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Essential oil of *Pulicaria vulgaris* (*prostrata*) and its biological activity

The investigation of the chemical composition, antioxidant and cytotoxic activities of the essential oil of *Pulicaria vulgaris* wild growing in Akmola region, Kazakhstan was the aim of the study. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC/MS). A total of 49 compounds were identified representing 86.4 % and the major components were patchoulane (37.4%), buddledin C (13.9 %), T-cadinol (4.7 %), trans-sesquibinene hydrate (4.1 %), dyhydro- β -agarofuran (2.7 %), (Z)- α -atlantone (1.8 %) and corymbolone (1.2 %). Six components were identified as unknown (2.6 %). The antioxidant activity was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and the essential oil demonstrated an average scavenging effect at 0.75 and 1 mg/ml concentrations compared with butylhydroxyanisole (BHA). The antiradical activity results of the *P. vulgaris* essential oil is published for the first time. Cytotoxic activity assay was studied against *Artemia salina* larvae and it can be concluded that the essential oil has a good lethal toxicity in all tested concentrations (10–1 mg/ml). The authors attribute this result to the presence of the patchoulane as a major component, which is known for its activity against ovarian cancer cells.

Keywords: *Pulicaria vulgaris* (*prostrata*), essential oil, water distillation, GC/MS, patchoulane, antioxidant, cytotoxic activities.

Introduction

Pulicaria Gaertn. is a plant genus in the family of *Asteraceae* (*Compositae*) with approximately 80 species which are widely distributed in Europe, North Africa and Asia [1]. A review of the literature showed that the genus *Pulicaria* has been associated with various biological activities, such as *P. inuloides* known as «sekba», is used in Yemen to treat wounds, *P. jaubertii* is distributed in the southern Arabian Peninsula, and is used traditionally as a diuretic and antipyretic, *P. stephanocarpa*, known as «derbeb» in Soqotra, has been traditionally used in a variety of health conditions including headache, abscesses, boils and sores, *P. undulata*, known as «kho'ah», is used in the central Sahara to treat chills, diabetes, cardiac disorders, skin diseases, and abscesses, and in Egypt to treat inflammation, as an insect repellent, and an herbal tea [2]. The essential oil of *P. inuloides* has showed antimicrobial and antioxidant (DPPH) activities and the main components were carvotanacetone (47.3 %) and palmitic acid (12.8 %) [3], the major component of *P. jaubertii* was carvotanacetone (64.0 %) and the oil was active against MCF-7 and Hep-G2 cells (IC₅₀= 3.8 and 5.1 $\mu\text{g ml}^{-1}$, respectively) [4], *P. stephanocarpa* contained α -cadinol (42.5 %), β -caryophyllen (10.8 %), spathulenol (6.8 %) and it had high antimicrobial and antioxidant (DPPH, IC₅₀= 330 $\mu\text{g ml}^{-1}$) [5] properties, *P. odora* L. contained thymol (47.8 %) and thymol isobutyrate (30.0 %) and it was active in antibacterial assay [6].

The essential oil of *P. undulata* was studied by different scientist from different countries and the results were significantly different from each other. For instance, the oil from Algeria contained mainly carvotanacetone (14.8 %), δ -cadinene (8.2 %), α -cadinol (4.7 %) and thujanol (4.7 %) [7]. The oil from Yemen contained carvotanacetone (91.4 %), 2,5-dimethoxy-*p*-cymene (2.6 %) and it has demonstrated good antimicrobial and moderate cytotoxic activities against MCF-7 cells (IC₅₀= 64.6 \pm 13.7 $\mu\text{g ml}^{-1}$) [8]. The oil from Egypt contained carvacrol (46.5 %), xanthoxylin (18.1 %), carvotanacetone (8.7 %) and it had a powerful antioxidant, a good antiacetylcholinesterase (IC₅₀ = 139.2 $\mu\text{g ml}^{-1}$), moderate cytotoxic against three cell lines (A375, T98G, HCT116) activities [9]. The oil from Iran contained 4-terpineole (20.1 %), 1S-*cis*-calamenene (13.4 %), junipene (8.7 %), *cis*-sabinene hydrate (8.3 %) and γ -terpinene (7.0 %) [10].

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Pulicaria vulgaris (*prostrata*) Gaertn (*Asteraceae*) is an herbaceous annual plant, erect, more than 30 cm high, much branched. The leaves are linear-oblong, subobtuse or subacute, mucronate, sessile, cordate semiamplexicaul, entire or denticulate. It grows on the wet banks of rivers and lakes, meadow depressions of bumpy sands. It is found in all areas of Kazakhstan, excepting mountains area. It is used as a remedy for dysentery in folk medicine [11]. Italian scientists studied before the essential oil of *P. vulgaris* and its antimicrobial activity, and the main components were hexadecanoic acid (21.7 %), β -caryophyllene (14.3 %) and geranyl propionate (8.2 %). The oil showed a quite good antimicrobial activity against gram positive bacterial strains [12]. According to the study of Iranian scientists, the main components of this essential oil were thymol (50.2 %), carvotanacetone (20.2 %), thymolisobutyrate (16.9 %), menthan-2-one (4.3 %), 1-methyl-1,2-propanedione (4.1 %), 2,5-dimethoxy-*p*-cymene (4.0 %), myrtenol (1.2 %) and it has showed antimicrobial and antifungal activities. Also in this study, the cytotoxic activity of essential oil was tested against MCF-7 and Hep-G2 cell lines (IC_{50} = 5.36 and 7.16 $\mu\text{g ml}^{-1}$ respectively) [13]. The results of other study have shown that essential oil of *P. vulgaris* may serve as an alternative or complementary treatment for leishmaniasis [14].

The purpose of this study is to determine the component composition of essential oil of *P. vulgaris* from Kazakhstan, which has great prospects, to test its cytotoxic and antiradical activity and compare with previous studies.

Experimental

The plant material of *P. vulgaris* (*Asteraceae*) was collected during the flowering period on September 1, 2017, near Eski Koluton village, in Astrakhan District, Akmola region, Kazakhstan. A voucher specimen (No. 1996.07.27.02.04.) was deposited in the Herbarium of the Biology and Geography Faculty, E.A. Buketov Karaganda State University.

The essential oil was distilled from the dried aerial parts using a Clevenger-type water distillation apparatus for two hours. Hexane was used as a trap for essential oil. Determination of chemical composition of the essential oil was carried out on the Clarus-SQ 8 (Perkin Elmer) Gas Chromatograph equipped with Mass spectrometer (GC/MS apparatus).

Preparation of sample: 25 mg of the essential oil were 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 \times 30 m \times 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 $^{\circ}\text{C}$, rise of 2 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$, temperature of evaporator — 280 $^{\circ}\text{C}$, mass spectrometric detection: temperature — 240 $^{\circ}\text{C}$, $E\text{I}^+$ = 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.

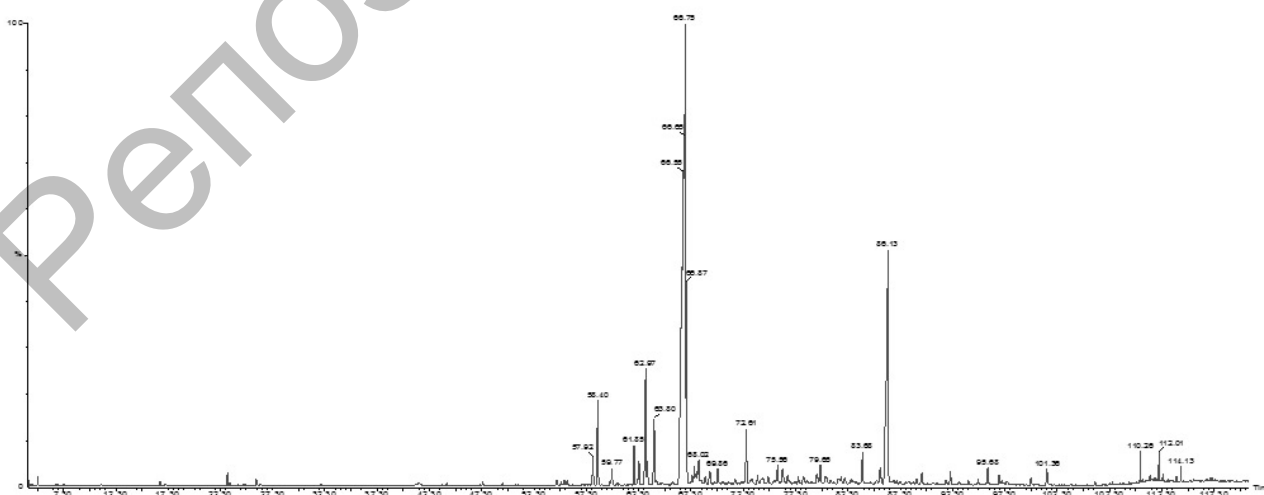


Figure 1. Chromatogram of GC/MS experiment of the essential oil of *P. vulgaris*

Antiradical activity of the essential oil

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

$$ARA(\%) = \frac{(A_0 - A_t)}{A_0} \times 100 \%,$$

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

DPPH molecule forms a free radical that is stable in different medium 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.

Cytotoxic activity of essential oil

The 55 ml separator funnel was filled with artificial seawater 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 nauplii, which moved on the bright side of the separator funnel, were removed with a Paster pipette.

20–40 nauplii were placed into each of the 24 micro titer plates with 990 μ l of seawater. Dead larvae were counted under a microscope. 10 μ l 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 control. After 24 hours 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:

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

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

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

Results and Discussion

The main components of the studied essential oil are presented in the Table 1. The analytical results revealed the presence of forty nine compounds representing 86.4 %. The essential oil of *P. vulgaris* has as the major compounds: patchoulane (37.4%), buddledin C (13.9 %), T-cadinol (4.7 %), *trans*-sesquisabinene hydrate (4.1 %), dihydro- β -agarofuran (2.7 %), (*Z*)- α -atlantone (1.8 %), dehydronerodiol (1.3 %) and corymbolone (1.2 %). These main compounds put together (65.8 %) of the total chemical composition.

Table 1

Chemical composition of essential oil of *P.vulgaris*

L _{literature}	R _{calc}	Compound	Area, %	R _{literature}	R _{calc}	Compound	Area, %
1	2	3	4	5	6	7	8
1103±2	1099	Thujone	0.3	1778±N/A	1762	α -Costol	0.6
1480±N/A	1464	Thymylisobutyrate	0.2	1754±10	1769	β -Acoradienol	0.7
1486±3	1503	β -Eudesmene	0.9	1778±4	1777	β -Costol	0.4
1496±0	1510	Dihydro-β-agarofuran	2.7	1777±N/A	1791	Eremophila-1,11-dien-9-one, 8 α -hydroxy-	0.3
1549±2	1530	Elemol	0.6	1803±4	1798	2-Naphthalenemethanol, 3,4,6,7,8,8a-hexahydro-5- methyl-8-(1-methylethyl)-, (8R,8aS)-	0.2

Continuation of Table 1

1	2	3	4	5	6	7	8
1562±N/A	1561	Dehydronerodiol	1.3	1818±11	1817	Zizanoic acid	0.4
1551±N/A	1568	Diepicedrene-1-oxide	0.9	1832±N/A	1822	trans-Valerenylacetate	0.7
1581±2	1577	trans-Sesquisabinenehydrate	4.1	1813±7	1828	α-Kessylacetate	0.2
1581±2	1580	Caryophylleneoxide	0.7	1845±N/A	1830	Cyperadione	0.3
1610±10	1590	Widdrol	2,0	1861±N/A	1851	(E)-Eremophila-1(10),7(11)-dien-12-yl acetate	0.3
1635±N/A	1633	Patchoulane	37.4	1899±N/A	1881	Corymbolone	1.2
1640±2	1635	T-Cadinol	4.7	1886±5	1906	Oplopanonylacetate	0.8
1649±2	1642	β-Eudesmol	0.3	2282±N/A	1916	Buddledin C *	13.9
1642±2	1646	α-epi-Muurolol	0.7	1939±N/A	1956	Verrucarol	0.2
1652±N/A	1649	Cedrane, 8-propoxy-	0.4		1964	Unknown 1	0.4
1639±0	1651	Oxacyclotetradeca-4,11-diyne	0.7		2005	Unknown 2	0.4
1679±4	1661	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, acetate, [1R-(1α,3α,4β)]-	0.3		2049	Unknown 3	0.2
1680±18	1667	Khusimylmethylether	0.5		2065	Unknown 4	0.7
1679±N/A	1678	(E)-α-Santalal	0.5	2073±N/A	2083	cis-10-Heptadecenoic acid	0.4
1693±3	1703	Germacrone	0.2		2134	Unknown 5	0.3
1717±4	1719	(Z)-α-Atlantone	1.8		2160	Unknown 6	0.6
1724±N/A	1726	Thujopsenal	0.3	2497±N/A	2511	Carbonic acid, eicosyl vinyl ester	0.2
1748±N/A	1734	5β,7βH,10α-Eudesm-11-en-1α-ol	0.3	2700	2695	Heptacosane	0.3
1763±3	1749	cis-Lanceol	0.2	2900	2892	Nonacosane	0.2
1752±4	1758	α-Sinensal	0.2			Total	86.4

Note. * — Compared with authentic compound.

The DPPH radical scavenging activity of the essential oil of *P. vulgaris* is shown in the Tables 2, 3. Based on the analysis of the data the essential oil *P. vulgaris* at concentration of 0.75 and 1 mg ml⁻¹ has an average antiradical activity compared to BHA.

Table 2

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	<i>P. vulgaris</i> (aerial part)	0.6328	0.5902	0.5380	0.4836	0.4288

Table 3

Antiradical activity of *P. vulgaris* essential oil

No.	Sample	Concentration 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	<i>P. vulgaris</i> (aerial part)	16.90	22.50	29.35	36.49	43.69

In the present study the essential oil of *P. vulgaris* with hexane was tested on cytotoxicity against *Artemia salina* nauplii. Based on the experiment (Table 4), it can be concluded that the essential oil *P. vulgaris* in all tested concentrations exhibited cytotoxicity; the mortality of nauplii was 96 %.

Cytotoxic activity of essential oil of *P. vulgaris*

Parallel	Amount of nauplii in control		Amount of nauplii in a sample			% surviving nauplii in control	% surviving nauplii in the sample	Mortality, P, %	Presence of neurotoxicity, %
	survivors	died	survivors	died	Parallel				
10.0 mg ml ⁻¹									
1	25	1	0	31	0	96	0	96	0
2	26	1	0	30	0				
3	30	0	0	27	0				
Average	25	1	0	29	0				
5.0 mg ml ⁻¹									
1	25	1	0	30	0	96	0	96	0
2	26	1	0	24	0				
3	30	0	0	24	0				
Average	25	1	0	26	0				
1.0 mg ml ⁻¹									
1	25	1	0	28	0	96	0	96	0
2	26	1	0	28	0				
3	30	0	0	26	0				
Average	27	1	0	27	0				

Conclusions

The results of the GC/MS experiment was showed that the main components of the essential oil of *P. vulgaris* from Akmola region (Kazakhstan) were patchoulane (37.4%), buddledin C (13.9 %), T-cadinol (4.7 %), *trans*-sesquisabinene hydrate (4.1 %) and dyhydro- β -agarofuran (2.7 %). Six components were identified as unknown (Σ 2.6 %). Antiradical activity test were showed that the essential oil has average activity at concentration 0.75 and 1 mg ml⁻¹ compared to BHA. It should be noted that in this study we investigated the antiradical activity of the essential oil for the first time. The results of cytotoxic activity assay on *Artemia salina* nauplii exhibited a good activity of the essential oil in all tested concentrations, mortality was 96 %. According to a literature review, the patchoulane is the main component of this essential oil and its derivatives were demonstrated moderate cytotoxic activity in human ovarian cancer cells [15].

It can be concluded that the essential oil of *P. vulgaris* from Kazakhstan is differed significantly by component composition of essential oils from Italy and Iran and it can be a good source of biological active compounds.

This research has been funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP08051842).

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Pulicaria vulgaris (*prostrata*) эфир майы және оның биологиялық белсенділігі

Зерттеудің мақсаты — Ақмола облысында (Қазақстан) өсетін *Pulicaria vulgaris* (*prostrata*) эфир майының химиялық құрамын, радикалға қарсы және цитоуыттылық белсенділігін зерттеу. Эфир майы гидродистилляция тәсілімен алынды және газды хроматография – масс спектрометрия (ГХ/МС) арқылы зерттелді. Нәтижесінде 86,4 % құрайтын 49 компонент анықталды, ал негізгі компоненттері — пачулан (37,4%), буддледин С (13,9 %), Т-кадинол (4,7 %), транс-сесквисабинен гидраты (4,1 %), дигидро-β-агарофуран (2,7 %), (Z)-α-атлантон (1,8 %) және коримболон (1,2 %) болды. Алты компонент белгісіз болып анықталды (2,6 %). Антиоксиданттық белсенділік DPPH еркін радикалдарды қолдану арқылы бағаланды және эфир майы 0,75 және 1 мг/мл концентрацияларда бутилгидроксианизолға (БГА) қарағанда орташа радикалға қарсы белсенділігін көрсетті. Бұл зерттеуде *P. vulgaris* эфир майының радикалға қарсы белсенділігінің нәтижелері алғаш рет жарияланды. *Artemia salina* дернәсілдеріне қарсы цитоуыттылық белсенділік нәтижелеріне сәйкес эфир майы барлық сыналған концентрацияларда өлімге әкелетін уыттылығы бар деген қорытындыға келді. Авторлар бұл нәтижені эфир майының негізгі компонентті — пачуланның аналық бездің қатерлі ісік жасушаларына қарсы белсенділігімен белгілі болуымен түсіндіреді.

Кілт сөздер: *Pulicaria vulgaris*, эфир майы, пачулан, антиоксиданттық, цитоуыттылық белсенділіктер.

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Эфирное масло *Pulicaria vulgaris* (*prostrata*) и его биологическая активность

Целью данного исследования является изучение химического компонентного состава, антирадикальной и цитотоксической активности эфирного масла *Pulicaria vulgaris* (*prostrata*), дико произрастающего в Акмолинской области (Казахстан). Эфирное масло было получено гидродистилляцией и исследовано методом газовой хроматографии – масс-спектрометрии (ГХ/МС). В результате было идентифицировано 49 компонентов, составляющих 86,4 %. Основными компонентами были пачулан (37,4 %), буддледин С (13,9 %), Т-кадинол (4,7 %), транс-сесквисабинен гидрат (4,1 %), дигидро-β-агарофуран (2,7 %), (Z)-α-атлантон (1,8 %) и коримболон (1,2 %). Шесть компонентов были определены как неизвестные (2,6 %). Антирадикальную активность оценивали с использованием свободных радикалов DPPH, и данное эфирное масло показало умеренную антирадикальную активность по срав-

нению с бутилгидроксианизолом (БГА) при концентрациях 0,75 и 1 мг/мл. В этом исследовании результаты антирадикальной активности эфирного масла *P. vulgaris* публикуются впервые. По результатам цитотоксической активности в отношении личинок *Artemia salina* было сделано заключение, что эфирное масло обладает хорошей летальной токсичностью при всех испытанных концентрациях. Авторы объясняют этот результат наличием основного компонента — пачулана, известного своей активностью в отношении раковых клеток яичников.

Ключевые слова: *Pulicaria vulgaris*, эфирное масло, пачулан, антирадикальная, цитотоксическая активность.

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