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Chemical composition of essential oil from two species of *Pulsatilla* growing wild in Northern Kazakhstan

The aim of the study was to investigate for the first time the chemical composition of the essential oil from plant species of the genus *Pulsatilla* of the family *Ranunculaceae* Juss. — *P. flavescens* (Zucc.) Juz. and *P. patens* (L.) Mill. growing wild in Northern Kazakhstan. The essential oil was obtained from the dried aerial parts of the plants (stems, leaves, flower heads) by hydrodistillation for 6 hours without steeping in distilled water and with preliminary steeping in distilled water for 14 hours. The qualitative and quantitative compositions of the specimens of the essential oils were analyzed by the method of GC-MS. The main constituents of *P. flavescens* and *P. patens* essential oil were tricosane (30.9–47.3 % and 45.6 % without steeping in distilled water and 40.4–50.1 % and 32.9 % with steeping in distilled water for 14 hours), heneicosane (22.1–31.8 % and 31.5 % without steeping in distilled water and 20.9–30.4 % and 26.6 % with steeping in distilled water for 14 hours), 2-pentadecanone (11.6–33.8 % and 10.8 % without steeping in distilled water and 6.3–10.1 % and 19.2 % with steeping in distilled water for 14 hours), respectively. The results suggested that the essential oil of *P. flavescens* and *P. patens* can have the antimicrobial properties.

Keywords: *Pulsatilla*, essential oil, tricosane, heneicosane, 2-pentadecanone.

Pulsatilla is a genus of the family *Ranunculaceae* Juss. The genus *Pulsatilla* contains about 38 species worldwide all of which occur in the Northern Hemisphere, mainly in Europe and Asia with two species in North America. Nine species occur in Europe [1]. There are 6 species of the genus *Pulsatilla* in Kazakhstan [2].

Pulsatilla flavescens (Zucc.) Juz. (*Pulsatilla patens* ssp. *flavescens* (Zucc.)) and *Pulsatilla patens* (L.) Mill. (*Pulsatilla patens* ssp. *patens*) are rare and endangered plant species in Kazakhstan [3]. They are ornamental, medicinal and venomous plants with yellow to yellowish-white flowers at *Pulsatilla flavescens* (Zucc.) Juz. (Fig. 1) and bluish-violet flowers at *Pulsatilla patens* (L.) Mill. (Fig. 2).



Figure 1. *Pulsatilla flavescens* (Zucc.) Juz.



Figure 2. *Pulsatilla patens* (L.) Mill.

P. flavescens and *P. patens* contain saponins, γ -lactones (anemonin) and flavonoids [4]. The official scientific literature survey showed that there is no previous report on the chemical composition of the essential oil of *P. flavescens* and *P. patens* but there is one for *Pulsatilla albana* (Stev.) Bercht. & Presl. The previous study of the essential oil obtained by hydrodistillation for 3 hours of the aerial flowering parts of *P. albana* exhibited that pulegone (39.1 %), piperitenone (17.2 %), menthone (16.1 %), 1,8-cineole (8.9 %) and p-mentha-3,8-diene (4.2 %) were the main compounds. There are oxygenated monoterpenes (87.9 %), monoterpene hydrocarbons (8.3 %) and sesquiterpenes (1.3 %) in this essential oil. Nonterpene hydrocarbons were not found among the identified components of the essential oil. Antibacterial screening of the essential oil showed moderate activity against certain strains of Gram-positive and Gram-negative bacteria [5].

The aim of the study was to investigate for the first time the chemical composition of the essential oil from rare and endangered plant species of *P. flavescens* (Zucc.) Juz. and *P. patens* (L.) Mill. growing wild in Northern Kazakhstan.

Materials and methods of research

Collection of the material was carried out in places of natural growth of *Pulsatilla flavescens* (Zucc.) Juz. and *Pulsatilla patens* (L.) Mill. on the territory of State National Natural Park «Burabay» (Northern Kazakhstan, Akmola Region, the town of Shchuchinsk). Specimens for the study were collected on 27–30 April 2015 in the stage of full blossoming. *P. patens* was observed on stony slopes of hills, in dry steppes; *P. flavescens* — on edges of pine forests, on steppe slopes of hills. Identification and documentation (certificates of the specimens) of the plant species were made by Dr. Tamara Stikhareva. The herbarium of the identified plants was placed at the Department of Breeding of Kazakh Research Institute of Forestry and Agroforestry in Shchuchinsk under herbarium code 27.04.2015/02 for *Pulsatilla flavescens* (Zucc.) Juz. and 29.04.2015/03 for *Pulsatilla patens* (L.) Mill. Drying to the air-dry condition of the raw material was done in a well-ventilated room, spread out on a paper by smooth thin layer (3–4 cm) and frequent turning.

Essential oil was obtained from the dried aerial parts of the plants (stems, leaves, flower heads) (100 g) by hydrodistillation in a Clevenger-type apparatus for 6 hours (samples Ia-IVa). The yield averaged 0.02–0.13 %. In samples Ib-IVb the dried aerial parts of the plants (stems, leaves, flower heads) (100 g) were preliminary steeped in distilled water for 14 hours and then the essential oil was isolated by hydrodistillation in a Clevenger-type apparatus for 6 hours. Steeping in distilled water was performed for the aim of destruction of the cell structure of plants and the release of components of the essential oils locating in the bound form. The yield averaged 0.02–0.06 %. The isolated essential oil was collected by ethyl acetate and then it was vaporized and weighed. The external characteristic of the essential oil — it is a transparent oil of a light-yellow colour with a slight specific smell.

The qualitative and quantitative compositions of the specimens of the essential oils were analyzed by the method of chromatate-mass-spectrometry on Agilent Technologies 7890A GC System gas chromatograph with Agilent Technologies 5975C mass selective detector. There was used the HP-5MS capillary column (5 % Phenyl Methyl Siloxane, 30 m \times 250 mm \times 0.25 mm) at the flow rate of the carrier gas of helium 1 mL/min. Temperature of the injector block was 230 °C. For 10 min the temperature of the column was 40 °C, with the programming of the temperature up to 240 °C at the rate of changing the temperature 2 °C/min, and then this column was set into isometric mode of operation for 20 min. The injection mode was splitless. The volume of the sample was 0.2 mL. Conditions of the recording of mass spectra were 70 eV, the range of mass was m/z 10–350. The percentage of the components was calculated automatically starting from the areas of peaks of the total chromatogram of ions. The components were identified on mass spectra and on retention time with the use of library Wiley 275.1, National Institute of Standards and Technology V. 2.0 GC/MS and literature [6].

Results and discussion

The qualitative and quantitative analyses of the essential oils of *P. flavescens* and *P. patens* showed that aliphatic hydrocarbons (64.0–96.9 %) were the major constituents. Table shows that the main constituents of *P. flavescens* and *P. patens* essential oil were tricosane (30.9–47.3 % and 45.6 % without steeping in distilled water and 40.4–50.1 % and 32.9 % with steeping in distilled water for 14 hours), heneicosane (22.1–31.8 % and 31.5 % without steeping in distilled water and 20.9–30.4 % and 26.6 % with steeping in distilled water for 14 hours), 2-pentadecanone (11.6–33.8 % and 10.8 % without steeping in distilled water and 6.3–10.1 % and 19.2 % with steeping in distilled water for 14 hours), respectively. Almost in all the studied specimens of

the essential oils of *P. flavescens* and *P. patens* there was revealed the content of tetradecane, pentadecane (except sample IVa) and nonadecane (except sample IIb).

Table

Constituent composition of essential oil from *P. flavescens* and *P. patens*

Constituent	RI calc.	Content, %							
		<i>P. flavescens</i>						<i>P. patens</i>	
		I		II		III		IV	
		<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
Tridecane	1300	0.5	–	–	2.2	–	–	0.7	2.3
Tetradecane	1400	1.4	0.8	1.6	4.5	1.9	0.8	1.3	4.7
Pentadecane	1500	5.4	3.4	2.2	5.1	3.8	2.5	–	3.1
β-Bisabolene	1500	–	0.7	–	–	–	–	–	–
δ-Cadinene	1514	–	0.7	–	–	–	–	–	–
2-Pentadecanone	1682	33.8	9.7	15.6	6.3	11.6	10.1	10.8	19.2
1-Pentadecanal	1693	–	–	–	–	–	1.0	–	–
Heptadecane	1700	–	0.8	1.2	–	–	1.1	–	2.3
2-Heptadecanone	1875	–	–	–	–	–	2.6	–	–
Nonadecane	1900	3.6	3.2	3.7	–	3.6	3.1	3.9	2.8
Hexadecanoic acid	1942	–	–	–	–	–	0.9	–	–
Eicosane	2000	–	–	–	–	–	1.3	–	–
Heneicosane	2100	22.1	22.6	29.5	30.4	31.8	20.9	31.5	26.6
Docosane	2200	–	3.1	3.3	–	–	4.6	3.1	–
Tricosane	2300	30.9	50.1	38.8	47.8	47.3	40.4	45.6	32.9
Pentacosane	2500	–	4.2	–	–	–	7.1	–	–
Total identified		97.7	99.3	95.9	96.3	100	96.4	96.9	93.9

Note: *a* — without steeping in distilled water; *b* — with steeping in distilled water for 14 hours.

It is known that waxes covering leaves and other plant organs are rich in hydrocarbons. We suppose that the probable origin of alkanes (tricosane, heneicosane and others) identified in the essential oils of *P. flavescens* and *P. patens* is related to the epidermis tissues and these alkanes were located in the cuticular waxes [7–9].

Tricosane and heneicosane have antimicrobial properties [10–15]. One can suppose that the essential oil of *P. flavescens* and *P. patens* can have the antimicrobial properties.

An interesting fact of this essential oil is the presence of methyl ketone — 2-pentadecanone. The methyl ketone activity provides protection of the plants from herbivores and fungal pathogens. 2-pentadecanone has the insect repellent properties.

The quantitative composition of the main components of the essential oils of *P. flavescens* and *P. patens* derived without the preliminary steeping in distilled water of the air-dried plant material differs from that one with the preliminary steeping in distilled water for 14 hours. In samples I and II of the essential oil, derived with the preliminary steeping in distilled water for 14 hours of the air-dried plant material, content of tricosane is 1.2–1.6 times higher, content of heneicosane is insignificantly higher and content of 2-pentadecanone is 2.5–3.5 times lower in comparison with the same samples of the essential oil derived without the preliminary steeping in distilled water. In sample III of the essential oil, derived with the preliminary steeping in distilled water of the air-dried plant material, content of tricosane is on the contrary 1.2 times lower, content of heneicosane is 1.5 times lower, content of 2-pentadecanone is insignificantly lower in comparison with the same sample of the essential oil derived without the preliminary steeping in distilled water. In sample IV of the essential oil of *P. patens*, derived with the preliminary steeping in distilled water of the air-dried plant material, content of tricosane is 1.4 times lower, content of heneicosane is 1.2 times lower and content of 2-pentadecanone is 1.8 times higher in comparison with the same sample of the essential oil derived without the preliminary steeping in distilled water. Habitats and the process of extraction have an influence upon the chemical composition of essential oils. So, some changes have been seen in the quantitative and qualitative compositions of the essential oils at extraction with the preliminary steeping in distilled water of samples collected from various habitats. Natural differences have not been identified.

According to the flora of Kazakhstan [2] the studied plants belong to two different species — *P. flavescens* (Zucc.) Juz. and *P. patens* (L.) Mill. However in European flora [1] the above-mentioned plants belong to one species — *P. patens*, but to different subspecies — *Pulsatilla patens* ssp. *flavescens* (Zucc.) with yellow to yellowish-white flowers and *Pulsatilla patens* ssp. *patens* with bluish-violet flowers. When comparing the essential oils of two species of *Pulsatilla*, one can see that the qualitative and quantitative compositions of the components do not have a considerable difference.

The results can be used in future investigations of *P. flavescens* and *P. patens*, to improve the new knowledge about these species.

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Солтүстік Қазақстанда өсетін *Pulsatilla* екі түрлерінің эфир майларының химиялық құрамы

Зерттеудің мақсаты Солтүстік Қазақстанда өсетін *Ranunculaceae* Juss. — *P. flavescens* (Zucc.) Juz. және *P. patens* (L.) Mill. тұқымдасының *Pulsatilla* туысы өсімдігінен алынатын эфир майының химиялық құрамын алғаш рет зерделеу болды. Эфир майын өсімдіктердің кепкен жерүсті бөліктерінен (сабактар, жапырақтар, гүл бастары) тазартылған суда сулаусыз 6 сағат бойы гидродистилляциялау және алдын ала тазартылған суда 14 сағат бойы сулау жолымен алынды. Эфир майлары үлгілерінің сапалық және сандық құрамын ГХ-МС әдісімен талданды. *P. flavescens* және *P. patens* эфир майларының негізгі компоненттері, сәйкес трикозан (30.9–47.3 % және 45.6 % тазартылған суда сулаусыз және 40.4–50.1 % және 32.9 % тазартылған суда 14 сағат бойы сулаумен), генейкозан (22.1–31.8 % және 31.5 % тазартылған суда сулаусыз және 20.9–30.4 % және 26.6 % тазартылған суда 14 сағат бойы сулаумен), 2-пентадеканон (11.6–33.8 % және 10.8 % тазартылған суда сулаусыз және 6.3–10.1 % және 19.2 % тазартылған суда 14 сағат бойы сулаумен) болды. Алынған нәтижелер *P. flavescens* және *P. patens* эфир майының микробтарға қарсы қасиеттері бар екендігін болжауға мүмкіндік берді.

Кілт сөздер: *Pulsatilla*, эфир майы, трикозан, генейкозан, 2-пентадеканон, микробтарға қарсы қасиет.

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Химический состав эфирного масла двух видов *Pulsatilla*, произрастающих в Северном Казахстане

Целью исследования являлось изучение впервые химического состава эфирного масла из растения рода *Pulsatilla* семейства *Ranunculaceae* Juss. — *P. flavescens* (Zucc.) Juz. и *P. patens* (L.) Mill., произрастающих в Северном Казахстане. Эфирное масло получали из высушенных надземных частей растений (стебли, листья, цветочные головки) путем гидродистилляции в течение 6 часов без замачивания в дистиллированной воде и с предварительным замачиванием в дистиллированной воде в течение 14 часов. Качественный и количественный состав образцов эфирных масел анализировали методом ГХ-МС. Основными компонентами эфирного масла *P. flavescens* и *P. patens* были трикозан (30.9–47.3 % и 45.6 % без замачивания в дистиллированной воде и 40.4–50.1 % и 32.9 % с замачиванием в дистиллированной воде в течение 14 часов), генейкозан (22.1–31.8 % и 31.5 % без замачивания в дистиллированной воде и 20.9–30.4 % и 26.6 % с замачиванием в дистиллированной воде в течение 14 часов), 2-пентадеканон (11.6–33.8 % и 10.8 % без замачивания в дистиллированной воде и 6.3–10.1 % и 19.2 % с замачиванием в дистиллированной воде в течение 14 часов), соответственно. Полученные результаты позволили предположить, что эфирное масло *P. flavescens* and *P. patens* может проявлять противомикробные свойства.

Ключевые слова: *Pulsatilla*, эфирное масло, трикозан, генейкозан, 2-пентадеканон, противомикробные свойства.

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