Research Paper

Chemical Composition and Antibacterial Properties of *Achillea micrantha*

O. ASTAFYEVA, L. SUKHENKO, E. KURASHOV[1,2], J. KRYLOVA[2,3], M. EGOROV, Y. BATAEVA AND A. BAIMUKHAMBETOVA

Department of Biotechnology, Faculty of Biology, Astrakhan State University, pl. Shaumyana 1, 414000, Astrakhan, [1]Laboratory of Hydrobiology of Institute of Limnology, Russian Academy of Sciences, ul.Sevastianova, 9, St. Petersburg, 196105, [2]Department of Ecology and Technosphere Safety, ITMO University, pr.Kronverskyi, 49, St. Petersburg, 197101, [3]Laboratory of Environmental Toxicology, Berg State Research Institute on Lake and River Fisheries, nab. Makarova, 26, 199053, St.Petersburg, Russia

The composition of detectable small organic compounds in the ethanol extract of *Achillea micrantha* was defined by means of gas chromatography-mass spectrometric analysis. There were 71 low molecular weight organic compounds observed, two of which remained unidentified. The antibacterial activity of the extract was studied in respect to *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* using the agar diffusion test and serial dilutions to define minimum inhibitory concentration. In order to compare the antibacterial activity of the herb and blossom truss extract of *Achillea micrantha*, the extracts of *Achillea millefolium* and *Achillea leptophylla* was used. In relation to the microorganisms tested, a significant inhibitory effect was observed with the aqueous alcoholic extract of *Achillea micrantha* at the minimum inhibitory concentration of 0.05 µg/ml.

Key words: *Achillea micrantha*, low molecular weight organic compounds, gas chromatography-mass spectrometry, antibacterial activity

The genus *Achillea* of compositae (Asteraceae) contains about 150 species. It vegetates mostly on the territory of Europe and Western Asia as well as in Australia, New Zealand and North America[1]. Different scientists managed to extract some chemical substances from the blossom truss and herb of yarrow, *Achillea millefolium* L., such as lactones, achillin, artillizin, grossmizin, michrantin, kaempferol 3-rhamnoside, campesterol[2,3], sesquiterpene lactones, sinthenin and micrantin[4,5], other various components of essential oils[6,7] and flavonoids[8].

The flavonoid content in the yarrow was considered to make antipyretic and haemostatic drugs[9]. In ethnomedicine, the herb and tips of flowering plants (anthodes) are used as a haemostatic agent in cases of internal haemorrhage, gastric disorders, external haemorrhage, inflammatory processes and metabolopathy[10-12]. There were reports of antioxidant activity of the extracts and essential oils of the plants sp. *Achillea*[13-15], as well as estrogenic[16], antiulcer[17,18], antitumoral[19], antisecretory (inhibiting intestinal motility)[20], immunomodulatory[21], fibrinogenic[22], antimicrobial[23-27], antifungal[28], and antiinflammatory[29,30] activities. In traditional medicine, in contrast to *A. micrantha* Willd., the herb and blossom truss of *A. millefolium*, the chemical composition of which has been properly studied is mostly used. Nevertheless, literature reported that the antimicrobial effect of the extracts *A. micrantha* was much higher than that of *A. millefolium*[23]. The aim of this paper was to examine the chemical composition of the extracts of *A. micrantha* vegetative elements and blossom truss and their antibacterial activity in comparison with the extracts of other *Achillea* species.

MATERIALS AND METHODS

Gas chromatography/mass spectrometry (GC/MS) analysis:

The low-molecular weight organic compounds (LMWOC) of the extracts were defined by methods of

***Address for correspondence**
E-mail: astra39@list.ru

Accepted 15 March 2018
Revised 03 August 2017
Received 07 September 2016
Indian J Pharm Sci 2018;80(3):434-441

GC/MS with the help of Shimadzu GC/MS (QP-5050A, Shimadzu, Japan) equipped with the software Class 5000 and chromato-mass spectrometer TRACE DSQ II (Thermo Electron Corporation) with quadrupole mass analyser. In the first case, the column of DBI, 30 m; 0.53 mm ID и 1.5 μm film (J&W Scientific) was used. As a mobile phase the carrier gas helium was applied. Ionization mode- EI, ionization voltage –70 ev. Temperature program: 5° (1 min)-150° (1 min) at 10°/min-250° (5 min) at 5°/min-270° (2 min) at 3.5°/min. Detector temperature was set at 250° and the injector temperature was set at 280°.

In the second one, before the analysis started the preparation was 200-fold attenuated and transformed into hexane. The constitution of the compounds in the solution obtained was detected by means of the chromato-mass spectrometer with quadrupole mass analyser. The column Thermo TR-5 ms SQC 15 м×0.25 mm phase ID 0.25 micron was applied. As the carrier gas helium was used. The mass spectra were tested in mode of scanning on mass full-scale range (30-580 m/z) at a programmed temperature range (35°-3 min, 2°/min to 60°-3 min, 2°/min to 80°-3 min, 4°/min to 120°-3 min, 5°/min to 150°-3 min, 15°/min to 240°-10 min) with the subsequent step-by-step operation of chromatograms. The agents detected were identified with the help of mass spectral libraries (NIST-2005 and Wiley). For more precise identification the Kavats's retention index, obtained due to the use of the standards of alkanes C_7-C_{30}, was applied. The quantitative analysis was made through the internal standards, decafluorobenzophenone and benzophenone.

Plant material:

The blossom trusses, leaves and footstalks of *A. micrantha* were picked in the spring-summer period (May-June) of 2010-11 at blossom-time on the territory of Volga Sands, Astrakhan region, Russia. Species identification was carried visually and microscopically by examining the morphology of blossoms in trusses, leaves and other characteristic features of the plants, and defined them with the determiner at the Botany Department, Astrakhan State University. While determining the antibacterial activity of *A. micrantha* extracts, the known medical plants of *Achillea* genus, *A. millefolium*, as well as *A. leptophylla* Bieb., growing in Astrakhan region were used for comparison.

The dried plant raw material (blossom trusses, leaves and footstalks) was extracted with 40 % aqueous solution of ethanol at an indoor temperature during 7 d while being constantly stirred. Then the extract was filtered, with the spirit evaporated, pasteurized in the dry-air sterilizer at a temperature of 85°. The final product was used to examine the antibacterial activity of the extract obtained. In the course of our pasteurization of extracts, some of the organic molecules could be destroyed due to the high temperature, and they were ultimately not taken into account in the analysis. However, this was done in order to obtain an extract completely free of microorganisms for further experiments.

Antibacterial assay:

The antibacterial activity of the extracts was tested using the agar diffusion test and counting the colony-forming units (CFUs) of test organisms, *Staphylococcus aureus* Rosenbach RNCIM B-1899, *Escherichia coli* Migula CK RNCIM B-1911, obtained from the Russian National Collection of Industrial Microorganisms, FSUE, State Research Institute of Genetics (Moscow, Russia); *Pseudomonas aeruginosa* Migula 1315, *Staphylococcus epidermidis* Rosenbach 537, obtained from the regional infectious hospital. The minimum inhibitory concentration (MIC) was tested by means of count method of CFUs on solid medium. Gentamycin was used as the standard drug.

RESULTS AND DISCUSSION

In the study of compositional analysis of aqueous alcoholic extracts of *A. micrantha* vegetative elements (leaves and footstalks) through GC/MS, there were a large number of LMWOC related to different groups of chemical compounds (Tables 1 and 2). Here with, aldehydes, alcohols and hydrocarbons were found prevailing (Table 2). Out of 55 defined compounds, one remained unidentified (fig. 1). The concentration of two compounds (butane-1,3-diol and hexanal) was the highest. Their proportion was about 63 % from the total LMWOC concentration in the solution. Hexanal participates in regulation of different plant reactions, among them the formation of plant defence mechanisms from external damage and plant-feeders[31-33]. This compound has antifungal and antimicrobial properties[34]. The substantial part of all LMWOCs were aromatic compounds and benzene derivatives (Table 1). The most abundant of them are given in fig. 2. All these compounds are bioactive.

It is interesting to have found some compounds, naphthalene derivatives (compounds 29, 30, 32, 33, Table 1), the share of which is 2.75 % of the

TABLE 1: PERCENT RELATIVE CONTENT OF LMWOC IN THE AQUEOUS ETHANOLIC (40 % V/V) EXTRACT OF VEGETATIVE PARTS OF *A. MICRANTHA*

Peak	Compound	Formula	RT	IK	C	%
1	hexan-2-one	$C_6H_{12}O$	2.55	796	183.47	1.93
2	butane-1,3-diol	$C_4H_{10}O_2$	2.72	803	2000.78	21.08
3	Hexanal	$C_6H_{12}O$	2.97	812	3980.44	41.93
4	Ethylbenzene	C_8H_{10}	4.11	853	68.31	0.72
5	1,4-dimethylbenzene	C_8H_{10}	4.34	861	279.39	2.94
6	3-methyloctane	C_9H_{20}	4.51	867	27.67	0.29
7	Ethenylbenzene [styrene]	C_8H_8	5.03	886	86.04	0.91
8	1,2-dimethylbenzene [o-xylene]	C_8H_{10}	5.08	888	89.39	0.94
9	5-methoxypentan-2-one	$C_6H_{12}O_2$	5.9	911	65.49	0.69
10	1-(4-methylpentan-2-yloxy)propan-2-ol	$C_9H_{20}O_2$	6.05	914	303.89	3.2
11	4,7,7-trimethylbicyclo[4.1.0]hept-3-ene [4-carene]	$C_{10}H_{16}$	6.58	925	31.59	0.33
12	1,2,3-trimethylbenzene	C_9H_{12}	9.38	986	144.93	1.53
13	(2S)-2-tert-butyl-5-methylidene-1,3-dioxolan-4-one	$C_8H_{12}O_3$	10	999	186.55	1.97
14	3,7,7-trimethylbicyclo[4.1.0]hept-3-ene [3-carene]	$C_{10}H_{16}$	10.26	1004	37	0.39
15	Unidentified compounds *m/z* 139 [M^+], 59 (100)		10.81	1013	70.48	0.74
16	1-methyl-3-propan-2-ylbenzene [m-cymene]	$C_{10}H_{14}$	11.08	1017	70.39	0.74
17	1-methyl-4-prop-1-en-2-ylcyclohexene [d-limonene]	$C_{10}H_{16}$	11.3	1021	25.81	0.27
18	5-ethyl-2,2,3-trimethylheptane	$C_{12}H_{26}$	11.36	1022	50.5	0.53
19	1-methyl-4-propan-2-ylcyclohexa-1,4-diene [γ-Terpinene]	$C_{10}H_{16}$	13.13	1052	25.88	0.27
20	2-methyldecane	$C_{11}H_{24}$	13.63	1060	51.34	0.54
21	3,7-dimethyldecane	$C_{12}H_{26}$	14.21	1070	37.98	0.4
22	(6S,7aS)-6-Ethyl-hexahydro-pyrrolizin-3-one	$C_9H_{15}NO$	14.43	1073	41.22	0.43
23	2,3,4-trimethyldecane	$C_{13}H_{28}$	15.08	1084	29.85	0.31
24	4-tert-butyl-1,3-thiazole	$C_7H_{11}NS$	15.76	1096	22.06	0.23
25	3,3-diethyl-4,5-dimethylhex-4-en-2-one	$C_{12}H_{22}O$	16.91	1111	42.16	0.44
26	1,1,3,3,5-pentamethylcyclohexane	$C_{11}H_{22}$	17.81	1123	25.9	0.27
27	1,5-diethyl-2,3-dimethylcyclohexane	$C_{12}H_{24}$	18.46	1131	54.05	0.57
28	1-methyl-2-pentylcyclohexane	$C_{12}H_{24}$	19.57	1145	40.84	0.43
29	2,3-dimethyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene	$C_{12}H_{22}$	22.17	1178	29.25	0.31
30	1,6-dimethyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene	$C_{12}H_{22}$	22.77	1186	15.36	0.16
31	dodecane	$C_{12}H_{26}$	23.9	1200	106.91	1.13
32	2,6-dimethyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene	$C_{12}H_{22}$	24.17	1204	119.9	1.26
33	1,5-dimethyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene	$C_{12}H_{22}$	24.58	1209	97.07	1.02
34	4,10-dimethylspiro[4.5]decane	$C_{12}H_{22}$	25.72	1224	24.26	0.26
35	tetradecane	$C_{14}H_{30}$	37.59	1400	60.17	0.63
36	dodecanoyl chloride	$C_{12}H_{23}ClO$	39.35	1443	12.64	0.13
37	2,6-ditert-butyl-4-methylphenol	$C_{15}H_{24}O$	41.8	1504	14.25	0.15
38	hexadecane	$C_{16}H_{34}$	45.93	1600	19.07	0.2
39	4-pyrrolidin-1-ylbenzene-1,3-diol	$C_{10}H_{13}NO_2$	47.06	1632	9.25	0.1
40	undecylcyclopentane	$C_{16}H_{32}$	47.68	1650	7.16	0.08
41	octadecane	$C_{18}H_{38}$	52.25	1800	14.7	0.15
42	bis(2-methylpropyl) benzene-1,2-dicarboxylate [diisobutyl phthalate]	$C_{16}H_{22}O_4$	54.31	1871	24.67	0.26
43	(z)-hexadec-11-enoic acid	$C_{16}H_{30}O_2$	55.72	1945	13.23	0.14
44	dibutyl benzene-1,2-dicarboxylate [dibutyl phthalate]	$C_{16}H_{22}O_4$	55.92	1960	71.54	0.75
45	hexadecanoic acid	$C_{16}H_{32}O_2$	56.02	1968	76.83	0.81
46	ethyl hexadecanoate	$C_{18}H_{36}O_2$	56.36	1993	14.62	0.15
47	eicosane	$C_{20}H_{42}$	56.45	2000	24.02	0.25

48	5-[(1S,4aS,8aS)-5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-1-en-3-ol; [manool]	$C_{20}H_{34}O$	56.87	2044	15.26	0.16
49	icos-1-ene	$C_{20}H_{40}$	57.04	2062	24.72	0.26
50	methyl 10-octadecenoate	$C_{19}H_{36}O_2$	57.69	2137	80.26	0.85
51	docosane	$C_{22}H_{46}$	58.19	2200	47.85	0.5
52	docos-1-ene	$C_{22}H_{44}$	58.63	2266	72.02	0.76
53	bis(2-ethylhexyl) hexanedioate	$C_{22}H_{42}O_4$	59.43	2392	41.6	0.44
54	bis(2-ethylhexyl) benzene-1,2-dicarboxylate [diethylhexyl phthalate]	$C_{24}H_{38}O_4$	60.45	2538	72.06	0.76
55	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene [squalene]	$C_{30}H_{50}$	63.65	2814	311.29	3.28

RT: Retention time, min, IK – Kovats retention index; C - concentration of the compounds in the extract, mg l^{-1}

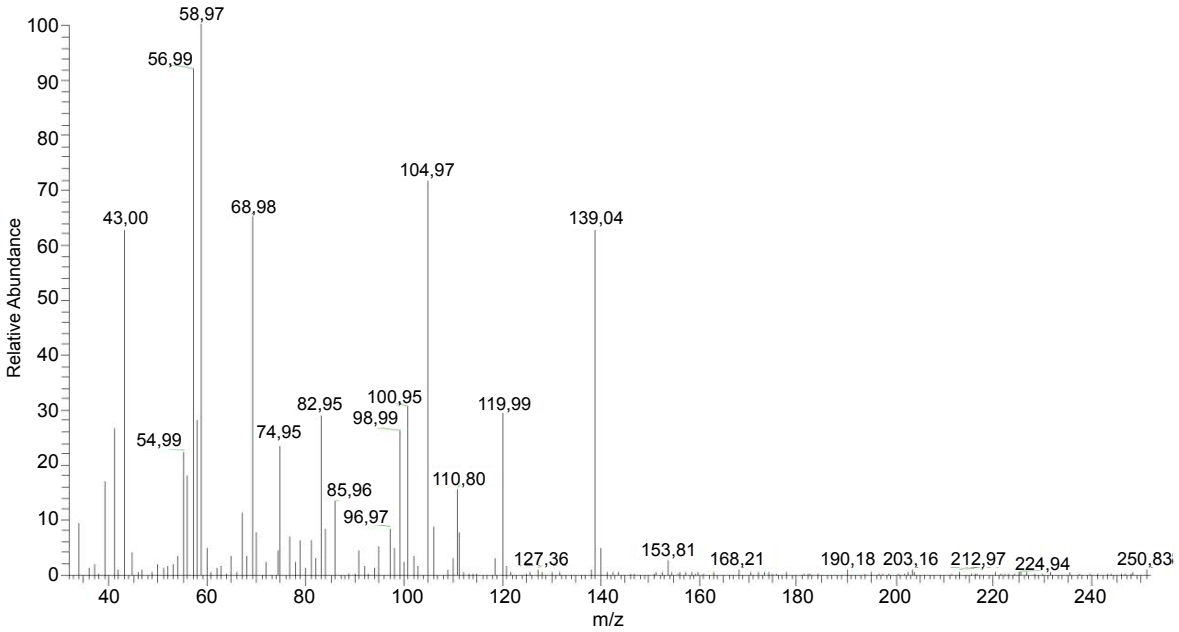

Fig. 1: Mass spectrometry of the unidentified compound RT= 10.81 min

TABLE 2: PERCENT RELATIVE CONTENT OF THE MAIN GROUPS OF SUBSTANCES IN THE *AQUEOUS ETHANOLIC* EXTRACT OF VEGETATIVE PARTS OF *A. MICRANTHA*

Group of substances	Relative content, %
Alcohols	21.24
Hydrocarbons	14.45
Esters	3.21
Ketones	2.37
Aldehydes	41.93
Diverse functional groups	6.29
Aromatic hydrocarbons	7.78
Nitrogen and sulphur containing compounds	0.76
Chlorine-containing compounds	0.13
Carboxylic acids	0.95
Phenols	0.15
Unidentified compounds	0.74

total concentration of LMWOC. Among LMWOCs of vegetative parts extract of *A. micrantha*, three compounds related to phthalates were identified (diisobutyl phthalate, dibutyl phthalate, diethylhexyl phthalate); herewith their total share was quite large (1.77 %). The given group of substances is often considered as pollutants. However, the recent findings have shown that actinomycetes, fungus and plants (terraneous and aquatic ones) are able to synthesize phthalates, which participate in allelopathic interacting and perform protective functions[35-39].

In ethanol extract of *A. micrantha* truss, 19 compounds were identified (Table 3). Out of the 19 compounds, identified in the truss extract of milfoil *Parviflorus*, 17 related to terpenes and their derivatives. This fact can be indicative of substantial antibacterial activity of this

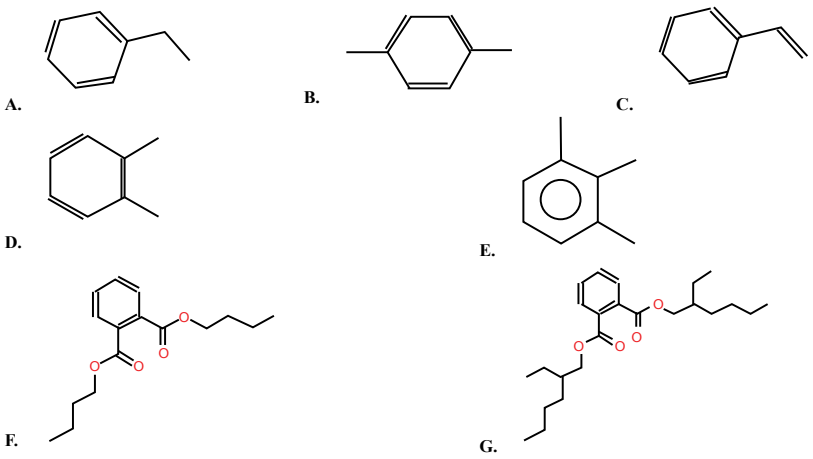

Fig. 2: The most abundant aromatic compounds and benzene derivatives in the aqueous ethanol extract of vegetative parts of *A. micrantha*

A. Ethylbenzene, B. 1,4-dimethylbenzene, C. ethenylbenzene, D. 1,2-dimethylbenzene, E. 1,2,3-trimethylbenzene, F. dibutyl phthalate, G. diethylhexyl phthalate

TABLE 3: PERCENT RELATIVE CONTENT OF LMWOC IN THE AQUEOUS ETHANOLIC (40 % V/V) EXTRACT OF INFLORESCENCES OF *A. MICRANTHA*

No.	Compound	Formula	IK	Relative content, %
1	4,6,6-trimethylbicyclo[3.1.1]hept-3-ene [α-pinene]	$C_{10}H_{16}$	939	2.46
2	3,3-dimethyl-2-methylidenebicyclo[2.2.1]heptane [camphene]	$C_{10}H_{16}$	945	0.5
3	4-methylidene-1-propan-2-ylbicyclo[3.1.0]hexane [sabinene]	$C_{10}H_{16}$	974	0.1
4	6,6-dimethyl-4-methylidenebicyclo[3.1.1]heptane [β-pinene]	$C_{10}H_{16}$	982	2.1
5	1-methyl-4-prop-1-en-2-ylcyclohexene [limonene]	$C_{10}H_{16}$	1029	1.3
6	2,2,4-trimethyl-3-oxabicyclo[2.2.2]octane [1,8-cineol]	$C_{10}H_{18}O$	1043	8.47
7	1-methyl-4-propan-2-ylcyclohexa-1,4-diene [γ-terpinene]	$C_{10}H_{16}$	1079	4.9
8	3,7-dimethylocta-1,6-dien-3-ol [linalool]	$C_{10}H_{18}O$	1097	0.56
9	Unidentified		1132	2.93
10	4,7,7-trimethylbicyclo[2.2.1]heptan-3-one [camphor]	$C_{10}H_{16}O$	1156	10.62
11	4,7,7-trimethylbicyclo[2.2.1]heptan-3-ol [borneol]	$C_{10}H_{18}O$	1169	1.37
12	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol [terpineol]	$C_{10}H_{18}O$	1174	1.53
13	(6S)-3-methyl-6-propan-2-ylcyclohex-2-en-1-one [piperitone]	$C_{10}H_{16}O$	1224	34.15
14	(4,7,7-trimethyl-3-bicyclo[2.2.1]heptanyl) acetate [bornyl acetate]	$C_{12}H_{20}O_2$	1237	1.32
15	2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one [carvone]	$C_{10}H_{14}O$	1242	24.93
16	dibutyl benzene-1,2-dicarboxylate [dibutyl phthalate]	$C_{16}H_{22}O_4$	1960	1.37
17	Octadecanoic acid	$C_{18}H_{36}O_2$	2100	0.45
18	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol [β-sitosterol]	$C_{29}H_{50}O$	3200	0.718
19	(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol [stigmasterol]	$C_{29}H_{48}O$	3230	0.24

IK: Kovats retention index

extract, as well as of vegetative parts extract where the proportion of terpenoids is also big, as the high antibacterial activity is intrinsic for terpenes and their derivatives[40-42].

The given chemical compounds, the majority of which refer to the group of biologically active terpenes and their derivatives were extracted with 40 % ethanol from the yarrow blossom trusses, were also the ones most commonly found in essential oils. The offered preparation method of extracting these compounds with the help of ethanol made it possible to obtain a complex of organic substances, which were the constituents of some essential oils with antimicrobial properties.

The complex of the detected compounds in the extracts *A. micrantha* had a notable inhibitory effect on the test strains studied. As evidenced from the Table 4, the

TABLE 4: THE COMPARATIVE ANTIMICROBIAL ACTIVITY OF THE PLANT EXTRACTS BY AGAR DIFFUSION TEST

Samples	Diameter of zone of inhibition M±m. mm			
	S. aureus	*P. aeruginosa*	*E. coli*	*S. epidermidis*
Gentamycin	42.0±1.4	26.0±0.8	16.0±0.5	33.2±0.3
A. millefolium Bl. truss	15.2±1.8	12.4±1.2	10.0±0.4	22.8±0.8
A. millefolium leaf	14.1±1.5	11.8±0.9	7.0±1.7	-
A. millefolium herb	13.4±2.3	13.0±1.3	-	-
A. micranta Bl. truss	39.0±0.4	21.0±0.8	14.0±1.5	25.2±1.5
A. micranta leaf	17.3±1.2	16.6±1.4	10.0±2.4	-
A. micranta herb	20.5±1.2	19.5±1.0	9.7±1.3	-

TABLE 5: MINIMUM INHIBITORY CONCENTRATION OF THE PLANT EXTRACTS IN TERMS OF *STAPHYLOCOCCUS AUREUS*

Concentration of extracts active agents	CFUs. $M\pm m_m$	
	A. millefolium	*A. micrantha*
Control without extract	158.4±1.3	213.2±2.5
0.25 µg/ml	50±0.6	26.8±0.9
0.5 µg/ml	15.8±0.7	17±0.9
2.5 µg/ml	3.6±0.2	8.6±0.5
5.0 µg/ml	1.7±0.2	4.7±1.03

CFU counting method -1.0×10^8

TABLE 6: THE COMPARATIVE ANTIMICROBIAL ACTIVITY (MIC) OF PLANT EXTRACTS IN RESPECT OF *STAPHYLOCOCCUS AUREUS*

Samples	$(M\pm m_m)$.mm		
	5.0 µg/ml	0.5 µg/ml	0.05 µg/ml
A. micrantha	39.0±0.4	20.0±0.5	25.2±0.8
A. millefolium	16.5±0.2	10.1±0.1	0
A. leptophylla	20.5±1.2	0	0
Gentamycin	42.0±1.4	22.5±0.1	10.0±0.1

most inhibitory effect on *S. aureus* was found in the *A. micrantha* blossom truss extract similar to the effect produced by gentamycin. The extracts of other parts of *A. micrantha* featured stronger antibacterial effect than the ones of *A. millefolium*. In this study the extracts of *A. micrantha* have demonstrated more antibacterial activity towards the strain *S. aureus*.

Besides, the study of effect of different concentrations (MIC) of the extracts *A. micrantha* and *A. millefolium* on the strain *S. aureus* (CFU counting method) showed more intense inhibiting effect of extract dilution *A. micrantha* (Table 5). The most active concentrations in MIC proved to be 5.0, 2.5, 0.25 µg/ml. It should be noted, that the antibacterial activity of the concentration of *A. micrantha* 0.025 µg/ml was 1.8 as high as that of the extract *A. millefolium* (Table 5). The study of different extract concentrations *A. millefolium*, *A. micrantha* and *A. leptophylla* by means of agar diffusion test also revealed more intense inhibiting effect of the extract *A. micrantha* in respect of *S. aureus* (MIC value= 5.0, 0.05 µg/ml; Table 6). As it was detected, the extracts of *A. micrantha* in all the concentrations studied 5.0, 0.5 and 0.05 µg/ml showed more antibacterial action than those of *A. millefoluim* and *A. leptophylla*. The inhibitory activity of the extract is similar (compared to) to that of gentamycin, and the antibacterial action of the extract with 0.05 concentrations is 2.5 times higher than that of gentamycin (Table 6).

As per the results of the study, the detected antimicrobial action of the extract *A. micrantha*, similar due to its activity to chemical specific antibiotic, is determined by the content of terpenic and phenolic compounds as its major constituents, and other detected in the course of study compounds.

Conflict of interests

None declared.

Financial support and sponsorship:

Nil.

REFERENCES

1. Khani A, Asghari J. Insecticide activity of essential oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* against two stored product pests, the flour beetle, *Tribolium castaneum* and the cowpea weevil, *Callosobruchus maculates*. J Insect Sci 2012;12:73.
2. Hatam NAR, Yousif NJ, Porzel A, Seifert K. Sesquiterpene lactones from *Achillea micrantha*. Phytochemistry 1992;21(6):2160-62.
3. Souza TM, Rangel VLBI, Pietro Rr RCL, Santos LE, Moreira RRD. Phytochemical screening of *Achillea millefolium* harvested at Araraquara-Sp. Rev Bras Plantas Med 2006;8:151-54.
4. Rustaiyan A, Sharif Z, Tajarodi A, Sadjadi AS. Sesquiterpene lactones from *Achillea micrantha*. Phytochemistry 1987;26(10):2856-57.
5. Mahmoud AA, Al-Shihry SS, Hegazy MEF. A new epimeric

sesquiterpene lactone from *Achillea liguistica*. Rec Nat Prod 2012;6(1):21-7.
6. Toncer O, Basbag S, Karaman S, Diraz E, Basbag M. Chemical composition of essential oils of some *Achillea* species growing wild in Turkey. Int J Agric Biol 2010;12:527-30.
7. Asimova SS, Glushenkova AI, Vinogradova VI. Lipids, lipophilic components and essential oils from plant sources. Berlin: Springer; 2012.
8. Valant-Vetschera KM. On the identity of five species of Achillea sect. *Millefolium subsect* Filipendulinae (Compositae, Anthemideae). Willdenowia 1999;29:141-46.
9. Benetis R, Radusiene J, Janulis V. Variability of phenolic compounds in flowers of *Achillea millefolium* wild populations in Lithuania. Medicina (Kaunas) 2008;44(10):775-81.
10. Priya KS, Gnanamani A, Radhakrishnan N, Babu M. Healing potential of *Datura alba* on burn wounds in albino rats. J Ethnopharmacol 2002;83:193-99.
11. Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. *In vitro* tests and ethnopharmacological investigations: wound healing as an example. J Ethnopharmacol 2005;100:100-07.
12. Saeidina S, Gohari AR, Mokhber-Derfuli N, Kiuchi F. A review on phytochemistry and medicinal properties of the genus Achillea. Daru 2011;19(3):173-86.
13. Konyalioglu S, Karamenderes CF. The protective effects of *Achillea* L. species native in Turkey against H2O2-induced oxidative damage in human erythrocytes and leucocytes. J Ethnopharmacol 2005;102:221-27.
14. Turkoglu I, Turkoglu S, Celik S, Kahyaoglu N. Antioxidant and antimicrobial activities of Turkish endemic *Achillea* species. African J Microbiol Res 2010;4(19):2034-42.
15. Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, Dall'Acqua S, *et al*. Phenolic compounds from *Achillea millefolium* L. and their bioactivity. Acta Biochim Pol 2011;58(2):203-9.
16. Innocentia G, Vegetob E, Dall-Acqua S, Cianab P, Giorgettia M, Agradib E, *et al*. *In vitro* estrogenic activity of *Achillea millefolium* L. Phytomedicine 2007;14:147-52.
17. Niazmand S, Khoshnood E. The effects of *Achillea wilhelmsii* extract on rat's gastric motility at basal and vagal stimulated conditions. Iran J Basic Med Sci 2010;14(2):151-57.
18. Potrich FB, Allemand A, da Silva LM, dos Santos AC, Baggio CH, Freitas CS, *et al*. Antiulcerogenic activity of hydroalcoholic extract of *Achillea millefolium* L. involvement of the antioxidant system. J Ethnopharmacol 2010;130:85-92.
19. Lopes FCM, Benzatti FP, Junir CMJ, Moreira RRD, Carlos IZ. Effect of the essential oil of *Achillea millefolium* L. in the production of hydrogen peroxide and tumor necrosis factor-α in murine macrophages. Rev Bras Cienc Farm 2005;31(3):401-05.
20. Babaei M, Abarghoei ME, Akhavan MM, Ansari R, Vafaei AA, Taherian AA, *et al*. Antimotility effect of hydroalcolic extract of yarrow (*Achillea millefolium*) on the guinea-pig ileum. Pak J Biol Sci 2010;10(20):3673-77.
21. Sharififar F, Pournourmohammadi S, Arabnejad M. Immunomodulatiry activity of aqueous extract of *Achillea wilhelmsii* C. Kosh. in mice. Indian J Exp Biol 2009;47:668-71.
22. Hemmati AA, Arzi A, Adinehvand A, Mostofi NE, Mozaffari AR, Jalali A. Yarrow (*Achillea millefolium* L.) extract impairs the fibrogenic effect of bleomycin in rat lung. J Med Plants Res 2011;5(10):1843-49.
23. Sukhenko LT. Biologically active substances of some plants and the mechanisms of their antimicrobial activity. Nat Sci 2010;3:166-76.
24. Magiatis P, Skaltsouis A-L, Haroutounian SA. Chemical composition and *in vitro* antimicrobial activity of the essential oils of three Greek *Achillea* species. Z Naturforsch C 2002;57:287-90.
25. Kharma A, Hassawi D. The Antimicrobial activity and genetic relationship of *Achillea* species. Biotechnology 2006;5(4):501-07.
26. Baser KHC, Demirci B, Demirci F, Kocak S, Akinci C, Malyer H, *et al*. Composition and antimicrobial activity of the essential oil of *Achillea multifidi*. Planta Med 2008;68:941-3.
27. Karaalp C, Yurtman AN, Yavasoglu NUK. Evaluation of antimicrobial properties of *Achillea* L. flower head extracts. Pharm Biol 2009;47(1):86-91.
28. Kordali S, Gakir A, Arkin TA, Mete E, Arkin A, Aydin T, *et al*. Antifungal and herbicidal properties of essential oils and n-hexane extracts of *Achillea gypsicola* Hub-Mor and *Achillea biebersteinii* Afan. (Asteraceae). Ind Crops Prod 2000;29:562-70.
29. Salvagnini LE, Migliato KF, Isaac VLB, Correa MA, Salgado NRN, Pietro RCLR. Evaluation of efficacy of preservatives associated with *Achillea millefolium* L. extract against *Bacillus subtilis*. Braz J Microbiol 2006;37:75-7.
30. Lakshimi T, Geetha RV, Roy A, Kumar SA. Yarrow (*Achillea millefolium* Linn.) a herbal medicanl plant with broad therapeutic use – a review. Int J Pharm Sci Rev Res 2011;9(2):136-41.
31. Fall R, Karl T, Hansel A, Jordan A, Lindinger W. Volatile organic compounds emitted after leaf wounding: On-line analysis by proton-transfer-reaction mass spectrometry. J Geophys Res 1999;104(13):15963-74.
32. Arimura G, Matsui K, Takabayashi J. Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant Cell Physiol 2009;50(5):911-23.
33. Jüttner F, Messina P, Patalano C, Zupo V. Odour compounds of the diatom *Cocconeis scutellum*: effects on benthic herbivores living on *Posidonia oceanica*. Mar Ecol Prog Ser 2010;400:63-73.
34. Lanciotti R, Belletti N, Patrignani F, Gianotti A, Gardini F, Guerzoni ME. Application of hexanal, (E)-2-hexenal, and hexyl acetate to improve the safety of fresh-sliced apples. J Agric Food Chem 2003;51:2958-63.
35. Xuan TD, Chung M, Khanh TD, Tawata S. Identification of Phytotoxic Substances from Early Growth of Barnyard Grass (*Echinochloa crusgalli*) Root Exudates. J Chem Ecol 2006;32:895-906.
36. Roy RN, Laskar S, Sen SK. Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2. Microbiol Res 2006;161(2):121-26.
37. Qiming X, Haidong C, Huixian Z, Daqiang Y. Chemical composition of essential oils of two submerged macrophytes, *Ceratophyllum demersum* L. and *Vallisneria spiralis* L. Flavour Fragr J 2006;21:524-26.
38. Qiming X, Haidong C, Huixian Z, Daqiang Y. Allelopathic activity of volatile substance from submerged macrophytes on *Microcystin aeruginosa*. Acta Ecologica Sinica 2006;26(11):3549-54.
39. Kurashov EA, Krylova JV, Mitrukova GG, Chernova AM.

Low-molecular-weight metabolites of aquatic macrophytes growing on the territory of Russia and their role in hydroecosystems. Contemp Probl Ecol 2014;7(4):433-48.

40. Solís C, Becerra J, Flores C, Robledo J, Silva M. Antibacterial and antifungal terpenes from Pilgerodendron uviferum (D. Don) Florin. J Chil Chem Soc 2004;49(2):157-61.
41. Togashi N, Inoue Y, Hamashima H, Takano A. Effects of two terpene alcohols on the antibacterial activity and the mode of action of farnesol against *Staphylococcus aureus*. Molecules 2008;13:3069-76.
42. Gupta N, Saxena G, Kalra SS. Antimicrobial activity pattern of certain terpenoids. Int J Pharm Biol Sci 2011;2(1):87-91.