %), terpinene-4-ol (3.0 %), α-terpineol (2.5 %) and bornyl acetate (2.0 %). The essential oil showed weak antifungal activity and effectiveness against a wide spectrum of microorganisms and *Candida albicans.* The authors explained this effect to the content the compounds — camphor and 1,8-cineole in the oil, which were known as antimicrobial agents [11].

The aim of present study is to investigate the component composition of essential oil of *A. salicifolia* Besser, collected in Kazakstan, and compare with the results from Turkey; and to make a conclusion how the differences of components have influence on biological activity. Also we included results from antiradical and cytotoxic activity of the essential oil, which have not been reported before.

Репозиторий КарГУ

Experimental

Aerial parts of *Achillea salicifolia* were collected in Akmola region of the Republic of Kazakhstan on August 19, 2017. The voucher specimen was prepared and deposited in Herbarium of the Biological and Geographical Faculty of Buketov Karaganda State University (N1984.08.14.01.01).

The essential oil was extracted from the dried leaves and flowers using a Clevenger-type water distillation apparatus for 2 hours. The yield was 0.34 %. Determination of component composition of *A. salicifolia* 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. salicifolia* 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 — Restek Rxi®-1 ms 0.25 mm × 30 m × 0.25 µm, sample volume: 1.0 µl, carrier gas — He, carrier gas speed: 1 ml min^{-1}, split ratio 1:25, temperature of column: 40 ºC, rise of 2 ºC min^{-1} to 280 ºC, temperature of evaporator — 280 ºC, mass spectrometric detection: temperature — 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 (Fig. 1). Components were identified by mass spectra and the retention times, with use of NIST library.

As shown in Table 1 the volatile composition of *A. salicifolia* contains α-thujone — 43.0 %, 1,8-cineole — 11.0 %, camphor — 5.3 %, terpinen-4-ol — 5.3 % as main components. Mostly main components belong to monoterpenoids.

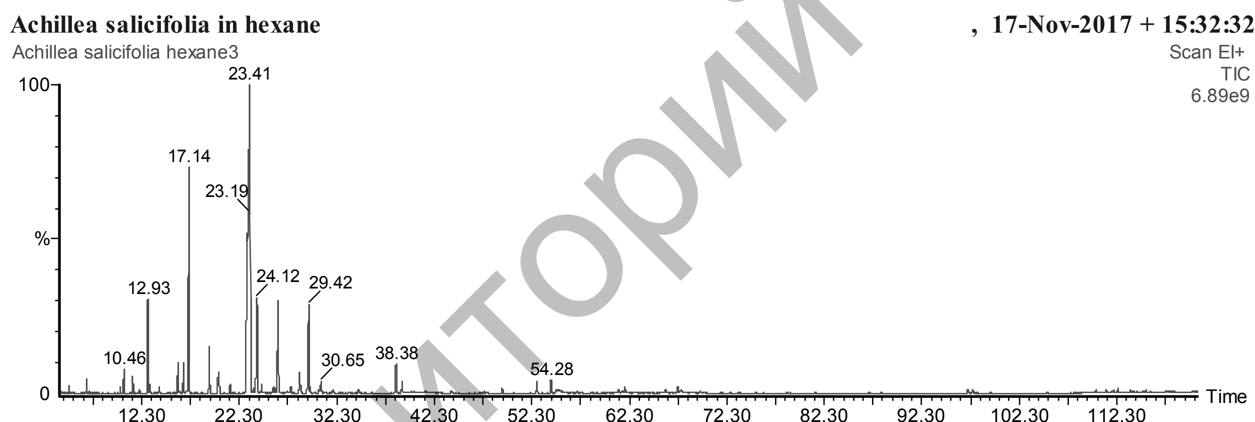

Figure 1. Chromotogram of GC/MS experiment of the essential oil of *A. salicifolia*

Table 1

Component composition of essential oil of *A. salicifolia*

R_{lit}	R_{calc}	Compound	Area, %	R_{lit}	R_{calc}	Compound	Area, %
1	2	3	4	5	6	7	8
800±2	797	*n*-Hexanal	0.1	1167±2	1160	Borneol	1.1
821	842	Cyclopropane, 1,1-dimethyl-2-(2-methyl-2-propenyl)-	0.3	1177±2	1170	**Terpinen-4-ol**	**5.3**
929±2	919	α-Thujene	0.2	1190±N/A	1171	α-Thujenal	0.2
929±7	925	α-Pinene	0.7	1193±3	1181	Myrtenal	0.1
952±2	939	Camphene	0.5	1189±2	1183	α-Terpineol	0.9
962±3	950	Benzaldehyde	0.1	1208±3	1197	*trans*-Piperitol	0.2
974±2	963	**Sabinene**	**3.1**	1239±3	1228	*p*-Isopropylbenzaldehyde	0.2
943	966	β-Pinene	0.3	1285±3	1275	Bornyl acetate	1.5
991±2	980	2,3-Dehydro-1,8-cineole	0.2	1297±N/A	1283	*trans*-Sabinyl acetate	0.5
1017±2	1009	α-Terpinene	1.2	1357±3	1343	Eugenol	0.1
1025±2	1016	*p*-Cymene	1.2	1419±3	1405	Caryophyllene	0.2
1032±2	1023	**1,8-Cineole**	**11.0**	1457±2	1445	*(E)*-β-Famesene	0.6

Репозиторий КарГУ

Continuation of Table 1

1	2	3	4	5	6	7	8
1060±3	1050	γ-Terpinene	1.9	1481±3	1462	Germacrene D	0.7
1070±4	1063	*cis*-Sabinene hydrate	1.6	1471±24	1475	Elixene	0.1
1088±2	1078	Terpinolene	0.4	1509±3	1491	β-Bisabolene	0.1
1103±2	1104	**α-Thujone**	**43.0**	1569 iu	1552	Longipinocarvone	0.2
1107±2	1108	2-Methylbutyl isovalerate	0.2	1576±2	1557	Spathulenol	0.1
1103±2	1112	**α-Thujone**	**5.3**	1581±2	1561	Caryophyllene oxide	0.3
1122±3	1117	*cis*-2-Menthenol	0.4	1637±4	1623	Caryophylladienol II	0.2
1139±2	1130	*trans*-Pinocarveol	0.3	1649±2	1641	β-Eudesmol	0.3
1143±0	1132	*cis*-Sabinol	0.2	1695±N/A	1674	*(1R,7S, E)*-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	0.1
1143±9	1136	Camphor	**5.3**	2092±4	2087	Methyl linoleate	0.2
1164±N/A	1150	Pinocarvone	0.3	2091±7	2096	Methyl oleate	0.2
Total							**91.2**

Determination of antiradical activity of essential oil

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

$$ARA\ (\%) = (A_0 - A_t) / A_0 * 100\ \%,$$

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

DPPH molecule forms a free radical that is stable in the different environments and wide range temperature, 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 may occur the isomerization 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 results of the experiment showed that the essential oil of *A. salicifolia* has low antiradical activity (Tables 2, 3).

Table 2

The change in optical density depending on the concentration

No	Sample	Values of optical density depending on concentration, mg·ml^{-1}				
		0.1	0.25	0.5	0.75	1.0
1	BHA	0.1362	0.1333	0.1257	0.1202	0.1145
2	*A. salicifolia* (aerial part)	0.7376	0.7106	0.6430	0.6130	0.5870

Table 3

Antiradical activity of essential oil in various concentrations, %

No	Sample	The concentrations of essential oil, mg·ml^{-1}				
		0.1	0.25	0.5	0.75	1.0
1	BHA	80.82	81.23	82.30	83.08	83.88
2	*A.salicifolia* (aerial part)	3.62	7.14	15.99	19.91	23.30

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

The 55 ml separator funnel was filled with artificial sea water and added 200 mg eggs of *Artemia salina*. Then, it was kept with a soft supply of air for three days, until the crustaceans hatch from eggs. The one side of funnel was covered with aluminum foil, and after 5 minutes, the larvae, which moved on the bright side of the separator funnel were removed with a Pasteur pipette.

20–40 Larvae were placed into each of the 24 micro titer plates with 990 µl of seawater. Dead larvae were counted under a microscope. 10 µl of dimethylsulfoxide solution per 10 mg·ml^{-1} sample was added. Actinomycin D or staurosporine was used as a standard comparison reagent, and DMSO was a negative con-

Репозиторий КарГУ

trol. After 24 h of incubation and further maintaining micro titer plates for 24 hours (to ensure immobility) the dead larvae were counted under the microscope.

Mortality P was determined by the following formula:

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

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

Results of the study the cytotoxic activity of essential oils are shown in Table 4.

Table 4

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

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, %	Mortal-ity, P,%	The percentage of neurotoxicity, %
	sur-vivors	died	sur-vivors	died	paralyzed				
10 mg·ml^{-1}									
1	24	1	0	24	0	96	0	96	0
2	26	2	0	26	0				
3	23	0	0	28	0				
Average	24	1	0	26	0				
5 mg·ml^{-1}									
1	24	1	0	26	0	96	0	96	0
2	26	2	0	23	0				
3	23	0	0	32	0				
Average	24	1	0	27	0				
1 mg·ml^{-1}									
1	24	1	0	21	0	96	0	96	0
2	26	2	0	27	0				
3	23	0	0	27	0				
Average	24	1	0	25	0				

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

The antimicrobial assay was performed a broth microdilution method against 3 bacterial strains; i.e. gram-positive — *Staphylococcus aureus* 6532, *Bacillus cereus*, gram-negative — *Salmonella enteridis* and *Candida albicans* SC5314. Ampicillin and fluconazole standard antibiotics were used as a positive and DMSO as a negative controls. The stock solution was prepared in DMSO and concentration was 50 mg ml^{-1}. The final concentration was varied from 2.5 mg·ml^{-1} till 0.2 mg·ml^{-1} for bacterial strains and from 1.25 mg·ml^{-1} till 0.1 mg ml^{-1} for *Candida albicans*. The lowest concentration that inhibits growth was determined as MIC value.

Table 5

Antimicrobial activity of essential oil of *A. salicifolia*, mg·ml^{-1}

Microorganisms	MIC		
	EO	Ampicillin	Fluconazole
Staphylococcus aureus 6532	1.25	0.25	-
Bacillus cereus	1.25	0.25	-
Salmonella enteritidis	0.63	0.25	-
Candida albicans SC 5314	0.31	-	0.066

The results of antimicrobial activity showed that against gram-positive bacterial strains MIC of essential oil was 1.25 mg·ml^{-1}, against gram-negative 0.63 mg·ml^{-1} and 0.31 mg·ml^{-1} against *Candida albicans* SC 5314 respectively. Thus, the essential oil was effective against *Candida albicans* SC 5314 (0.31 mg·ml^{-1}).

Репозиторий КарГУ

Conclusions

We have investigated the chemical composition, antiradical, cytotoxic and antimicrobial activities of the essential oil of *A. salicifolia* wild growing in Akmola region (Kazakhstan). The essential oil possessed quite different chemical composition as compared with the oil composition of the same species reported in previously published study. The main constitute in the essential oil were α-thujone — 43.0 %, 1,8-cineole — 11.0 %, camphor — 5.3 %, terpinen-4-ol — 5.3 % while the main constitute of the oil from other study was camphor — 55.3 %, 1,8-cineole — 22.8 %, 2,5,5-trimethyl-3,6-heptadien-2-ol — 4.4 %, camphene — 3.2 %, artemisia alcohol — 3.2 %, terpinene-4-ol — 3.0 %, α-terpineol — 2.5 % and bornyl acetate — 2.0 % [11]. The yield of essential oil was 0.34 %, it was higher than essential oil from Turkey (0.08 %). Antimicrobial tests provided information that essential oil had low activity against gram-positive (MIC — 1.25 $mg \cdot ml^{-1}$) and gram-negative (MIC — 0.63 $mg \cdot ml^{-1}$) bacterial strains and significant effective against *Candida albicans* SC 5314 (MIC — 0.31 $mg \cdot ml^{-1}$) as comparing with previous study. It can be assumed that low antimicrobial activity is associated with a low content of camphor and 1,8-cineole.

In present study antioxidant and cytotoxic tests results of the essential oil of *A. salicifolia* were reported for the first time. The essential oil showed lethal toxicity on *Artemia salina* larvae; and low antiradical activity in all concentrations tested. These studies confirmed that the essential oil of the plant can be different in quantity and quality according to geographical and environmental conditions and the period of plant growth and proceeding from this fact the different level biological activity.

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Репозиторий КарГУ

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Achillea salicifolia Besser эфир майының компоненттік құрамы және оның биологиялық белсенділігі

Мақалада *Achilllea salicifolia* Besser (*Asteraceae* тұқымдастығы) өсімдігі эфир майының компоненттік құрамы және биологиялық белсенділігінің зерттеу нәтижелері келтірілген. *Achilllea salicifolia* Besser өсімдік шикізаты гүлдену кезеңінде Қазақстан Республикасының Ақмола облысында жиналған. Эфир майы сулы дистилляция әдісімен алынды, шығымы 0,34 % құрады. Эфир майының компоненттік құрамы Clarus-SQ 8 (PerkinElmer) масс-спектрометриялық детекторымен қамтылған хроматограф көмегімен анықталған. Сонымен қатар эфир майы радикалға, микробқа қарсы және цитоуыттылық белсенділігі зерттелді. Эфир майының цитоуыттылық белсенділігі *Artemia salina* дернәсілдерінде анықталды. Радикалға қарсы белсенділігі 2,2-дифенил-1-пикрилгидразил затына қатысты зерттелді, салыстыру реагенті ретінде галл қышқылы және бутилгидроксианизол қолданылды. Микробқа қарсы белсенділік үш түрлі патогенді бактерияларға: грамоң — *Staphylococcus aureus* 6532, *Bacillus cereus*; грамтеріс — *Salmonella enteridis* және *Candida albicans* SC5314 қолдана анықталды. Зерттеулер нәтижесінде *Achilllea salicifolia* Besser эфир майының негізгі компоненттері (47 компонент) 91,2 % құрайтын, туйон (43,0 %), 1,8-цинеол (11,0 %), терпинен-4-ол (5,3 %), камфора (5,3 %) және сабинен (3,1 %) болды. Эфир майынның *Artemia salina* дернәсілдеріне қатысты барлық сыналған концентрация мәндерінде (1–10 мг·мл$^{-1}$) жоғары улылық және төмен радикалға қарсы белсенділікті көрсетті.

Кілт сөздер: *Achillea salicifolia* Besser, эфир майы, газ хроматография–масс-спектрометрия, микробқа қарсы, цитоуыттылық және радикалға қарсы белсенділіктер, *Artemia salina,* 2,2-дифенил-1-пикрилгидразил.

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Компонентный состав эфирного масла *Achillea salicifolia* Besser и его биологическая активность

В статье даны результаты исследования компонентного состава и биологической активности эфирного масла *Achilllea salicifolia* Besser (семейства *Asteraceae*). Растительное сырье было собрано в период цветения в Акмолинской области Республики Казахстан. Эфирное масло было получено методом гидродистилляции, выход продукта составил 0,34 %. Компонентный состав эфирного масла изучен с помощью газового хроматографа с масс-спектрометрическим детектором Clarus-SQ 8 (Perkin Elmer). Также была изучена антирадикальная, антимикробная и цитотоксическая активность эфирного масла. Определение антирадикальной активности эфирного масла проводили по отношению к 2,2-дифенил-1-пикрилгидразилу, в качестве реагента сравнения использовали галловую кислоту и бутилгидроксианизол. Цитотоксическая активность проведена с использованием теста на личинках рачков *Artemia salina*. Антимикробную активность эфирного масла оценивали против трех видов патогенных бактерий: грамположительные — *Staphylococcus aureus* 6532, *Bacillus cereus*, грамотрицательные — *Salmonella enteridis* и *Candida albicans* SC5314. В результате проведенных исследований установлено, что основными компонентами эфирного масла (47 компонентов, составляющих 91.2 %), были α-туйон (43.0 %), 1.8-цинеол (11.0 %), терпинен-4-ол (5.3 %), камфора (5.3 %) и сабинен (3.1 %). Эфирное масло *Achilllea salicifolia* Besser проявляет летальную токсичность в отношении личинок *Artemia salina* во всех испытанных концентрациях (1–10 мг·мл$^{-1}$) и обладает низкой антирадикальной активностью.

Ключевые слова: Achillea salicifolia Besser, эфирное масло, газовая хроматография–масс-спектрометрия, антимикробная, цитотоксическая и антирадикальная активности, *Artemia salina,* 2,2-дифенил-1-пикрилгидразил.

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