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COMPONENT COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS OF GENUS *DRACOCEPHALUM* L.

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This article presents data on the study of the chemical composition, antiradical and cytotoxic activity of three species of the genus *Dracocephalum* L., which grow in East Kazakstan. Essential oils are obtained from aerial parts of plants *D. nutans, D. ruyschiana, D. thymiflorum* and the average yield of oil was 0,2%. The chemical composition of essential oils were determined by the GC/MS.

Cytotoxic activity of essential oils was determined by the survival of *Artemia salina* aquatic crustaceans. Essential oils of D. nutans, D. ruyschiana and D. thymiflorum in all concentrations (1–10 mg/mL) tested exhibit acute lethal toxicity – all larvae were died. Antiradical activity was determined by the method based on colorimetry of free radicals (DPPH). The experimental results showed that essential oils from D. nutans, D. ruyschiana and D. thymiflorum at all tested concentrations (0,1–1,0 mg/mL) exhibited low antiradical activity compared with the standard drug – butylhydroxyanisole.

Keywords: Dracocephalum L., essential oils, chemical composition, GC/MS, cytotoxic activity, antiradical activity.

Introduction

The nature of East Kazakstan land is diverse and in many respects unique. The unique geographical position of the East Kazakstan region is that it is located in the depths of the largest continent of Eurasia within its central part, on the border of the Great Plains – Western Siberia, Central Asia and Kazakstan. In the territory of the region there is continental pole of the planet and the geographical center of Eurasia. East Kazakstan occupies the south-western part of the Altai (Kazak Altai), Zaysan hollow, Kalba highlands, Saur-Tarbagatay ridges, Irtysh valley and eastern Kazak hills, it covers an area of 283,3 thousand km^2. It is bordered on the North by Russia, on the East by China, on the South the border pass with Almaty, on the West – with the Pavlodar and Karaganda regions. On the territory of East Kazakstan is the law clear vertical zones of climate, vegetation and soils [1].

Dracocephalum L. (dragonhead) are widely distributed around the world. The genus includes about 70 species. In flora of East Kazakstan among the genuses of the family Lamiaceae the genus Dracocephalum occupies a leading position, including 9 species, representing 12% of the total number of species of the family Lamiaceae. Dracocephalum grow across all territory of East Kazakstan in various ecological conditions, rising up to 2500 m above sea level. They are part of the various phytocenoses [2]. The genus dragonhead (Dracocephalum) is widely distributed in the flora of East Kazakstan and is used in folk medicine. The aerial

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part of dragonhead contains large amounts of essential oils, tannins, flavonoids, vitamins [3].

According Budantsev A.L. the genus includes about 70 species [2]. In "Flora of Kazakstan" (1964) described 22 species of this genus found on the territory of the Republic of Kazakstan [4].

To investigate we have been selected and collected plant materials of the following species of dragonhead: *D. nutans* L., *D. ruyschiana* L. and *D. thymiflorum* L.

Previously it was defined that the essential oil *D. nutans* consists of about 25 monoterpenoids, such as pinocamphone (56,4%), β-pinene (12,7%), isopinocamphone (4,3%), α-phellandrene (4,6%) and isopinocampheol (3,7%) [5].

The composition of essential oil of the Kazak species *D. nutans* is very different from the Indian species. It is indicated that the main components of the essential oil of the Kazakstan species *D. nutans* are decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol, (14,2%), caryophyllene oxide (11,3%), 3,7-dimethyl-1,6-octadien-3-ol, (7,7%), myrtenyl acetate (5,7%), 1,2,3,4,4A,5,6,8A-octahydro-7-methyl-4-methylene-naphthalene (5,3%), spathulenol (4,8%), 1-methoxy-4-(1-propenyl)-benzene (4,7%), decahydro-1,1,4,7-tetra-[ethenyl]-4ah-cycloprop[e]azulen-4A-ol (4,7%), germacrene D (4,4%), 3(10)-caren-4-ol (4,2%), 4,11,11-trimethyl-8-methulene-bicyclo[7.2.0]undec-4-ene (3,7%), α-caryophyllene (3,6%), 1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (2,1%) [6].

D. ruyschiana is cultivated in Baksa, Marosvásárhely/Tîrgu Mureş (Transylvania, Romania). The predominant compounds in the essential oil of *D. ruyschiana* (0,2%) are oxygenated bicyclic monoterpenes, such as pinocamphone (43,6%) and isopinocamphone (21,5%). Besides the main components, also identified monoterpenes: myrcene (3,1%), limonene (0,7%), p-cymene (1,5%) and β-pinene (0,9%); sesquiterpenes: β-caryophyllene (3,8%), caryophyllene oxide (1,6%), β-cubebene (1,6%), germacrene-D (3,6%) and elemol (4,4%); and a phenylpropane: methyl chavicol (0,6%). Pinocamphone and isopinocamphone also have been identified as main constituents of essential oil of *D. nutans* [7, 8].

The chemical composition and biological activity of essential oil of *D. thymiflorum* or *Moldavica thymiflora* is poorly investigated, according to the database of Dr. Duke main components of the essential oil of *D. thymiflorum* are pulegone, caryophyllene, isomenthone, β-elemene and 1,8-cineole [9].

Experimental

Plant material. For study the chemical composition and biological activity of essential oils, we have collected vegetable raw materials of three types of dragonhead: *Dracocephalum nutans, D. ruyschiana, D. thymiflorum*.

D. nutans – rhizomatous perennial. It grows in forests, shrubby thickets, on gravelly, sandy and rocky slopes, taluses and steppe pastures, often comes up to the timber line [4]. The plant for research was collected in the territory of East Kazakstan, Katon-Karagay district, the neighborhood of the village Enbek, the mountain Shagyl (N 49°12,200′; E 086°10,320′ at the height of 990 m above sea level).

D. ruyschiana – a puberulent perennial plant with height 20–60 cm. It blossoms in the summer, in June-July, fruiting in July-August [4]. Samples of plants were also collected in the territory of East Kazakstan, in Katon-Karagay district, on the ridge Sarymsakty, tract Akimbay (N 49°12,407′; E 086°11,415′ at the height of 1383 m above sea level).

D. thymiflorum – an annual plant 15–50 cm high. It grows in bushes, on edges of forests, often as thicket of weed on raw farm lands [4]. The plant was collected in the East Kazakstan region, in Katonkaragay District, on the rocky slopes of the ridge Sarymsakty, along the road to the pass Burhat (N 49°09,617′; E 086°02,198′ at the height of 1012 m above sea level).

All plants were collected in the second decade of July, 2014. Plant samples are stored in the herbarium of the Department of Biology of the S. Amanzholov East Kazakstan State University (Oskemen).

Extraction of essential oil. Essential oils were obtained from dried, crushed aerial parts of plants (~100 g) by steam distillation in a Clevenger apparatus for 3 h according to the method by the Pharmacopoeia [10]. During receiving essential oil used hexane as a trap. The essential oil was collected by decantation, dried over Na_2SO_4, weighed and stored in sealed dark glass vials at 4–5 °C until analysis.

The yields for all samples averaged 0,1%.

Analytical GC. The constituent composition of essential oils were determined on a Clarus-SQ 8 GC/MS (PerkinElmer) with a mass-spectrometric detector. An essential oil sample (25 mg) was placed into a 25-mL volumetric flask, dissolved in hexane (15 mL), adjusted to the mark, and stirred until the oil was fully mixed.

Chromatographic conditions: capillary column Restek Rxi®-1ms (0,25 mm × 30 m × 0,25 μm); sample volume 1,0 μL; He carrier gas at 1 mL/min; flow division 1 : 25; column temperature 45 °C increasing at 1,5 °C/min to 200 °C, than at 15 °C/min to 280 °C and isotherm at 280 °C for 10 min; vaporizer temperature 280 °C; mass spectrometric detector at 240 °C with EI+ 70 eV; scan time 4–120 min; scan range 39–500 *m/z*.

The percent contents of constituents were calculated automatically from peak areas in the total ion chromatogram. Constituents were identified from mass spectra and retention times, using the NIST library. Retention indices were calculated relative to *n*-alkanes.

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 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 [11].

Mortality P determined by the following formula:

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

$P = \frac{A-N-B}{Z} 100\%$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 essential oil 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 [12, 13].

For determination of inhibition of DPPH to 0,1 mL of the test sample in the range of concentration of 0,25; 0,5; 0,75; 1 mg/mL 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:

$$ARA(\%) = (A_0 - A_t)/A_0 * 100\% \quad (2)$$

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 essential oil, we compared with butylhydroxyanisole (BHA).

Antiradical activity is defined in relation to standard – of butylhydroxyanisole (BHA).

Results and Discussion

Chemical composition . As shown in the Table 1, in essential oil of *D. nutans* mail components are: *cis, cis*-nepetalactone – 35,0%, germacrene D – 6,3%, β-cyclocitral – 4,0%, β-bourbonene – 3,1% and *cis, trans*-nepetalactone – 2,9%. The main components of the essential oil of *D. ruyschiana* found α-pinene – 3,8%, 3-carene – 3,2%, β-pinene – 2,7% and 3-octanol acetate – 2,5%, and in essential oil of *D. thymiflorum* – β-caryophyllene oxide – 12,2%, spathulenol – 9,4%, palustrol – 3,9%, 1,8-cineol – 3,7% and humulene-1,2-epoxide – 3,7%.

Cytotoxic activity. Results of the study the cytotoxic activity of essential oils from areal parts of *D. nutans, D. ruyschiana* and *D. thymiflorum* are shown in Tables 2–4.

Based on this experiment it can be assumed that the essential oils of *D. nutans, D. ruyschiana* and *D. thymiflorum* in all concentrations tested exhibit acute lethal toxicity – all larvae are died.

Antiradical activity. Values of the antiradical activity of essential oils of *D. nutans, D. ruyschiana* and *D. thymiflorum* calculated by the formula (2) are given in Table 5.

The experimental results showed that essential oils of *D. nutans, D. ruyschiana* and *D. thymiflorum* at all tested concentrations exhibited low antiradical activity compared with the reference drug butylhydroxyanisole.

Table 1. Chemical composition of the aerial parts essential oils of *D. nutans, D. ruyschiana* and *D. thymiflorum*

RI	Compound	Composition (%)		
		D. nutans	*D. ruyschiana*	*D. thymiflorum*
1	*2*	*3*	*4*	*5*
781	Hexanal	–	1,6	1,1
819	2-Hexenal	–	0,6	–
921	Benzaldehyde	–	0,4	–
924	**α-Pinene**	0,8	**3,8**	0,8
959	**β-Pinene**	0,4	**2,7**	0,8
960	1-Octen-3-ol	1,0	–	–
962	6-Methylheptan-3-on	–	0,6	–
973	2-Pentyl- furan	–	–	0,4
980	β-Myrcene	0,6	2,1	1,0
997	**3-Carene**	–	**3,2**	0,7
997	α-Ocimene	1,0	–	–
1001	Benzeneacetaldehyde	–	1,0	0,6
1006	*o*-Cymene	–	0,7	–
1012	**1,8-Cineol**	0,5	1,9	**3,7**
1014	Limonene	–	0,4	0,9
1014	3-Ethyl-4,5-dimethyl-1,4-hexadiene	0,6	–	–
1025	*cis*-β-Ocimene	1,3	–	–
1035	*trans*-β-Ocimene	0,3	–	–
1080	Nonanal	–	0,7	–
1081	Linalool	0,6	1,1	2,1
1094	3,4-Diethylthiophene	1,5	0,8	–
1111	**3-Octanol acetate**	–	**2,5**	–
1124	Pinocarvone	0,3	1,5	0,4
1133	Isopinocamphone	–	–	0,4
1134	*trans*-Pinocamphone	0,3	1,0	–
1138	Cryptone	–	–	2,2
1140	Naphthalene	0,4	1,9	–
1148	Terpinen-4-ol	–	0,5	–
1153	Myrtenal	–	0,5	–
1159	α-Terpineol	–	0,9	0,6
1172	**β-Cyclocitral**	**4,0**	–	–
1191	*p*-Ethylbenzyl alcohol	0,4	–	–
1193	*p*-Cumic aldehyde	–	–	1,4
1194	5,5-Dimethyl-2-propyl-1,3-cyclopentadiene	0,4	–	–
1196	Pulegone	–	1,4	0,6
1209	*cis*-3-Hexenyl-α-methylbutyrate	–	–	1,2
1214	*cis*-3-Hexenyl isovalerate	0,3	–	–
1229	Phellandral	–	–	0,5
1233	*cis*-Chrysanthenyl acetate	–	–	0,5
1257	Isocarveol	–	0,5	–
1263	Dihydroedulan II	2,0	–	–
1295	n-Nonyl acetate	–	0,4	–
1295	Myrtenyl acetate	–	–	0,9
1305	p-Methoxy thiophenol	0,9	0,6	–
1306	***cis, cis*-Nepetalactone**	**35,0**	0,5	0,4
1319	Eugenol	–	0,5	–
1332	***cis, trans*-Nepetalactone**	**2,9**	1,3	1,8
1350	*trans*-β-Damascenone	–	0,4	–
1355	*cis*-Jasmone	0,3	–	–
1359	α-Copaene	0,5	–	–
1364	**β-Bourbonene**	**3,1**	0,5	2,3
1373	β-Elemene	–	–	0,6

End of Table 1

1	*2*	*3*	*4*	*5*
1376	α-Dihydroionone	0,3	–	–
1395	β-Caryophyllene	1,1	–	0,7
1396	Aristolene	–	0,6	–
1406	β-Copaene	0,6	–	–
1422	Geranyl acetone	–	0,8	–
1440	*trans*-β-Farnesene	1,4	–	–
1446	*trans*-β-Ionone	0,3	1,8	0,5
1450	**Germacrene D**	**6,3**	0,4	1,1
1455	α-Curcumene	0,6	–	–
1460	γ-Muurolen	0,7	–	–
1464	Bicyclogermacrene	–	–	0,4
1464	δ-Guaiene	–	–	0,4
1470	Unknown 1	–	0,5	–
1471	α-Cedrene	0,8	–	–
1471	Cubebol	–	–	0,4
1479	γ-Cadinene	0,5	–	–
1480	β-Bisabolene	1,3	–	–
1491	δ-Cadinene	2,0	–	–
1500	Pentadecane	–	–	0,4
1503	Norbourbonone	–	–	0,5
1505	Caryophyllene oxide isomer	–	–	0,7
1521	1,5-Epoxysalvial-4(14)-ene	–	–	0,5
1528	**Palustrol**	–	–	**3,9**
1533	**Spathulenol**	0,3	–	**9,4**
1538	**β-Caryophyllene oxide**	1,0	0,6	**12,2**
1541	β-Spathulenol	–	–	0,8
1547	Isoaromadendrene epoxide	0,5	–	0,4
1547	Salvial-4(14)-en-1-one	0,3	–	–
1553	Aromadendrene oxide-(2)	–	–	0,4
1563	α-Guaiol	1,0	2,3	–
1564	**Humulene-1,2-epoxide**	–	–	**3,7**
1590	Isospathulenol	–	–	1,7
1600	Hexadecane	–	–	0,6
1618	δ-Cadinol	–	–	0,5
1633	Bulnesol	0,9	2,4	–
1635	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	–	–	2,1
1646	ent-Germacra-4(15),5,10(14)-trien-1β-ol	0,6	–	–
1649	Unknown 2	–	0,8	1,1
1824	Phthalic acid, isobutyl octyl ester	–	0,7	0,5
1835	Perhydrofarnesyl acetone	0,3	0,6	0,6
1949	*n*-Hexadecanoic acid	0,5	1,2	0,7
2066	Biformen	0,3	–	–
2097	*trans*-Phytol	0,4	0,7	–
2346	Larixol	0,5	–	–
2700	*n*-Heptacosane	0,4	–	–
2900	*n*-Nonacosane	–	0,9	0,5

Table 2. Cytotoxic activity of essential oil from areal parts of *D. nutans*

Parallel	Number of larvae in control		Number of larvae in sample			The amount of surviving larvae in the control, %	The amount of surviving larvae in sample, %	Mortality, P,%	The percentage of neurotoxicity, %
	survived	died	survived	died	paralyzed				
10 mg/mL									
Medium	25	1	0	28	0	96	0	96	0
5 mg/mL									
Medium	25	1	0	27	0	96	0	96	0
1 mg/mL									
Medium	25	1	0	27	0	96	0	96	0

Table 3. Cytotoxic activity of essential oil from areal parts *D. ruyschiana*

Parallel	Number of larvae in control		Number of larvae in sample			The amount of surviving larvae in the control, %	The amount of surviving larvae in sample, %	Mortality, P,%	The percentage of neurotoxicity, %
	survived	died	survived	died	paralyzed				
10 mg/mL									
Medium	25	1	0	23	0	96	0	96	0
5 mg/mL									
Medium	25	1	0	24	0	96	0	96	0
1 mg/mL									
Medium	25	1	0	25	0	96	0	96	0

Table 4. Cytotoxic activity of essential oil from areal parts *D. thymiflorum*

Parallel	Number of larvae in control		Number of larvae in sample			The amount of surviving larvae in the control, %	The amount of surviving larvae in sample, %	Mortality, P,%	The percentage of neurotoxicity, %
	survived	died	survived	died	paralyzed				
10 mg/mL									
Medium	25	1	0	28	0	96	0	96	0
5 mg/mL									
Medium	25	1	0	25	0	96	0	96	0
1 mg/mL									
Medium	25	1	0	28	0	96	0	96	0

Table 5. Antiradical activity of various concentrations of essential oils from *D. nutans, D. ruyschiana* and *D. thymiflorum*, %

No	Sample	Essential oil concentration, mg/mL				
		0,1	0,25	0,5	0,75	1,0
1	Butylhydroxyanisole (BHA)	80,82	81,23	80,30	83,08	83,88
2	*Dracocephalum nutans* L.	5,53	6,46	6,59	7,79	7,32
3	*Dracocephalum ruyschiana* L.	3,51	4,57	3,89	4,97	4,26
4	*Dracocephalum thymiflorum* L.	5,65	7,57	7,83	8,64	8,70

Conclusion

Thus, during the researches the chemical composition, cytotoxic and antiradical activity of essential oils from *D. nutans, D. ruyschiana* and *D. thymiflorum* were determined.

The experimental data show that the essential oils of *D. nutans, D. ruyschiana* and *D. thymiflorum* showed high cytotoxic and low antiradical activities.

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