

4.3 Selection of optimal conditions for obtaining biologically active compounds in *pulsatilla alba* extracts

One of the main tasks of modern pharmacy is to expand the range of medicines, including drugs of natural origin, as phytopreparations contain several groups of biologically active substances and have a more sparing effect on the human body. In this regard, the study of the plant family Ranunculaceae is promising, as they are sources of biologically active compounds that have long been used in folk medicine as drugs with diverse pharmacological action. Plants of this family contain such valuable biologically active substances as the glycoside ranunculin, triterpene saponins, essential oil, tannin, vitamin C, organic acids, flavonoids, alkaloids, volatile acids, tannins, resins and about 20 different macro-and micronutrients. The buttercup family unites dicotyledonous free-petalled flowering plants. This group of plants includes about 50 genera and more than two thousand species of plants that grow mostly in areas with temperate and cold climates on all continents. [309] A special place among these plants is occupied by *Pulsatilla*, a genus of flowering plants in the Ranunculaceae family of 30 species. Among them, 7 species grow in Ukraine: *Pulsatilla alba* (Fig. 1), *P. grandis*, *P. halleri*, *P. patens*, and Meadow (blackening) (*P. pratensis*), Crimean dream (*P. Taurica*) and Common dream (*P. vulgaris*).

For our research, we chose *Pulsatilla alba*, which has a very broad pharmacological action and is not represented in the pharmaceutical market.



Figure 1. *Pulsatilla alba*

Botanical description of the plant. Hemicryptophyte. Perennial, herbaceous plant. Develops a strong taproot and numerous branches of the caudex, from which diverge stolon-like underground shoots. The leaves appear at the end of flowering, on long petioles and with deeply 2-parted pointed lobes, basal leaves long-petiolate with three times trifoliate plates. Leaves bracts on extended at the base of the free petioles, twice or trifoliate. Generative shoots 7-25 cm tall, axillary. Leaves, shoots densely pubescent, as well as the petals on the outside. Perianth wide open, white, solitary flowers, erect, petals 2-2.5 cm long, blooms in April and May, and on the highest peaks even until mid-July [310]. The fruit of many nuts, nuts with feathery pubescent spines. Propagated by seeds and vegetatively, using stolons.



Figure 2. Distribution of *Pulsatilla alba* on the territory of Ukraine

Distribution. It grows in the meadows of the subalpine and alpine belts (1650 - 1960 m), in some places reaches the peaks, in rocky places, mainly on carbonate substrate. Known habitats in Chivchyny, Marmaros massif, Chornohora, Svydovka, Gorgany, occasionally in the Beskids. A small area of the plant in 2012 was discovered in the newly created Blue Oaks Reserve in Kyiv region. Distribution area: the mountains of Central Europe, from northern Spain to Central Yugoslavia and the Carpathians.

Habitat conditions. It grows in the subalpine zone on the slopes of various exposures, mainly on poorly turfed areas, rocky outcrops and screes, in the associations *Cystopteridetum fragilis*, *Salicetum herbaceae*, *Salicetum retuso-reticulatae*,

Festucetum curicavium, . (cl. *Asplenietea trichomanis*, *Elyno-Seslerietea*, *Saliceta herbaceae*). Mesophyte. [311-315]

Security status. Protected in the Carpathian Biosphere Reserve and the Carpathian National Nature Park, the plant is listed in the Red Book of Ukraine (III category). [316] The plant is ornamental, so it can be thoughtlessly gathered by tourists for bouquets. It is known that at present this plant has started to be bred in the nursery of the Plodsad company in Melitopol (Ukraine) and the planting material is coming to the market.

Chemical composition. The plant contains such valuable biologically active substances as the glycoside ranunculin, which is broken down into glucose and protoanemonin (anemonol), with further cleavage gives anemone (anemone camphor), and then with inactive anemone acid. In addition to ranunculin, it contains triterpene saponins, essential oil, tannin, vitamin C, organic acids, flavonoids, traces of alkaloids, volatile acids, tannins, resins and about 20 different macro- and micronutrients. In particular, the herb contains: essential oil, γ -lactones (ranunculin, protoanemonin), triterpenoids (p-amyrin acetate), sterols (sitosterol), chelidonic acid, saponins, coumarins, vitamin C; leaves contain: camphor and glycoside ranunculin. In addition, the leaves include alkaloids, volatile acids, vitamins, saponins, tannins and resinous substances, organic acids; the seeds contain fatty oil.

Roots and leaves are used in medicine.

The plant is nonofficial [312].

Pharmacological action. Internally, the plant is used as an antitumor, hypnotic, antimicrobial, antifungal, anti-inflammatory and analgesic.

Use in folk medicine. *Pulsatilla alba* is a strong antitumor agent and is used to treat cancer of various localizations (cancer of the uterus, breast, gastrointestinal tract, prostate, lung, etc.). Considered a powerful tool for the treatment of functional diseases of the nervous system: neuralgia, migraine, spasmophilia, insomnia, has hypnotic, antimicrobial and antifungal effects, lowers blood pressure, intracranial pressure, dilates the vessels of the fundus, treats cataracts, glaucoma, inflammation. Alcohol tincture, decoction of roots and herbs are recommended for eczema, fungal skin

lesions. Decoction of herbs in folk medicine is used for bronchitis, asthma, headaches, gout, rheumatism.

In China, a decoction of shot is used to treat dysentery, edema of renal and cardiac origin, the juice of herbs with honey is used to treat glaucoma. The decoction is drunk for pulmonary tuberculosis, measles, abdominal disruption, toothache, sexually transmitted and gynecological diseases, and the decoction is given to women in difficult childbirth (as an analgesic and facilitates childbirth). Juice from fresh herbs, evaporated in the bath is used to treat burns, rubbing numb parts of the body. Caution should be exercised when ingested, because fresh plants in overdose can cause inflammation of the intestinal mucosa, kidneys. And in large doses - paralysis. At glomerulonephritis it is contraindicated.

Used in folk cosmetics as: face mask for inflamed skin and acne; to add satin smoothness to the skin; for washing; as a hair conditioner.

In veterinary medicine, it is used as a tincture, which has fungicidal and bactericidal properties. [312-314, 317]

Therefore, the object of our study was selected aboveground part of *Pulsatilla alba* plants. The collection of raw materials was carried out in ecologically clean regions of Western Ukraine during the flowering period in March - April 2019-2020. The plant was dried by air-shadow method without artificial heating of raw materials. The raw material was cleaned of impurities, defective and discolored parts of plants were removed. The herb *Pulsatilla alba* was ground and standardized according to the requirements of analytical and normative documentation. The identification of plant raw materials was carried out on the basis of macroscopic signs. [318]

Dried and crushed raw materials were sifted through a sieve with a hole diameter of 7; 5; 3; 2; 1; 0.5; 0.25 mm.

The exact sample was selected from each faction. 2.0 g of raw material was placed in a round-bottomed flask with a capacity of 100 ml, purified water was added in a ratio of raw material to the extractant of 1:50 and heated on a water heater at the temperature of boiling with the liquid for 15 minutes. The extract was cooled, filtered,

if necessary, brought to the initial volume with water and determined the content of polyphenols and extractives.

During extraction, biologically active substances (BAS) are transferred from medicinal plant raw materials to the extractant. It is known that the yield of extractives is influenced by factors that determine the completeness and speed of extraction: the nature of the extractant, the degree of grinding of raw materials, the ratio between raw material and extractant, the reagent, the hydrodydrant. [319] In order to work out the optimal conditions for obtaining the sum of biologically active substances from the studied object, the degree of crushing of raw materials, the nature of the extractant, the extraction time, the co-ratio were taken into account in their work. The amount of polyphenols was determined according to the pharmacopoeial method of SPU. [318]

To facilitate the penetration of the extractant, plant raw materials are ground in accordance with the requirements of the SPU. [318]

The results of the study of the influence of the degree of grinding on the completeness of BAS extraction are given in Table 1.

Table 1.

*Dependence of completeness of BAS extraction on the degree of grinding of dry
Pulsatilla alba herb*

Degree of grinding of raw materials	Content of BAS and extractives, %, $\bar{x} \pm \Delta\bar{x}$, n = 3	
	The amount of polyphenols	Extractives
> 7 mm	2,02± 0,01	7,05 ± 1,24
5 - 7 mm	2,14± 0,01	7,73 ± 0,81
3 - 5 mm	2,52± 0,01	7,88 ± 0,16
2 - 3 mm	2,12 ± 0,01	7,01 ± 0,16
1 - 2 mm	2,62± 0,01	7,94 ± 1,01
0,5 – 1mm	1,82± 0,02	7,50 ± 1,26
0,25 - 0,5 mm	2,22± 0,01	7,15 ± 0,94

As can be seen from Table 1, the optimal degree of grinding for *Pulsatilla alba* is 1 - 2 mm.

Table 2.

Influence of the nature of the extractant on the completeness of the extraction of the BAS complex from Pulsatilla alba raw materials

Extractant	Content of BAS and extractives, %, $\bar{x} \pm \Delta\bar{x}$, n = 3	
	The amount of polyphenols	Extractives
Purified water	$2,12 \pm 0,01$	$7,33 \pm 1,1$
20% alcohol	$1,82 \pm 0,01$	$7,04 \pm 1,3$
40% alcohol	$2,52 \pm 0,02$	$7,73 \pm 1,3$
70% alcohol	$2,02 \pm 0,02$	$7,14 \pm 1,3$
90% alcohol	$1,74 \pm 0,02$	$6,99 \pm 1,1$

During the selection of the extractant, the quality and quantity of the finished extract were taken into account. We used water-alcohol mixtures with an alcohol content of 20%, 40%, 70% and 90% as extractants (Table 2).

The results of the research show that the optimal extractant for *Pulsatilla alba* is 40% aqueous-alcoholic solution.

The amount of extractant must be such as to ensure complete extraction of the active substances. In order to establish the optimal ratio between raw materials and extractants, we used the following ratios 1: 5, 1:10 and 1:20. The results of the selection of the optimal ratio between plant raw materials and extractants are given in Table 3.

Table 3.

Selection of the optimal ratio between raw material (Pulsatilla alba) and extractant

The ratio between raw material and extractant	Content of BAS and extractives, $\%, \bar{x} \pm \Delta\bar{x}, n = 5$	
	The amount of polyphenols	Extractives
1:5	$2,02 \pm 0,01$	$7,57 \pm 0,04$
1:10	$2,04 \pm 0,04$	$7,60 \pm 0,06$
1:20	$2,12 \pm 0,07$	$7,63 \pm 0,02$

As a result of the conducted researches it was determined that the optimal ratio between raw materials and extractants for *Pulsatilla alba* is 1:20, using 40% ethanol. The determination of the optimal time of one-time extraction of biologically active substances from the investigated raw material was carried out by the optimal extractant, adjusting the raw material in the selected ratios, in the ratios of raw materials, in the amount of 90 to 60, 30 to 30 extra extracts. The results of the dependence of BAS content in the obtained extracts on the time of extraction are given in Table 4.

Table 4.

Influence of extraction time on the yield of BAS from Pulsatilla alba raw materials

Extraction time, min	Content of BAS and extractives, $\%, \bar{x} \pm \Delta\bar{x}, n = 3$	
	The amount of polyphenols	Extractives
30	$1,92 \pm 0,02$	$7,97 \pm 0,06$
60	$2,00 \pm 0,01$	$7,04 \pm 0,04$
90	$2,11 \pm 0,02$	$7,33 \pm 0,01$
120	$2,02 \pm 0,01$	$7,10 \pm 0,08$

According to the obtained results of the study, the optimal time for a single extraction with complete BAS isolation for *Pulsatilla alba* is 90 min.

The results of the conducted researches on establishment of optimum conditions of reception of extracts from the investigated raw materials are given in Table 5.

Table 5.

Optimal conditions for extraction of BAS from the investigated Pulsatilla alba raw material

Raw material	Degree of grinding, mm	Extractant	The ratio between raw material and extractant	One-time extraction time, min
<i>Pulsatilla alba</i>	1 – 2	40% alcohol	1:20	90

The results of the research showed that the optimal conditions for the release of BAS is the extraction of grinded to 1-2 mm *Pulsatilla alba* plant with 40% alcohol in a flask with reflux in a water bath for 90 minutes, the optimal ratio between raw material and extractant is 1:20.

The obtained extracts were standardized in accordance with the requirements of the State Pharmacopoeia of Ukraine [318].

Description. *Pulsatilla alba* herb extract is a clear brown-green liquid with a faint odor.

Dry residue. The tests were performed in accordance with SPU 2.8.16. The dry balance of the extract was not less than 1.7%.

Heavy metals. For all studied extracts not more than 0.01% (SPU, 2.4.8, method A).

A preliminary assessment of the quality of the extracts obtained was performed.

Thus, in particular, several qualitative reactions were performed to detect phenolic bonds. It was found that the addition of 1% solution of ferric chloride to the extract shows the appearance of violet color. Moreover, the addition to 1 ml of the

extract of 3-5 drops of freshly prepared mixture of equal volumes of solutions of 1% iron III chloride and 1% potassium ferricyanide and 10 drops of concentrated nitric acid leads to the appearance of dark brown color. And when adding 2–4 drops of 1% solution of vanillin in concentrated hydrochloric acid to 1 ml of extract, a red color was observed.

To detect acids in the extract, 2-3 drops of 0.05% bromothymol blue solution were added to 1 ml of the extract, and a yellow color was observed.

Therefore, *Pulsatilla alba* is a promising and valuable raw material for the production and production of phytochemicals, and we have determined the optimal conditions for the extraction process.