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Current state of populations, resources, and biological activity *Pulsatilla patens* (Ranunculaceae) in Northern Kazakhstan

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Abstract. *Aidarkhanova GS, Kubentayev SA, Kukhar YV, Urazalina AS. 2022. Current state of populations, resources, and biological activity* Pulsatilla patens *(Ranunculaceae) in Northern Kazakhstan. Biodiversitas 23: 2311-2320. Pulsatilla* (Ranunculaceae Juss.) is one of the complex groups in systematic and nomenclature terms, which has more than 30 taxa. *Pulsatilla* species are widely used against many pathologies. Anti-inflammatory, antibacterial, antifungal, antimicrobial, antimalarial, and antitumor properties of these plants are known. The object of our research was *Pulsatilla patens* (L.) Mill. (Ranunculaceae) from the allopathic complex *P. patens* with yellow flower color in the Northern regions of Kazakhstan. The article provides detailed information on environmental and biological features, morphology, geographical distribution, phylogenetic characteristics, and raw materials of *P. patens* in Northern Kazakhstan. In addition to these studies, the antimicrobial and fungicidal activity of extracts from leaves and roots of the species is shown. It was established that the species grows in 6 administrative regions of Central and Northern Kazakhstan and 7 floristic regions. In the studied region, *P. patens* are often found along rocky foothill terraces (mainly on the southern slopes), forest edges, along the southern slopes and tops of the hills of steppes, and among shrubs. Data on the study of *P. patens* biomorphology showed that quantitative parameters are characterized by the greatest intra- and inter-population variability CV (number of individuals per 1 m^2 (38-69%); the number of basal leaves (44-61%); the number of generative shoots per bush (32-53%). Metric parameters such as height during flowering (17-27%), height during fruiting (12-18%), flower diameter (14-24%), leaf blade diameter (11-26%) are characterized by low and medium variability, both within the population and at the inter-population level. In the phenological aspect, the species begins to grow in the first half of April. Blooms from mid-April to early May. Bears fruit from the second half of May to early June. The complete end of vegetation falls at the end of September. Analysis of biological activity of stem extracts and leaves of *P. patens* of Kazakhstan population showed that they have pronounced fungicidal properties in relation to conditionally pathogenic yeast *Candida parapsilosis* and molds *Aspergillus niger*. Water decoction *P. patens* was also active against *Escherichia coli*, indicating that it has antibacterial properties.

Keywords: Antimicrobial activity, fungicidal properties, medicinal plant, phytocenosis, *Pulsatilla uralensis*, windflower

INTRODUCTION

Currently, ephemeroids of the genus *Pulsatilla* are very valuable and interesting representatives of the flora in Central and Northern Kazakhstan, where three species grow according to literary data – *Pulsatilla patens* (L.) Mill., (=*P. uralensis* (Zămels) Tzvel., =*P. flavescens*), *P. Multifida* (Pritz.) Juz. (Karamysheva and Rachkovskaya 1973; Sultangazina and Kupriyanov 2012; Kupriyanov et al. 2016; Kupriyanov 2020). At the same time, the genus *Pulsatilla* is one of the most complex groups, due to the high morphological variability of species, which has more than 30 taxa (Stepanov 2014; Sramko et al. 2019).

In this work, we examined the species from the allopathic complex *P. patens* (Ranunculaceae) with yellow flowers, which is specified as *P. flavescens* (Zuccar.) Juz in Kazakhstan Flora (Gamaiunova 1961). However, the combination made by Juzepczuk (1937) in «USSR Flora» – *P. flavescens* (Zuccar.) Juz turned out to be a late homonym, since the species epithet "*flavescens*" in the genus *Pulsatilla* has already been used for another species (Tzvelev 2001) and is an illegal name (Somlay 2000). Thus, lampoon flowers with yellow flowers were of the perianth narrow wedge-shaped leaflets to the base used to designate *P. uralensis*. The *P. patens* are characterized by a blue-violet (different saturation) color of the perianth, wider lobes of leaf plates (Tzvelev 2001, 2012; Egorova et al. 2017). In common habitats, these species form transitional populations with intermediate trait values (Figure 1) (Sushencov 2007). According to the modern nomenclature of plants (POWO 2021; WCVP 2021), *P. uralensis* is given as a synonym for *P. patens* based on phylogenetic studies (Li et al. 2019; Sramko et al. 2019; Valuyskikh et al. 2020). In the studied region, the coenopopulations of *P. patens* are characterized by high indicators of genetic diversity (Beishov et al. 2021).

According to the classification of life forms by Serebryakov (1962), *P. patens* is a perennial herbaceous short-rod multi-headed polycarpic. It grows in the steppe, pubescent phytocenoses on soils of light mechanical composition (Naumenko 2008; Kulikov 2010). In Northern Kazakhstan, it is found in pine forests on the sand and sandy loam (Pugachev 1994). The species is listed in the Red List of Kazakhstan, status: category III - the number of

species is reduced (Red Data Book of Kazakhstan 2014).

According to literature data population, *P. patens* in Northern Kazakhstan is confined to forest edges and steppe communities (Kupriyanov 2020; Sultangazina et al. 2020). However, according to our data, *P. patens* has higher ecological plasticity, in the studied region, it is often found on rocky foothill terraces (mainly on the southern slopes), forest edges in open well-warmed areas, among shrubs. This feature is confirmed by some authors (Juzepczuk 1937; Egorova et al. 2017).

Various plants that come to the attention of researchers, both medicinal official and wild, are regularly analyzed for the presence of bactericidity, fungicality, helminthocidity in BAV (Jaca and Kambizi 2011). Species from the genus *Pulsatilla* produce many secondary metabolites with biological activity. These plants play a special role in phytotherapy and are used in traditional folk medicine to treat many diseases. Due to their numerous therapeutic properties, they are also widely used as homeopathic drugs (Laskai et al. 2017).

Fresh samples of Pulsatilla contain protoanemonin, bicyclic lactone (anemonin), saponins - in dry. Preparations of the *Pulsatilla* plant are used as a calming and sleeping pill. In folk medicine, windflower is used for many diseases. A decoction of grass is drunk in small doses for coughing and female diseases. Vodka tincture on grass is used as rubbing for rheumatism, burns are treated with juice of it (Belov 2005). Most available studies on the detection of Pulsatilla biological activity have been conducted on *Pulsatilla chinensis* (Bunge) Regel and *Pulsatilla cernua* (Thunb.) Bercht. & J.Presl (=*Pulsatilla Koreana* (Y.Yabe ex Nakai) T.Mori). Data on the composition and biological properties of *P. patens* growing in Kazakhstan, fragmentary, relate only to the antimicrobial properties of essential oils (Kirillov et al. 2018).

The *P. patens* have recently attracted great interest from many researchers and the local population, because of its pharmacological properties and decorative qualities. Therefore, the work aims to study the distribution, phenology, the number of populations, and stocks of raw materials of *P. patens* in Northern Kazakhstan, including ecological and phytocenotic characteristics of habitats. In addition to the conducted research, one of the tasks was to study the features of antimicrobial, fungicidal properties of various plant extracts (aboveground and underground parts).

Figure 1. Polymorphic population *Pulsatilla patens* in Northern Kazakhstan (Photo by Kubentayev S.A.)

MATERIALS AND METHODS

The research was carried out on the territory of the Kokshetau Upland, which is located on the northwestern outskirts of the Kazakh Upland and consists of poorly connected massifs (watershed is well expressed only in the central part) (Karamysheva and Rachkovskaya 1973). Five *P. patens* populations (P) were examined (Figure 2), describing the ecological-phytocenotic structure of communities and morphological-quantitative indicators of the species in the studied region. The description of the populations was carried out using special description forms. In each population, 15 study sites were laid, the area of the site was: 10 x 10 m (100 m^2). A total of 75 sites were taken into account. The GPS device recorded the marginal points of the community boundaries to identify the area. First, general information was written in the form: description number, geographical location, date, coordinates, height, site size, photo number, then the following main sections are reflected in the form: The name of the vegetation type based on dominant species; the floral composition of the community with an indication of the occurrence of species. To do this, we selected a section in the redevelopment of a homogeneous con-tour. The GPS device determined the coordinates and the absolute height. The research was carried out between 2019-2021, each population was visited during flowering (10.04-30.04) and during fruiting (25.05-05.06).

The distribution of the species in Kazakhstan was studied according to herbaria of Moscow State University (MW; Seregin 2020), herbarium of the Komarov Botanical Institute (LE), herbarium of the Astana Botanical Garden (NUR), and on literary sources and observations. For the convenience of identifying the location of the growth point of the species, we cite modern administrative areas, and inside the region's administrative areas. The distribution of species by region is based on the floristic zoning of Kazakhstan, proposed by Academician Pavlov (1956) in summary "Flora of Kazakhstan", where the following floristic region (FR) and subregion belong to the studied territory: 2-Tobolsk-Ishimskiy; 3-Irtyshskiy; 4-Semipalatinskiy borovoy; 5-Kokchetavskiy; 9-Turgayskiy; 10-Western upland (Figure 2). Linking to geomorphological areas was carried out according to the Physical Map of Kazakhstan.

The study of *P. patens* populations was carried out by standard geobotanical methods (Bykov 1970). The structure of each specific population was studied according to the methods of Rabotnov (1964) and Smirnova (1976). The basis for studying the ecological and biological features of the species in the field is the developed methodological guidelines by Golubev and Molchanov (1978). Nomenclature names of plants are given according to WCSP (wcsp.science.kew.org).

The raw material reserves were recorded on specific thickets in the studied CP with laying of test areas and further extrapolation of the obtained data for the entire area. The value of the yield, operational reserve (OR), and the volume of potential annual blanks (VPAB), taking into account the recovery period of the studied species according to Shreter (1986). The populations' area was determined using a GPS navigator-Garmin eTrex 10. The areas of *P. patens* populations were determined by establishing coordinates along the perimeter of the population. Statistical processing of biometric parameters of individuals was carried out according to the recommendations of Zaitcev (1973). The level of variability of morphometric features is calculated from the value of the coefficient of variation - CV.

Oil and water infusions, alcohol tinctures, water decoctions at the rate of 1:10 were prepared from the underground and aboveground parts of *P. patens* plant biomass. Infusions and tinctures were infused for 2 weeks in a dark place, decoctions were prepared immediately before use. Sterilization filtration of the preparations was carried out using filters with a pore diameter of 0.45 nm. The extracts were stored at 4-6°C for no more than 24 hours (State Pharmacopeia of Kazakhstan Republic 2008).

Analysis of antimicrobial and antifungal activity of plant raw materials was carried out using serial dilutions in agar and disco-diffusion method. The determination of minimum suppressive concentrations (MSC) and minimum bactericidal concentrations (MBC) of aqueous plant extracts was carried out by sequential micro divisions in MüllerHinton broth (WHO 2009; Adwan et al. 2010; Abdallah 2011; Valieva 2016).

The biological activity assessment of P. patens extracts was evaluated sequentially with several steps. To detect bactericidal minimum suppressive concentration, extracts were tested against *Escherichia coli*, antifungal minimum suppressive concentration against conditionally pathogenic strains, opportunistic mycosis pathogens, yeast *Candida parapsilosis* strain 398.2 and mold *Aspergillus niger*. The minimum suppressive concentration (MPC) is taken visually from the absence of visible growth of microorganisms (Table 3). Standard data were used to interpret the results of determining the sensitivity of microorganisms to antimicrobials (EUCAST 2018).

RESULTS AND DISCUSSION

Geographical distribution of *Pulsatilla patens* in Kazakhstan territory

According to the results of the analysis of herbarium materials (NUR, MW, LE), observations (Serpova 2012), and literary sources (Gamaiunova 1961; Ismailova 2020; Kupriyanov 2020), 47 locations of *P. patens* (with the yellow color of flowers) in Kazakhstan have been identified. It is established that the species is observed in 6 administrative regions of Central and Northern Kazakhstan: Pavlodar Region, North Kazakhstan Region, Kostanay Region, Akmola Region; Karaganda Region, East Kazakhstan Region (partially, border areas with Pavlodar regions), and in 7 floristic regions of Kazakhstan: Tobolsk-Ishimskiy, Irtyshskiy, Kokchetavskiy, Western upland, Karkaralinsky, Turgayskiy, Semipalatinsky borovoy. The largest number of localities of the species have been registered in Akmola region (26), Kostanay region (11), North Kazakhstan region (6). The species is less frequently

recorded in Pavlodar (1 without exact localization), Karaganda (2), and East Kazakhstan regions (2). (Figure 2).

Ecologic-phytocenotic characteristics of populations of *Pulsatilla patens*

In the study area, the species begins to grow immediately after snowfalls, in the first half of April, usually blooms from mid-April to early May (Figure 3 A-B), it bears fruit from the second half of May to early June. Leaves appear in early May and persist until mid-September. It forms the buds of renewal in August-September, the complete end of vegetation falls at the end of September (Figure 3 C-D).

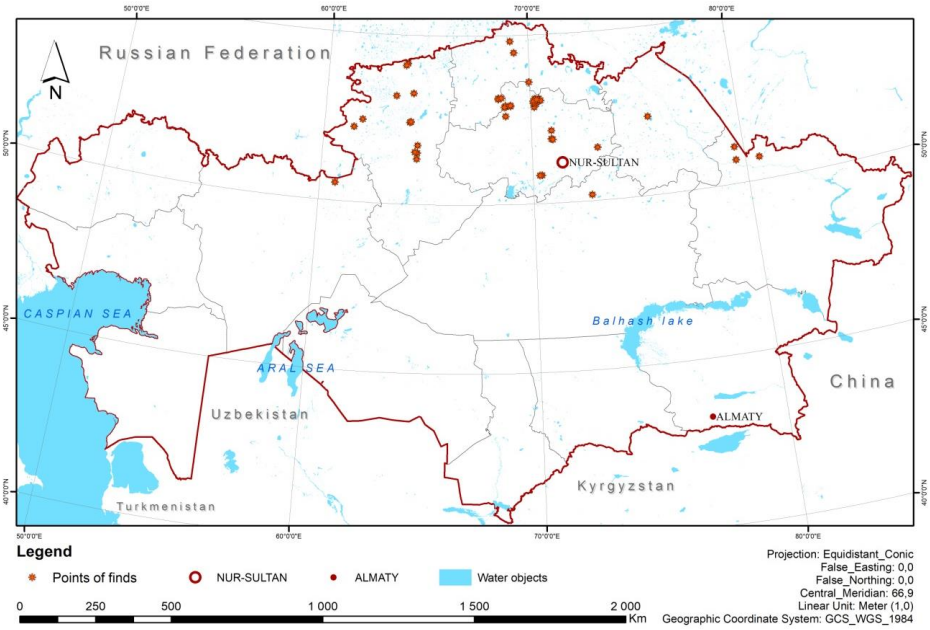

Figure 2. Scheme map of the distribution of the *Pulsatilla patens* in East Kazakhstan (Scheme map was obtained by ArcMap)

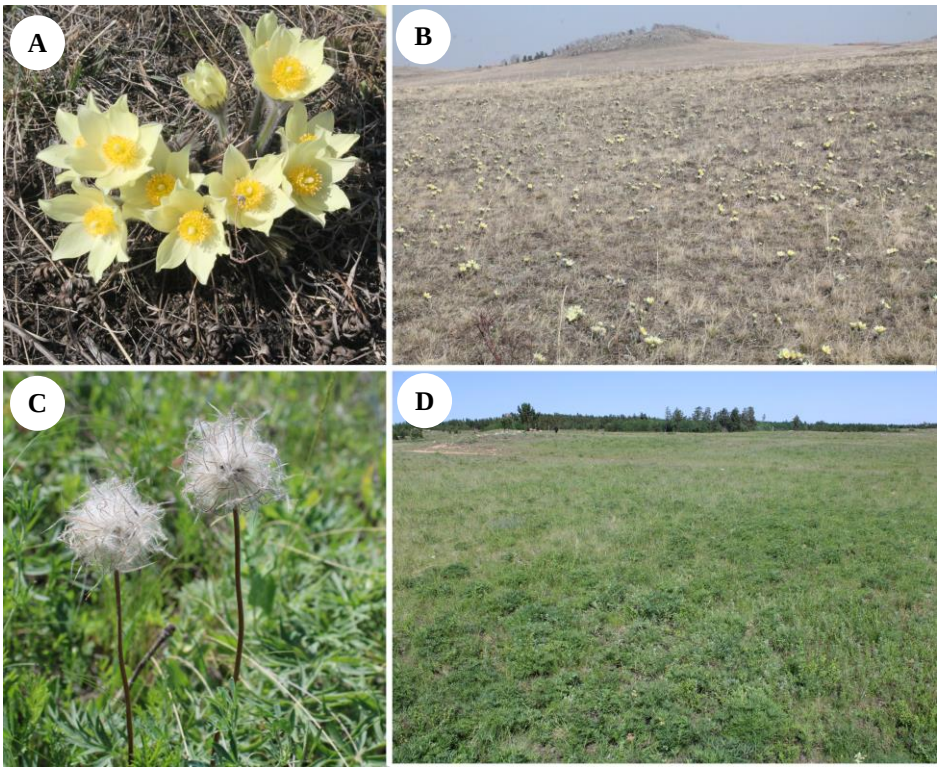

Figure 3. Population *Pulsatilla patens* (A-B. during blossom (26.04.2020); C-D. During fruiting 22.05.2020

According to the results of the conducted research, it is discovered, that *P. patens* are more frequently met in communities with the participation of *Antennaria dioica* (L.) Gaertn., *Artemisia marschalliana* Spreng., *Artemisia frigida* Willd., *Carex humilis* Leyss., *Festuca valesiaca* Schleich. ex Gaudin, *Filipendula vulgaris* Moench, *Helictotrichon desertorum* (Less.) Pilg., *Peucedanum morisonii* Besser ex Schult., *Spiraea hypericifolia* L., *Stipa capillata* L., *Stipa pennata* L., *Stipa zalesskyi* Wilensky ex Grossh., that earlier conducted research in this region prove (Sultangazina et al. 2019; 2020). However, the predominance of xeropetrophytic species in *P. patens* communities has not been previously cited before. The characteristics of 5 populations of *P. patens* on the Kokchetav upland are given below.

1 Population (*F. valesiaca + P. patens – A. frigida + F. vulgaris*) have been studied on foothill terraces, with a slight slope (>10º) and the exit of granite outcrops. The CP was examined in Akmola Region, Zerendinsky District, In Dist. Karsak (Figure 4). The territory belongs to the Kokshetau SNNP, a branch of Ormanda-bulak (52°56'42.1"N, 68°46'32.0"E), height 1117 m above sea level. Soils are low-power, steppe chernozems with a significant content of small granite crumbs. The soil cover is weakly expressed - 0.5-1.4 cm thick. Vegetation is formed mainly by steppe petroxerophytic species. The total projective coating is about 75%. In the community, there are often: *S. capillata*, *Verbascum thapsus* L., *C. humilis*, *Galium verum* L., *A. dioica*, *Fragaria vesca* L., *Tragopogon ruber* S.G.Gmel., *Achillea millefolium* L., *Artemisia glauca* Pall. ex Willd., *Sibbaldianthe bifurca* (L.) Kurtto & T.Erikss., *Koeleria macrantha* (Ledeb.) Schult., *Pilosella echioides* (Lumn.) F.W.Schultz & Sch.Bip., *Phlomoides tuberosa* (L.) Moench, *Salvia dumetorum* Andrz. ex Besser, *Clausia robusta* Pachom., *Polygala comosa* Schkuhr, *Thymus pulegioides* subsp. *pannonicus* (All.) Kerguélen, *Hypochaeris maculata* L., *Thalictrum flavum* L., *Myosotis micrantha* Pall. ex Lehm., *Alyssum desertorum* Stapf, *S. hypericifolia* and *Rosa acicularis* Lindl is rare met among shrubs. The vegetation cover of this site experiences significant anthropogenic loads during grazing, mainly cereals, legumes and young shoots of *S. hypericifolia* are eaten in the spring. It should be noted that plants of pasqueflower are not eaten by cattle in view of the content of poisonous substances, and a dense dernina of the species is resistant to trampling.

2 Population (*S. hypericifolia + S. pennata – F. valesiaca + P. patens*) coincided with the edges of the forest and the southwestern slopes of the low mountains. Population was examined in the Akmola region, Burabai district, northwest of the village Burabay, at the foot of mountain Bolectau. The territory belongs to the "Burabay" SNNP, Borovsky forestry (53º05'31" N, 70º13'21"E), height 345 m above sea level. The microrelief is heterogeneous, the slope of the mountain with the exits of fragments of granite rocks. The third tier is weakly expressed, plantations are rarely noted *Betula pendula* Roth and *Pinus sylvestris* L. Common plant cover is – 60%. *Ranunculus polyrhizos* Stephan ex Willd., *Veronica incana* L., *Phedimus hybridus* (L.) Hart, *S. capillata*, *A. millefolium*, *Allium lineare* L., *P. tuberosa*, *Fragaria viridis* Weston, *G. verum*, *Potentilla virgata* Lehm., *Calamagrostis epigejos* (L.) Roth, *Medicago falcata* L., *A. glauca*, *A. sericea* Weber ex Stechm, and *F. vulgaris* are often found among the accompanying species in the community. The population of the pasqueflower in this community can be considered degrading, due to the strong recreational impact of the Borovoye Resort Complex. The population is dominated by adult generative and senile individuals.

3 Population (*S. zalesskyi + A. frigida – C. humilis + A. dioica – P. patens*) are marked by rocky, southern slopes of low mountains, steepness of 20-25°. It is examined in the North Kazakhstan Region, Airtau District, Imantau District, Mountain Imantau. The territory belongs to the Kokshetau SNNP Shalkar forestry (52°55'01"N 68°12'18"E), height of 1450 m above sea level. The species is a part of petrophytic cereal-grass communities. Low-power soils, horizon 5-15 cm. Population is characterized by petroxerophytic species: *Echinops ritro* L., *G. verum*, *Potentilla chrysantha* Trevir., *Pedicularis physocalyx* Bunge, *T. ruber*, *Seseli libanotis* (L.) W.D.J.Koch, *A. desertorum*, *Orostachys spinosa* (L.) Sweet, *Phleum phleoides* (L.) H.Karst., *Hedysarum gmelinii* Ledeb., *A. glauca*, *A. latifolia* Ledeb., *Koeleria pyramidata* (Lam.) P.Beauv., *S. bifurca*, *P. comosa*, *Thymus mongolicus* (Ronniger) Ronniger, *F. vulgaris*, *S. hypericifolia*, *Cotoneaster laxiflorus* J.Jacq. ex Lindl. are rare met from the bushes. Populations *P. patens* from dense multipurpose bushes in these communities are found scattered, single individuals. A weak seed reproduction is noted.

4 Population (*F. vulgaris + S. zalesskyi + S. hypericifolia+ P. patens*) are noted in the forest-steppe zone, among sparse bush formations and along the vast edges of pine forests (*P. sylvestris)* with little participation *B. pendula.* CP is examined in Akmola Region, Zerendy Area, Podlesnoye District. The territory belong to SNNP «Kokshetau», forestry Ormandybulak (52º53'43" N, 68º48'24" E), height is 1206 meters above sea level. Soils are ordinary chernozems with a significant content of granite crumbs, the soil horizon is 30-50 cm, at the tops of elevations no more than 15 cm. The soil cover is formed by lichens and fall, with a cover of 80-85%. The total projective coating is 70%. Steppe xerophytes prevail in the community, less often petrophytes: *P. tuberosa*, *A. glauca*, *H. desertorum*, *P. phleoides*, *Trifolium lupinaster* L., *Pedicularis abrotanifolia* M.Bieb. ex Steven, *T. p.* subsp. *pannonicus*, *H. maculata*, *Onosma simplicissima* L., *M. falcata*, *A. dioica*, *Iris glaucescens* Bunge, *C. humilis*, *Epilobium angustifolium* L., *F. valesiaca*, *Thalictrum foetidum* L., *Tanacetum vulgare* L., *R. acicularis*, *C. laxiflorus* are met among bushes. Pasqueflower species in this CP are scattered, good seed renewal is noted. Anthropogenic exposure is not observed in a normal type population.

5 Population (*S. capillata*, *P. morisonii*, *H. desertorum*, *A. marschalliana* and *P. patens*) is examined in Akmola Region, Burabay Area, Zelenyi Bor Village District. The territory belongs to SNNP «Burabay», Zelenoborsk forestry (53º08'34" N, 70º22'38" E), height is 313 m above

sea level. The species is part of cereal-trivial steppe communities on ordinary chernozems. The soil cover is weakly expressed, presented in the form of a fall, with a cover of 35-40%. The total projective cover is 55%. Such communities are characterized by xerophytic and petroxerophytic species: *Stipa lessingiana* Trin. et Rupr., *Adonis volgensis* Steven ex DC., *F. valesiaca, V. incana, P. tuberosa, G. verum, M. falcat, E. ritro, Campanula sibirica* L., *Linaria vulgaris* Mill., *Echium vulgare* L., *Psephellus sibiricus* (L.) Wagenitz, *S. dumetorum, A. millefolium, Aster alpinus* L., *O. simplicissima.* The *S. hypericifolia* is very rarely noted in separate groups among the shrubs. In the data of the community, the pasqueflower population is found by isolated microphytocenoses, 500-800 m^2 each, mainly along the southern slopes or closer to the top of the hills, where cereals do not form continuous thickets. There is an anthropogenic effect in the form of grazing, however, the state of the populations of the pasqueflower does not have a significant influence. The population is a normal type, all age states in ontogenesis are noted.

Morphological quantitative parameters of *Pulsatilla patens* in Northern Kazakhstan

Data on the study of *P. patens* biomorphology showed that quantitative parameters are characterized by the greatest intra- and inter-population variability (number of individuals per 1 m^2 (CV: 38-69%); the number of basal leaves (CV: 44-61%); the number of generative shoots per bush (CV: 32-53%)). Metric parameters such as height during flowering (CV: 17-27%), height during fruiting (CV: 12-18%), flower diameter (CV: 14-24%), leaf blade diameter (CV: 11-26%) are characterized by low and medium variability, both within the population and at the inter-population level (Table 1).

Natural reserves of raw materials *Pulsatilla patens* in Northern Kazakhstan

Due to the possibility of using the populations *P. patens* as a gene pool for the reproduction of the species and obtaining raw materials for the manufacture of biological products, the stocks of raw materials of the species in Northern Kazakhstan have been studied (Table 2). The (OR) and the VPAB were calculated taking into account the restoration of aboveground mass for 5 years, roots for 10 years.

It should be recognized that populations P1 and P5 showed relatively high yields of leaves (748.2 and 765.6 g/h and roots (963.2 and 985.6 kg/ha). It was established what *P. patens* in P1 and P2 forms industrial reserves (or more than 10 tons). This is due to optimal ecological conditions for the growth of *P. patents* in P1 and P2, confined to steppe grass-grass communities on the slopes of low hills. These populations can serve as a base for the conservation and reproduction of the gene pool of the species, as well as for harvesting in limited quantities. The total amount of dry raw materials in all studied items. The population of the patent was 33.56 tons of leaves and 43.21 tons of roots, with a VPAB volume of 6.69 tons and 4.3 tons, respectively, on a total area of 46.7 hectares.

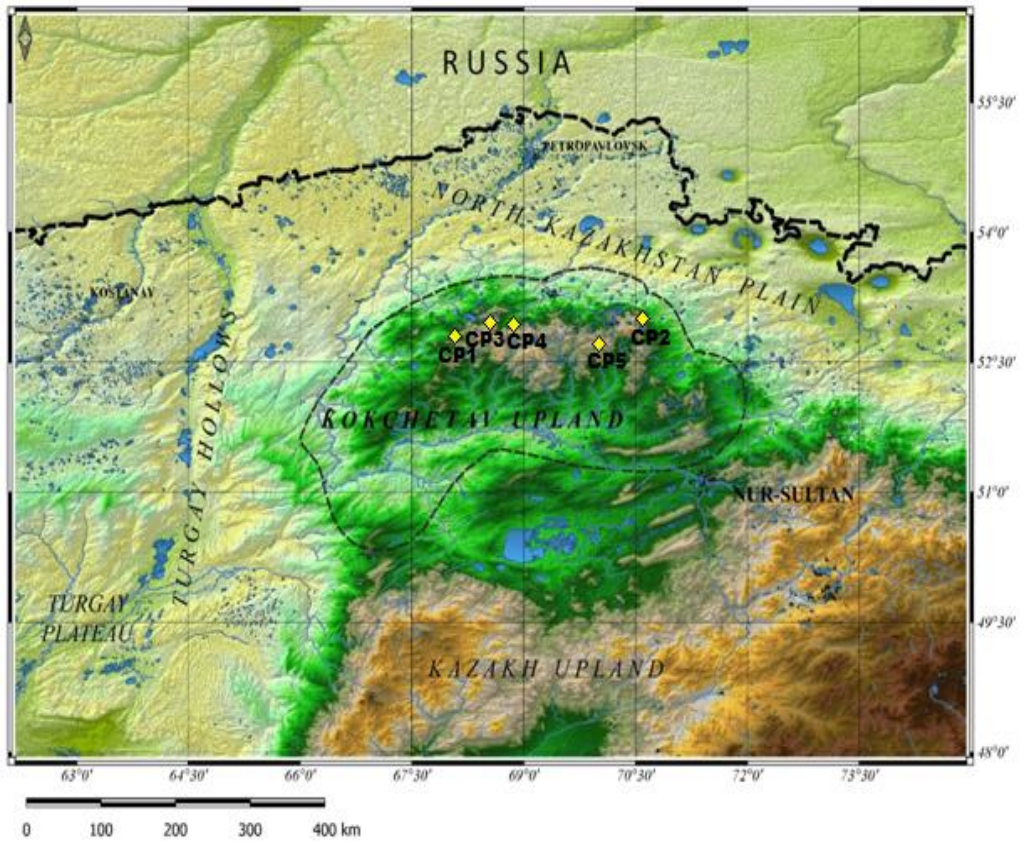

Figure 4. Map of examined population of *Pulsatilla patens* in Northern Kazakhstan

Table 1. Morphological quantitative characteristics of studied populations *Pulsatilla patens*

Morphological quantitative parameters	P1		P2		P3		P4		P5	
	M±SD	CV	M±SD	CV	M±SD	CV	M±SD	CV	M±SD	CV
Species` number in 1 m^2	4.3±1.31	38	0.5±0.09	65	2.2±0.41	69	3.2±0.59	47	4.4±0.57	49
Height at the time of blossom	11.5±0.80	27	12.4±0.69	24	9.8±0.85	19	14.5±0.72	21	9.6±0.42	17
Height at the time of fruiting	16.6±0.69	16	21.1±1.03	18	19.7±0.59	16	23.7±0.77	13	18.1±0.32	12
Number of generative shoots per bush	8.2±0.68	37	9.1±1.12	47	3.4±0.38	32	13.5±1.23	36	4.2±0.84	53
Number of basal leaves	34.3±3.42	61	28.6±1.94	49	54.1±6.14	44	36.7±63.2	56	14.5±0.59	47
Flower diameter	4.8±0.12	14	3.8±0.17	15	4.2±0.22	24	4.5±0.43	17	5.4±0.19	14
Diameter of leaf plate	6.3±0.17	15	6.8±0.94	16	7.1±0.21	11	6.5±0.43	26	7.6±0.18	17

Table 2. Raw materials of *Pulsatilla patens* in Northern Kazakhstan

Population	Area. ha	Crop yield. кg/ha		OR. t		VPAB. t	
		leaves	roots	leaves	roots	leaves	roots
P1	15.6	748.2	963.2	11.67	15.02	2.33	1.50
P2	1.3	87.2	112.4	0.11	0.14	0.02	0.01
P3	0.8	382.6	492.8	0.30	0.39	0.06	0.03
P4	3.4	556.5	716.8	1.89	2.43	0.37	0.24
P5	25.6	765.6	985.6	19.59	25.23	3.91	2.52

Biological activity

It can be seen from Table 3 that among all the extracts of *P. patens* pronounced bactericidal properties, preparations obtained from the aboveground part differ, except oil. The oil extract of the roots showed activity in the dilution of lg^7, and the aqua-alcohol tincture of lg^5. Aqueous and alcoholic extracts from the aboveground part of *P. patens* showed marked bactericidal activity in dilution from lg^8 to lg^{10}.

From the total number of analyzed plants, only aqueous extracts of *P. patens* are isolated by higher rates of fungicidal activity against conditionally pathogenic yeast *C. parapsilosis*. Analysis of fungicidal MBC showed that the aquatic decoctions of the *P. patens* plant of the aboveground part and root on the first day actively suppressed: 1:32, 1:64, 1:128, the growth of *A. niger*, which indicates their fungistatic properties against this micromycete.

A disco diffusion method determined the MBC aqueous digestions against three strains of the microorganisms selected by us. To do this, a bacterial suspension was applied to the agar surface in a Petri dish with a density equivalent to the McFarland turbidity standard of 0.5. Disks containing 0.01 ml of each extract were placed on the daily culture. Diffusion of the extract into the agar led to the formation of a zone of inhibition of the growth of microorganisms around the disks in the presence of antimicrobial properties. The results were recorded 24 hours after incubation of the plates in a thermostat at a temperature of 35-37°C (Figure 5).

As can be seen from Figure 6, extracts from the aboveground portion and roots of *P. patens* showed a pronounced fungicidal activity against opportunistic fungi yeast *C. parapsilosis* (diameter of the growth inhibition zone of micromycetes 30 mm and 15 mm) and mold *A. niger* (20 mm and 10 mm, respectively) stem and leaf decoctions were also active against *E. coli* (10 mm).

Detection of fungicidity of aquatic decoctions of the aboveground part and roots *P. patens* against conditionally pathogenic molds *A. niger* showed that micromycetes are quite sensitive to the number of digs *P. patens* (roots) (diameter of the lysis zone is from 16 to 24 mm). The *C. parapsilosis* yeast was highly sensitive to *P. patens* aquatic decay (aboveground). At the same time, the diameter of the yeast growth delay on the first day was 25-40 mm, on the second day - 20-30 mm, on the third day the fungicidal activity of the water decoction practically disappeared.

Comparative analysis of the fungicidity of the aqueous decoction of the aboveground part *P. patens* with known fungicides on *C. parapsilosis* showed that it is not inferior to the known preparations (26 mm) (Figure 6).

According to the standard values for determining the sensitivity of microorganisms to extracts, the presence of a zone of no growth indicates the following: 0-6 mm - resistant; 7-15 mm - slightly sensitive; 16-24 mm - sensitive; 25 mm or more are highly sensitive. Thus, the obtained experimental data indicate a high sensitivity of the yeast *C. parapsilosis* to the components of aqueous decoctions of *P. patens* (26 mm). The results of experiments show a high activity of extracts from the aboveground part and roots of *P. patens*, which allows us to conclude that it can be used in biotechnological and medical practice for the manufacture of domestic antifungals.

About antibacterial features *Pulsatilla vulgaris* Mill. it was informed earlier (Newall et al. 1996; Hoffmann 2002; Foster and Johnson 2006). In the experiments, made by Łaska et al. (2019), extracts species *Pulsatilla* were tested for antifungal, antimicrobial, and antimalarial activity, as well as cytotoxicity to mammalian cell lines. The results showed that biologically active secondary metabolites of

rare plant species *Pulsatilla patens* subsp. *patens* and the cultivated *Pulsatilla vulgaris* subsp. *vulgaris* isolated from the leaves and roots of these plants had antifungal and cytotoxic activity. Both plants *P. patens* subsp. *patens* and *P. vulgaris* subsp. *vulgaris* were active against fungus *Candida glabrata* with the meanings of half-maximum inhibitory concentration (IC_{50}) 9,37 mcg/mL and 11 mcg/mL, respectively (Łaska et al. 2019). Similar data when studying extracts from the aboveground part and roots of *P. patens* fungicidal activity against conditionally pathogenic yeast *C. parapsilosis* were obtained by our studies. Additional data was obtained on the pronounced fungicidal activity of *P. patens* against mold fungi *A. niger*.

Table 3. Minimal suppressive bactericidal concentration of *P. patens* extracts against bacteria, yeast, mold fungi

Raw material type	Oil extracts	Aqua-alcohol extracts	Aqueous extracts	Aquatic decoctions
Escherichia coli				
Pulsatilla patens, roots	1:64	1:16	-	-
Pulsatilla patens, aboveground part	-	1:128	1:512	1:128
Control	-	-	-	-
Candida parapsilosis				
Pulsatilla patens, roots	-	1:8	native	1:128
Pulsatilla patens, aboveground part	-	1:32	1:64	1:32
Control	-	-	-	-
Aspergillus niger				
Pulsatilla patens, roots	-	1:128	1:64	1:32
Pulsatilla patens, aboveground part	1:8	1:32	---	1:512
Control	-	-	-	-

Note: "-" - does not inhibit the growth of microorganisms; "---" - stimulates the growth of micromycete

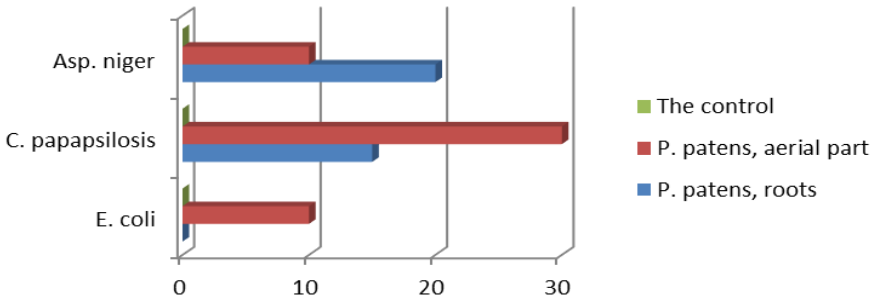

Figure 5. Sensitivity of microflora to aqueous decoctions *Pulsatilla patens* (signed axes of values in millimeters)

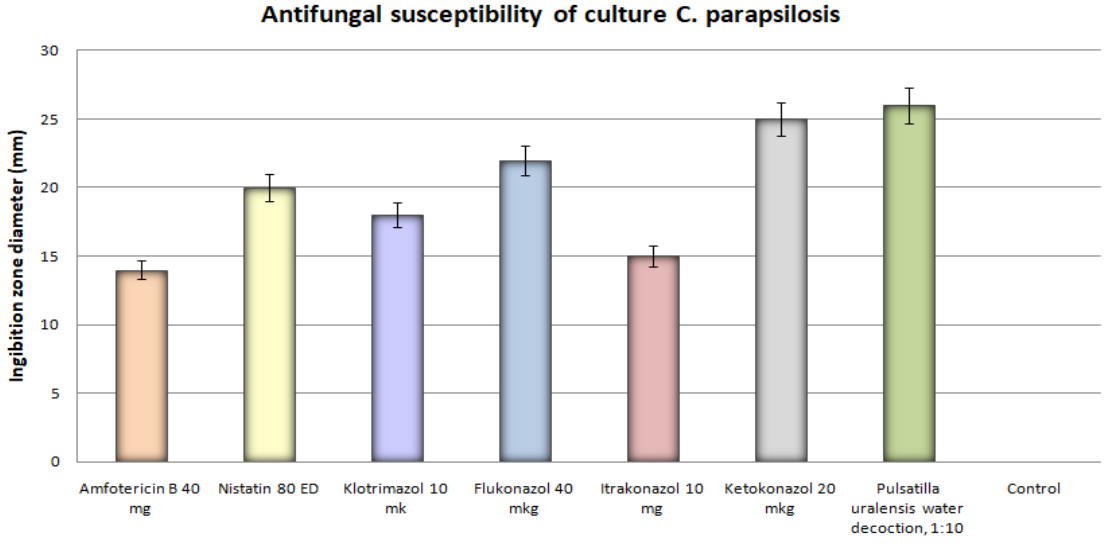

Figure 6. Comparative sensitivity of culture *Candida parapsilosis* to antifungal drugs and water decoction *Pulsatilla patens*

Belov N. (2005) reported on the strong bactericidal and fungicidal effect of the water extract of pasqueflower grass growing in Russia. Our results on the effectiveness of an aqueous decoction of the stem and leaves of the Kazakhstan population against *E. coli* with a growth retardation diameter of up to 10 mm are consistent with his data.

Also about the presence of antimicrobial properties of the essential oil of wild-growing in Northern Kazakhstan two species of plants of the genus *Pulsatilla* – *P. flavescens* (Zucc.) Juz. and *P. patens* reported by Kirillov et al. (2018). The authors found in the essential oils of *P. flavescens* and *P. patens* tricosan and geneucosan, which have antimicrobial properties. Also, the presence of methyl ketone, 2-pentadecanone, was found in the essential oil, the activity of which ensures the protection of plants from herbivorous and fungal pathogens.

The detected antimicrobial and fungicidal activity of *P. patens* extracts make further research in this direction to obtain domestic pharmacological agents based on biologically active substances of this species of Kazakh population

In conclusion, the study of ecological and biological features, morphology, phenology, geographical distribution, phytocenotic characteristics and raw materials of *P. patens* in Northern Kazakhstan allowed us to establish that quantitative parameters are characterized by high intra- and inter-population variability in all populations of *P. patens*. Quantitative biomorphological parameters of *P. patens* are characterized by the greatest intra- and inter-population variability: CV variability (number of individuals per 1 m^2 (38-69%); number of basal leaves (44-61%); number of generative shoots per bush (32-53%)). At the same time, the parameters height during flowering (17-27%), height during fruiting (12-18%), flower diameter (14-24%), leaf blade diameter (11-26%) are characterized by low and medium variability, both within the population and at the inter-population level. In the phenological aspect, the species begins vegetation in the first half of April, flowering - from mid-April to early May, fruiting - from the second half of May to early June, the end of vegetation falls at the end of September.

The study of antimicrobial and fungicidal activity of extracts of stems and leaves of *P. patens* of the Kazakh population showed that they have pronounced fungicidal properties against opportunistic yeast *C. parapsilosis* and mold fungi *A. niger*. An aqueous decoction of *P. patens* has antibacterial properties against *E. coli*. The results obtained make it possible to characterize the Kazakh population of *P. patens* as promising for the study and development of new pharmaceuticals with antifungal and antimicrobial properties.

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REFERENCES

Abdallah EM. 2011. Plants: An alternative source for antimicrobials. J Appl Pharm Sci 1 (6): 16-20.

Adwan G, Abu-Shanab B, Adwan K. 2010. Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug resistant *Pseudomonas aeruginosa* strains. Asian Pac J Trop Med 3 (4): 266-269. DOI: 10.1016/S19957645(10)60064-8.

Beishov RS, Sultangazina G, Beishova IS, Ulyanov VA, Beltyukova NN. 2021. Genetic state of *Pulsatilla Patens* (L.) Mill. coenopopulations based on DNA labeling. OnLine J Biol Sci 21 (1): 161-171. DOI: 10.3844/ojbsci.2021.161.171.

Belov NV. 2005. Calendula, Althea, Celandine and other Folk Medicinal Plants in a Large Encyclopedia of Herbalism. ACT, Harvest, Moscow.

Bykov BA. 1970. Introduction to Phytocenology. Publishing House, ANKazSSR, Alma-Ata.

Egorova NY, Egoshina TL, Sushentsov OE. 2017. Population variability of *Pulsatilla patens* S.L. (Ranunculaceae) in the Vyatka river basin. Rastitel'nye Resursy 53 (2): 237-254.

European Committee on Antimicrobial Susceptibility testing (EUCAST). 2018. Breakpoint tables for interpretation of MICs and zone diameters. Ver. 8.0 Available at: www.eucast.org/clinical_breakpoints/.

Foster S, Johnson R. 2006. National Geographic Desk Reference to Natural Medicine. National Geographic, Washington.

Gamaiunova AP. 1961. The *Pulsaltilla* Genus. Flora of Kazakhstan (Vol. 4), Alma-Ata.

Golubev VN, Molchanov EF. 1978. Methodical instructions for population-quantitative and ecological-biological study of rare, endangered and endemic plants of Crimea. Yalta 41.

Hoffmann D. 2002. Complete Illustrated Guide to the Holistic Herbal. Element, London.

Ismailova FM. 2020. Plants of State National Natural Park "Buiratau", listed in the red list. Current Problems of Our Time 2 (28): 163-166.

Jaca TAPI, Kambizi L. 2011. Antibacterial properties of some wild leafy vegetables of the Eastern Cape Province, South Africa. J Med Plants Res 5 (13): 2624-2628.

Juzepczuk SV. 1937. *Pulsatilla* Adans. In: Komarov VL, Schischkin BK (eds.). Flora of the USSR. Volume 7. Editio Academiae Scientiarum URSS, Moscow, Leningrad.

Kirillov VYu, Stikhareva TN, Serafimovich MV, Mukasheva FT, Gering AV, Sarsenbaeva LA, Atazhanova GA, Adekenov SM. 2018. Chemical composition of essential oil from two species of *Pulsatilla* growing wild in Northern Kazakhstan. Bull Karaganda Univ Biol Med Geogr 2 (90): 29-34.

Kulikov PV. 2010. Determinator of Vascular Plants in Chelyabinsk Region. Bot Garden RSA, Yekaterinburg.

Kupriyanov AN, Schegoleva NV, Ebel AL. 2016. Review of buttercup family (*Ranunculaceae* Juss.) flora of Kazakh hillocky area. Syst Notes 113: 51-63. DOI: 10.17223/20764103.113.5.

Kupriyanov AN. 2020. Conspectus of Flora of Kazakh Hillocky Area. Academical Publ, Novosibirsk.

Karamysheva ZV, Rachkovskaya EI. 1973. Botanical Geography of Steppe Part in Central Kazakhstan. Nauka Publ., Leningrad.

Łaska G, Sienkiewicz A, Stocki M, Zjawiony JK, Sharma V, Bajguz A, Piotrowska-Niczyporuk A, Jacob M, Khan S. 2019. Phytochemical screening of *Pulsatilla* species and investigation of their biological activities. Acta Soc Bot Pol 88 (1): 3613. DOI: DOI: 10.5586/asbp.3613.

Laskai G, Sienkiewicz A, Zjawiony JK, Jacob M, Khan S. 2017. Antifungal activity of the root extracts of *Pulsatilla patens* against *Candida glabrata*. Acta Pol Pharm 74 (1): 179-185.

Li Q, Wang X, Wang J, Su N, Zhang L, Ma Y, Chang Z-y, Zhao L, Potter D. 2019. Efficient identification of *Pulsatilla* (Ranunculaceae) using DNA barcodes and micro-morphological characters. Front Plant Sci 10: 1196. DOI: 10.3389/fpls.2019.01196.

Naumenko NI. 2008. Flora and Vegetation of Southern Transurals. Publishing House at Kurgan State University, Kurgan.

Newall CA, Anderson LA, Phillipson JD. 1996. Herbal Medicines - a Guide for Health-Care Professionals. Pharmaceutical Press, London.

Pavlov NV. 1956. Flora of Kazakhstan. Publishing House of Science Academy of Kazakhskaya SSR, Alma-Ata.

Plants of the World Online (POWO). 2021. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/.

Pugachev PG. 1994. Pine Forests of the Turgai Depression. Kustanai.

Rabotnov TA. 1964. Determination of the Age Composition of Species Populations in a Community. Field Geobotany. M-L Publishing, House of the Academy of Sciences of the USSR, Moscow.

Red Data Book of Kazakhstan. 2014. Vol. 2, 2nd ed.. Ltd., XXI, Astana.

Serebryakov IG. 1962. Ecological Morphology of Plants. Life Forms of Angiosperms and Conifers. Higher School, Moscow.

Seregin AP. 2020. Digital herbarium of MSU: Electronic resource. MSU, Moscow.

Serpova E. 2012. Image of *Pulsatilla*. Plantarium: Open on-line atlas and key to plants and lichens of Russia and neighbouring countries. 2007-2021. https://www.plantarium.ru/page/image/id/165035.html

Shreter AI. 1986. Procedure for determination of medicinal plant reserves. USSR on Forestry, in Medical and Microbiological Industry. State com, Moscow.

Smirnova OV. 1976. The Volume of the counting units in the study of coenopopulations of plants of various biomorph. Coenopopulation of plants: Basic concepts and structure. M.

Somlay L. 2000. A *Pulsatilla flavescens* (Hazsl.) Borb. Emend. Borosprioritasanakvedelmeben. Kitaibelia 5: 245-248.

Sramko G, Laczko L, Volkova PA, Bateman RM, Mlinarec J. 2019. Evolutionary history of the Pasque-flowers (*Pulsatilla*, Ranunculaceae): Molecular phylogenetics, systematics and rDNA evolution. Mol Phyl Evol 135: 45-61. DOI: 10.1016/j.ympev.2019.02.015.

State Pharmacopeia of Kazakhstan Republic. 2008. V.1. Almaty, Kazakhstan.

Stepanov NV. 2014. Notes about some species *Pulsatilla* L. (Ranunculaceae) from the Near–Yenisey Sayans Mountains: Systematic notes on the materials of P.N. Krylov Herb Tomsk State Univ 109: 6-19.

Sultangazina GJ, Kupriyanov AN, Kupriyanov OA, Raimbekov EB. 2020. The ontogenesis and structure of coenopopulations of *Pulsatilla uralensis* in the conditions of Northern Kazakhstan. Bull Karaganda Univ Biol Med 3 (99): 134-140. DOI: 10.31489/2020BMG3/140-148.

Sultangazina GJ, Kupriyanov OA, Kupriyanov AN, Beyshov RS. 2019. Coenoflora *Pulsatilla patens* (L.) Mill. s.l. in Northern Kazakhstan. Bull Natl Acad Sci Repub Kazakhstan 4 (380): 83-92. DOI: 10.32014/2019.2518-1467.95.

Sultangazina GZ, Kupriyanov AN. 2012. Floristic findings on the territory of national park «Burabay». KemSU Sci Rev 1 (49): 23-26.

Sushencov OE. 2007. Population structure of *Pulsatilla* (Ranunculaceae) species in the Urals. Bot J 92 (4): 493-505.

Tzvelev NN. 2001. The genus *Pulsatilla* Mill. In: Tzvelev NN (eds.). Flora of Eastern Europe. Mir i Semja, Saint Petersburg.

Tzvelev NN. 2012. *Pulsatilla*. In: Tzvelev NN, Geltman DV (eds.). Conspectus Florae Europae Orientalis, Volume 1. KMK Scientific Press, Moscow & St. Petersburg.

Valieva LA. 2016. Quantification of Antimicrobial Activity of New Drug Infusions and Extracts. Ufa.

Valuyskikh OE, Teteryuk LV, Pylina YI, Sushentsov OE, Martynenko NA, Shadrin DM. 2020. Phylogenetic relationships and status of taxa of *Pulsatilla uralensis* and *P. patens* s.str. (Ranunculaceae) in North-eastern European Russia. PhytoKeys 162: 113-130. DOI: 10.3897/phytokeys.162.53361.

World Checklist of Vascular Plants (WCVP). 2021. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://wcvp.science.kew.org/ Retrieved 10 January 2021.

World Health Organization (WHO). 2009. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products [Electronic resourse] N 953. Mode of access: http:// apps.who.int/medicinedocs/documents/s19133en/ s19133en.pdf.

Zaitcev GN. 1973. Biometric Calculation Methodology. Science, Moscow.