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THE COMPONENT COMPOSITION OF THE *TRAGOPOGON ORIENTALIS* VOLATILE CONSTITUENTS AND ITS BIOLOGICAL ACTIVITY

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The purpose of this study is the chemical investigation of the composition of *Tragopogon orientalis* volatile constituents (VC) and studying of its biological activity.

This article presents the results of research on the study of the chemical composition of species *Tragopogon,* as well as data of antiradical and cytotoxic activity. To study the dried and crushed aboveground part of the plant (leaves, flowers and stems) was taken. The raw material was collected in the flowering phase, growing in Northern Kazakstan. The VC was isolated from the plant of *Tragopogon orientalis* by hydrodistillation method on the Clevenger type apparatus. The chemical composition of the VC was determined by GC/MS.

The cytotoxic activity of the VC was determined by the survival rate of sea shrimp *Artemia salina*. VC of *T. orientalis* in all studied concentrations (1–10 mg ml^{-1}) exhibit acute lethal toxicity - all larvae die. Antiradical activity was determined by the method of colorimetry of free radicals (DPPH). The experimental results showed that the VC of *T. orientalis* at all tested concentrations (0.1–1.0 mg ml^{-1}) exhibit low antiradical activity compared with the butylhydroxyanisole standard drug.

Keywords: Tragopogon orientalis L., volatile constituents, hydrodistillation method, GC/MS-analysis, component composition, biological activity.

Introduction

We continued our research in the field of studying the component composition of essential oils and their biological activity of plants of Kazakhstan [1–10] and selected *Tragopogon orientalis* L. as the object.

Tragopogon orientalis L. (Asteraceae Family) widespread in the territory Central, Eastern and Northern Kazakstan, Russia (Central Black Earth), grows in meadows and on dry slopes, in glades, sandy soils in pine forests [11, 12]. This type is widely used in folk medicine as a choleretic, antiseptic, expectorant, antiscorbutic. In folk medicine in Siberia, the *T. orientalis* herb is used for hysteria, rheumatism, and gonorrhea [13], relieves headache, soothes and normalizes the state of health under stress [14, 15].

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Previously, its anatomical structure was studied [15], a number of flavonoids were detected spectrophotometrically [16]. As noted above, the plant is widely used in traditional medicine, and therefore we decided to investigate the component composition of the volatile compounds of the plant. In the literature we have not found any information on *T. orientalis* volatile compound, therefore, in this article we presented a study component composition its volatile constituents (VC).

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Experimental part

Plant material. The elevated portion of raw materials *T. orientalis* (leaves, flowers and stems) was collected in the flowering phase in 20 June 2018 at surrounding of Astana city (under the bridge on Akzhol Street).

The well-developed samples of plants, without external signs of damages cut off with secateurs at the level of 4–5 cm from the surface of the soil; taking the top parts of stems, leaves and inflorescences. Collecting was carried out in the 1st half of day from 9 to 11 o'clock. The cut-off raw materials were packed into paper package.

The collected raw material was dried and crushed. Samples of this plant are stored in the herbarium fund of the Faculty of Biology and Geography of Academician E.A. Buketov Karaganda State University. Voucher specimen is 1996.07.04.01.03.

Isolation of volatile constituents. VC was obtained from crushed dried masses (125 g) plants (without coarse stems) by the method of water distillation on the Clevenger apparatus within 2 hours [17]. Hexane is used as a trap for taking the VC. Isolated VC was dried over Na_2SO_4, weighed and stored in sealed dark glass bottles in a cool and dark place at 5 °C temperature.

Analytical GC. GC/MS-analysis of *T. orientalis* VC made 3 times under the same conditions [8]. Determination of the composition of VC carried out on a gas chromatograph Clarus-SQ 8 with a mass spectrometric detector. Chromatographic conditions: capillary column RestekRxi®-1 ms 0.25 mm × 30 m × 0.25 μm; sample volume: 1.0 μl; carrier gas He (purity 99.9999); carrier gas rate: 1 ml/min; stream division 1 : 25; t columns: 45 °C (2 min), rise 1.5 °C/min to 200 °C, then 15 °C / min to 280 °C, isothermal mode at 280 °C for 10 min; evaporator t – 280 °C, mass spectrometry detector: t – 240 °C, EI+ = 70 eV; scan time from 4 to 120 minutes; ion scan mode 39–500 m/z. The percentage of components was calculated automatically, based on the peak areas of the total ion chromatogram. Components were identified by mass spectra and retention times using the NIST library. The retention time of the components was recalculated with respect to saturated hydrocarbons.

Cytotoxic activity. Separating funnel filled with 55 mL of artificial sea water and 200 mg of *Artemia salina* eggs. 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 with Pasteur pipette.

20–40 larvae were placed in 990 μL of seawater into each of the 24 micro titer plates. Dead larvae were counted using a microscope. Added 10 μL of dimethylsulfoxide solution of 10 mg ml^{-1} sample. As a comparison, the drug actinomycin D or staurosporine was used. 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 by the microscope [18].

Mortality P determined by the following formula:

$$P = \frac{A - N - B}{Z} \cdot 100\% \quad (1)$$

Where 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.

Antiradical activity. Determination of antiradical activity of VC was carried out by the known technique of the colorimetry of free radicals based on reaction of the radical a 2,2-diphenyl-1-picrylhydrazil (DPPH) with standard of antioxidant [19, 20].

For determination of inhibition of DPPH to 0,1 mL of the test sample in the range of concentration of 0.1; 0.25; 0.5; 0.75; 1 mg mL^{-1} added 3 ml of 6×10^{-5} M solution of radical. Centrifuge test tubes were in a support, wrapped in black polyethylene. After intensive mixing, solutions were left in the dark and after 30 minutes were measured absorbance of solutions at 520 nm.

The values of antiradical activity (ARA) were calculated using the formula shown below:

$$ARA\ (\%) = (A_0\text{-}A_t)/A_0 \cdot 100\% \quad (2)$$

Where A_0 – absorbance of control; A_t – absorbance of the working sample.

The optical density of the investigated samples measured on a spectrophotometer Cary 60 UV-Vis. Antiradical activity of volatile constituents, we compared with butylhydroxyanisole (BHA).

The discussion of the results

As can be seen from the table 1 and figure 1, the main components of the VC of *T. orientalis* are predominantly hydrocarbonsheneicosane – 15.6%, (E)-15-heptadecenal – 11.2%, hentriacontane – 7.9%, nonacosane – 3.2%, heptacosane – 2.9%, 3,7,11,15-tetramethyl-2-hexadecen-1-ol – 2.7% and nonanal – 2.4%.

These components, mostly hydrocarbons – typical of plants in the family Asteraceae, subfamily Lactucoideae, the genus Tragopogon belongs to them, are usually characterized by the presence of milky juice (latex) contained in the glandules, that was confirmed by earlier anatomical studies of a number of authors [15].

We carried out the determination of the cytotoxic activity of the *T. orientalis* volatile constituents. Determination of activity was carried out according to the well-known method of survival of marine shrimp *Artemia salina* [18]. Based on the experiment, it was found that the VC of *T. orientalis* in all tested concentrations (10, 5 and 1 $mg \cdot ml^{-1}$) exhibits acute lethal toxicity (96%) – all larvae die (tabl. 2).

The determination of the antiradical activity of volatile constituents was carried out according to the method [19, 20]. According to the results of the experiment, it was found that the VC of *T. orientalis* has low antiradical activity compared with butylhydroxyanisole (tabl. 3).

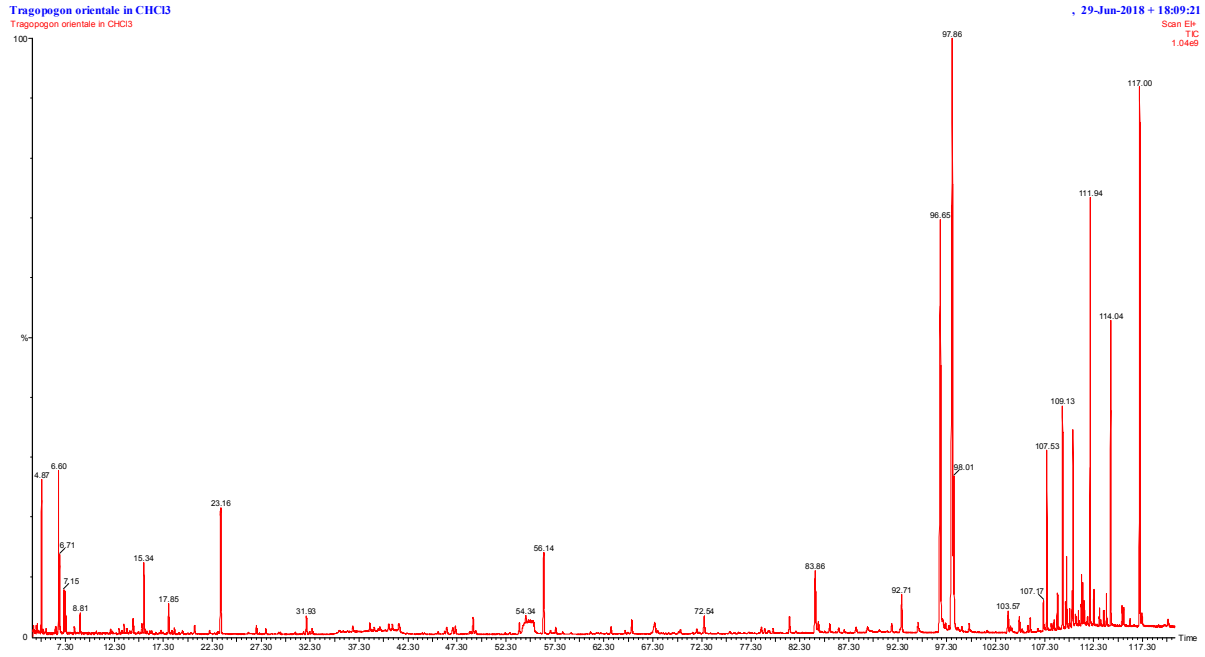

Fig. 1. Chromatogram of VC of *T. orientalis*

Table 1. Chemical composition of *T. orientalis* volatile constituents

No	Retention time	R_{lit}	R_{calc}	Component	Probability	Content,%
1	*2*	*3*	*4*	*5*	*6*	*7*
1	4.866	800	798	Octane	838	1.5±0.2
2	6.605	854±3	843	*(E)*-2-Hexanal	942	1.4±0.2
3	6.711	857±3	845	*(Z)*-Hex-3-en-1-ol	940	0.7±0.1
4	7.148	868±4	857	*cis*-2-Hexene-1-ol	930	0.4±0.1
5	7.313	846±8	861	Isohexyl alcohol	870	0.4±0.1
6	8.211	891±2	884	2-Heptanone	805	0.1±0.1
7	8.809	901±2	899	Heptanal	8 47	0.3±0.1
8	13.3	979±2	969	1-Octen-3-on	883	0.1±0.1
9	15.12	1003±2	997	*n*-Caprylic aldehyde	921	0.2±0.1
10	15.336	1005±2	1000	*cis*-3-Hexenyl acetate	948	1.1±0.2
11	17.853	1045±4	1033	Benzaldehyde	924	0.6±0.1
12	18.436	1038±2	1040	β-*cis*-Ocimen	894	0.1±0.1
13	20.494	1071±3	1067	1-Octanol	787	0.2±0.1
14	**23.162**	**1104±2**	**1101**	**Nonanal**	**924**	**2.4**±0.3
15	27.777	1162±3	1152	*(E)*-2-Nonenal	893	0.1±0.1

End of table 1

1	*2*	*3*	*4*	*5*	*6*	*7*
16	31.926	1206±2	1197	Decanal	885	0.4±0.1
17	32.524	1220±3	1204	β-Cyclocytral	875	0.2±0.1
18	36.655	1252±2	1254	(Z)-2-Decenal	839	0.4±0.1
19	38.402	1293±N/A	1276	Dihydroedulan	838	0.3±0.1
20	38.834	1318±0	1281	Dihydroedulan II	810	0.2±0.1
21	39.264	1302±4	1286	2,6,10,10-Tetramethyl-1-oxaspiro [4.5] dec-6-ene	760	0.2±0.1
22	39.421	1294±N/A	1288	α-Limonen diepoxide	637	0.3±0.1
23	40.317	1300	1299	Tridecane	837	0.5±0.1
24	40.68		1303	*Not identified 1*		0.5±0.1
25	46.26	1386±5	1369	β-Damascenon	652	0.2±0.1
26	46.84	1354±N/A	1376	1,2-Dihydro-1,5,8-trimethylnaphthalene	670	0.2±0.1
27	47.133	1373±0	1380	1,2-Dihydro-1,4,6-trimethylnaphthalene	721	0.2±0.1
28	48.942		1401	*Not identified 2*		0.4±0.1
29	53.66	1449±1	1455	2,6,10-Trimethyltridecan	886	0.2±0.1
30	54.339	1457 iu	1462	β-Ionone	835	1.3±0.2
31	56.14	1492±1	1483	1-Pentadecene	927	1.8±0.3
32	57.398	1512±5	1497	Tricanal	825	0.2±0.1
33	65.15	1613±2	1609	Tetradecanal	923	0.4±0.1
34	70.107	1684±3	1682	α-Bisabolol	739	0.1±0
35	71.78	1700	1706	Heptadecan	817	0.1±0.1
36	72.539	1715±3	1718	1-Pentadecanal	908	0.4±0.1
37	79.579	1817±6	1821	Palmitaldehyde	849	0.1±0.1
38	81.238	1844±4	1845	Hexahydrofarnesilacetone	879	0.4±0.1
39	83.864	1880±3	1883	1-Hexadecanol	894	1.6±0.3
40	84.195	1871±N/A	1888	Methyl-6,9,12-hexadecatrienoate	715	0.2±0.1
41	85.347	1900	1905	*n*-Nonadecane	871	0.2±0.1
42	86.267	1900±N/A	1919	1,2-Epoxyoctadecane	854	0.1±0.1
43	89.225	1968±7	1962	*n*-Hexadecanoic acid	849	0.2±0.1
44	91.69	2000	1998	Eicosane	875	0.2±0.1
45	92.706	2021±11	2015	Octadecanal	914	0.9±0.2
46	94.375	2052±N/A	2043	*(9Z, 12Z)*-9,12- Octadecadiene -1-ol	854	0.3±0.1
47	**96.646**	**2085±1**	**2081**	***(E)*-15-Heptadecanal**	**858**	**11.2**±0.4
48	97.175	2098±3	2090	Methyllinolenat	809	0.1±0.1
49	**97.864**	**2100**	**2102**	**Heneicosane**	**933**	**15.6**±0.4
50	**98.015**	**2116±2**	**2104**	**3,7,11,15-Tetramethyl-2-hexadecen-1-ol**	**913**	**2.7**±0.3
51	98.829	2114±5	2118	*trans*-Phytol	766	0.1±0.1
52	99.589	2141±11	2131	Oleic acid	692	0.2±0.1
53	103.569	2200	2197	Docosane	882	0.5±0.1
54	104.707		2236	*Not identified 3*		0.4±0.1
55	105.587	2299±N/A	2268	Octadecenyl vinyl ester carboxylic acid	816	0.2±0.1
56	105.829		2277	*Not identified 4*		0.3±0.1
57	107.168	2281±8	2326	1-Eicosanol	909	0.4±0.1
58	107.528		2340	*Not identified 5*	911	1.9±0.3
59	108.617	2363±2	2379	2-Methyltricosane	858	0.3±0.1
60	109.127	2400	2398	Tetracosane	889	1.9±0.3
61	109.443	2430±2	2427	*n*-Docosanal	808	0.2±0.1
62	109.56	2442 iu	2439	9-Octylheptadecane	867	0.5±0.1
63	110.21	2500	2505	Pentacosane	892	1.1±0.2
64	111.032	2582 iu	2591	Heptyl octadecyl ether	816	0.1±0.1
65	111.068	2600	2595	Hexacosane	882	0.3±0.1
66	111.197		2608	*Not identified 6*		0.3±0.1
67	**111.945**	**2700**	**2688**	**Heptacosane**	**916**	**2.9**±0.3
68	112.921	280 0	2793	Octacosane	856	0.2±0.1
69	113.328	2832±2	2827	Hexacosanal	786	0.1±0.1
70	**114.044**	**2900**	**2885**	**Nonacosane**	**863**	**3.2**±0.3
71	115.214		2979	*Not identified 7*		0.3±0.1
72	**117.004**	**3100**	**3125**	**Gentriacontane**	**901**	**7.9**±0.4
73	119.892		3242	*Not identified 8*		0.3±0.1
				Total		**75.5**

Table 2. Cytotoxic activity of *T. orientalis* volatile constituents

Paral-lel	Number of larvae in control		Number of larvae in the sample			% of surviving larvae in control	% of surviving larvae in the sample	Mortality A,%	Presence of neurotoxicity, %
	surv.	dead	surv.	dead	par.				
10 mg ml^{-1}									
Med.	24	1	0	25	0	96	0	96	0
5 mg ml^{-1}									
Med.	24	1	0	28	0	96	0	96	0
1 mg ml^{-1}									
Med.	24	1	0	27	0	96	0	96	0

Table 3. Antiradical activity (%) of *T. orientalis* volatile constituents in different concentrations

Test substances	Concentration of solutions (mg ml^{-1})				
	0.1	0.25	0.5	0.75	1.0
Butylhydroxyanisole (BHA)	80.82	81.23	80.30	83.08	83.88
VC of *Tragopogon orientalis* (Torient-1)	6.13	6.71	7.48	7.62	7.87

Conclusion

Thus, we first investigated the component composition of the volatile constituents of *T. orientalis*, as well as studied cytotoxic and antiradical activity. As a result of researches it is revealed that the volatile constituents of *T. orientalis* show high cytotoxic and low antiradical activity.

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