

## Phytochemical Analysis of Biotechnological Raw Materials of Representatives of the Genus *Potentilla* L.

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**Abstract**—The genus *Potentilla* (*Potentilla* L.) is a member of the family Rosaceae, widespread in temperate, arctic, and alpine zones of the northern hemisphere. This genus has been known since ancient times for its curative properties. Recent pharmacological studies have confirmed the traditional use of extracts of the *Potentilla* species against different diseases. The phytochemistry of the members of *Potentilla* L. remains poorly studied. A biotechnological technique for the production of renewable raw materials has been developed only for *Potentilla alba* L. The goal of this work is to conduct the phytochemical analysis of biotechnological raw materials of *Potentilla alba* L. and *Potentilla fragarioides* L. and to identify the features of the elemental composition and accumulation of biologically active substances in regenerants, compared to the intact plants. The research has led to the development of a biotechnological technique for the production of *P. fragarioides* phytomass. The intensity and the specificity of accumulation of chemical elements by organs of regenerated plants of *P. alba* from the nutrient media in tissue culture have been evaluated. The elements of vigorous accumulation (Ca, Mg, Fe, Mn, Zn, Mo, and Cu), as well as the element of strong accumulation, Co, are noted. The features of the elemental composition of *P. alba* raw materials, depending on the production method, are noted. The quality, the content of water- and alcohol-soluble extractives and of some groups of biologically active substances have been estimated. It is revealed that *P. alba* and *P. fragarioides* are concentrators of flavonoids and tannins. At the same time, the indicators of accumulation of biologically active substances in *P. fragarioides* are higher than those for *P. alba*, in both conventional and biotechnological plant raw materials.

**Keywords:** *Potentilla alba* L., *Potentilla fragarioides* L., plant material, biotechnological technique, elemental composition, phytochemical characteristics

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### INTRODUCTION

Drug preparations based on plant raw materials have a major drawback: the scarcity of natural sources, the industrial use of which implies certain ecological concerns [1]. One of the most important tasks in the development of the pharmaceutical and food industries is to ensure the availability of renewable raw materials with the required properties.

The genus *Potentilla* L. is one of the large polymorphic genera of the Rosaceae family in Western Siberia and Gorny Altai [2]. Some members of this genus have been used by folk and official medicine for a long time, but their phytochemical composition has not been studied enough. At present, only a few members of the genus *Potentilla* L., such as septfoil (*Potentilla erecta* L.) and hoary cinquefoil herb (*Potentilla argentea* L.), are officially applied as raw materials.

Recent pharmacological studies have generally confirmed the traditional use of the *Potentilla* species and their extracts from the aerial and/or underground

parts in treatment of inflammation, colitis, some cancer types, viral and bacterial infections, immune system disorders, diabetes, and liver diseases [3].

The most studied chemically and pharmacologically is white cinquefoil (*Potentilla alba* L.) [4–6], a perennial herbaceous medicinal plant, 8–25 cm high, with a long thick, little-branched and black-brown rhizome. It is known that drugs based on *P. alba* affect the thyroid gland, regulating its function, eliminating diffuse changes, and eliminating numerous toxic effects. Furthermore, *P. alba* is used in the prevention and treatment of liver diseases, diseases of cardiovascular system and gastrointestinal tract (in particular, ulcers), and also as an antiseptic and wound-healing agent [4].

A phytochemical analysis of nonconventional renewable raw material of *P. alba* grown on the basis of the developed biotechnological technique was carried out. The results indicate that according to the content of extractives and main groups of biologically active substances, the biotechnological raw material is not inferior to the traditional (and according to some

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parameters, it was even superior). It has been found that the intact and regenerated plants of *P. alba* exhibit comparable biological activity against the herpes virus [6, 7].

One more promising species, the phytomass of which can be used as a new raw material, is strawberry cinquefoil (*Potentilla fragarioides* L.). The stems of *Potentilla fragarioides* L. are from 5 to 25 cm high, weak and low-leafy, and are, like the leaf petioles, covered with long and separated hairs often seated on tubercles. Basal leaves are pinnate, with 2–3 pairs of toothed leaflets. The leaflets of the upper pair, including the terminal leaflet, are 1–6 cm long, 0.6–3 cm wide. It has a fibrous root system. It blooms in June–July and grows in dry and forest meadows, sparse forests, and river valleys; on meadow-steppe slopes and idle fields in the administrative oblasts of Siberia, Tomsk, Novosibirsk, and Kemerovo oblasts, Altai region, Altai Republic, Krasnoyarsk oblast, Khakassia, Tuva, Irkutsk oblast, Buryatia, Chita oblast, at the Far East, in Yakutia, Mongolia, and Manchuria [8]. The plant contains tannins (6.2%) and flavonoids.

The antioxidant properties of flavonoids are well known. Numerous hypotheses about the effects of flavonoids on human health, including a positive effect on the cardiovascular system, anticarcinogenic effects, etc., are also based on their antioxidant properties [9]. Flavonoids, along with other antioxidants (for example, vitamins E and C) that are ingested with food, are important components of the cell antioxidant system [10–13].

Flavonoids are not synthesized in animal and human cells, and the presence of flavonoids in the tissues is completely dependent on the consumption of plant products. Due to the prospective application of flavonoids in medicine, the interest in studies on their effect on the human body is significantly increased. For the past two decades, the number of studies in this area has grown more than tenfold and reaches about five thousand a year. This is approximately equal to the number of publications on targeted drug delivery and twice the number of publications on gene therapy. The description of flavonoids is present in most of the works where the chemical composition of plants used in traditional medicine is analyzed. The healing properties of some plants are often explained by the presence of certain flavonoids [14].

Folk medicine commonly uses the leaves of *P. fragarioides*. The leaf decoction is taken as an astringent for diarrhea and for rinsing against gingivitis. A strong decoction of rhizomes with roots is used for washing burns and purulent wounds, for rinsing mouth and throat in treatment of stomatitis and sore throat. The herb decoction is drunk in treatment of hemoptysis in pulmonary diseases and against gynecological bleeding [15].

The goal of this work is to conduct the phytochemical analysis of biotechnologically produced raw mate-

rials of *Potentilla alba* L. and *Potentilla fragarioides* L., and to identify the features of the elemental composition and accumulation of biologically active substances in regenerants, compared to intact plants.

## EXPERIMENTAL

**Plant material.** Samples of biomass of regenerated plants (regenerants) of *P. alba* and *P. fragarioides* were obtained at the Laboratory of Plant Biotechnology of the South-Siberian Botanical Garden of Altai State University [16, 17]. The regenerants of *P. alba* and *P. fragarioides* were propagated on a nutrient agar medium containing Murashige and Skoog (MS) mineral salts and were grown in a CuttingBoard 27 system (GHE, France) under hydroponic conditions.

We studied the samples of rhizomes of intact *P. alba* used in the production at ZAO Evalar (Biysk, Russia) and the samples of the aerial and underground parts of conventional *P. fragarioides* raw material grown under field conditions in the Tyumentsevsky district of Altai krai (collection: end of summer 2016).

**Research method.** Elemental analysis was performed with an Optima 7300 DV ICP atomic emission spectrometer (AES) (PerkinElmer, United States). For the ICP-AES analysis, the samples of plant material were preliminarily ground. A sample weight of 1 g was treated with nitric acid diluted in a 1 : 1 ratio with distilled water and placed in a microwave oven.

The precooled vessel with the mineralized sample was placed in a fume hood and left until no visible brown fumes were evolved. The mineralizate was clear. In case if the volume decreased, it was adjusted to the required level with distilled water. The resulting solution was transferred to a quartz vessel for the elemental identification and quantification (Table 1).

The intensity of accumulation of chemical elements from the nutrient media by the organs of regenerants was evaluated using bioaccumulation factors (BAF) defined as the ratio of the element content in plant organs to that in the media. The elements were classified based on this factor, using A.I. Perelman's classification groups: (1) vigorous accumulation ( $100 > \text{BAF} \geq 10$ ); (2) strong accumulation ( $10 > \text{BAF} \geq 1$ ); (3) weak accumulation/medium uptake ( $1 > \text{BAF} \geq 0.1$ ); (4) weak uptake ( $0.1 > \text{BAF} \geq 0.01$ ); (5) very weak uptake ( $0.01 > \text{BAF} \geq 0.001$ ) [18, 19].

The moisture content was determined with a MX-50 moisture analyzer at 105°C [20]. The ash content was determined by burning the samples in a muffle furnace at 600°C. The extractives were isolated from the plant material by sequential treatment of the samples with various solvents (hexane, chloroform, 96% ethanol, and water). The extraction was carried out in a Soxhlet apparatus at a raw material to extractant ratio of 1 : 15. For the treatment with water, the sample was kept in a solvent at 60°C.

**Table 1.** Concentrations of the elements in the organs of intact and regenerated plants of *P. alba*

Elements	Intact plants		Regenerants	
	rhizomes with roots, ZAO Evalar	3-year old rhizomes [34]	rhizomes with roots	herb
<i>Macro-elements, g</i>				
K	3.2 ± 0.1	3.4	*	*
Ca	*	11.5	*	1.6 ± 0.5
Mg	1.6 ± 0.5	2.3	1.4 ± 0.4	1.2 ± 0.3
P	1.4 ± 0.4	1.2	3.4 ± 0.1	3.3 ± 0.9
Na	*	0.029	*	0.50 ± 0.01
Fe	0.50 ± 0.04	0.09	0.94 ± 0.06	0.20 ± 0.06
<i>Trace elements, mg</i>				
Al	600 ± 60	110	55 ± 4	16 ± 4
Mn	40 ± 12	47	122 ± 36	192 ± 57
Zn	29 ± 6	34	243 ± 49	141 ± 28
Ti	15 ± 5	<0.5	<5.0	<5.0
Ba	15 ± 4	27	12 ± 4	<5.0
Cu	8 ± 2	6.3	23 ± 4	1.6 ± 0.3
Sr	2.7 ± 0.6	94	30 ± 9	6 ± 1
V	2.3 ± 0.6	0.6	1.1 ± 0.3	0.21 ± 0.05
Ni	1.1 ± 0.4	0.49	0.8 ± 0.3	0.4 ± 0.1
Mo	0.9 ± 0.3	0.61	–	4 ± 1
Co	0.3 ± 0.1	0.085	1.3 ± 0.5	0.3 ± 0.1
<i>Ultratrace elements, mg</i>				
Sb	<0.1	<0.5	0.14 ± 0.07	<0.1
Sn	<0.1	12	0.14 ± 0.05	0.24 ± 0.09
Se	<0.1	<0.034	<0.1	<0.1
Ag	<0.1	<0.5	<0.1	<0.1
Cr	<0.1	0.17	<0.1	<0.1
Be	<0.05	<0.005	<0.05	<0.05

\*The concentration of the element exceeded the maximum sensitivity threshold of the instrument. “–” No available data.

**Determination of the content of some groups of biologically active substances.** The flavonoid content in the cinquefoil extracts was determined using a method based on the formation of a colored aluminum chloride complex. Ethanol (90%) containing a 10% sulfuric acid solution was used as an extracting agent. The absorbance of the resulting solution was measured with a UV-Vis Cary 60 spectrophotometer at 430 nm using a 10 mm pathlength cuvette. The extract solution in ethanol (95%) was used as a reference solution [21, 22]. The total flavonoid content was expressed in quercetin equivalents. The content of tannins was determined with permanganometric method [21].

**Biotechnology for the production of *P. alba* and *P. fragarioides* raw materials.** The developed technique for the preparation of medicinal raw material of *Potentilla* combines clonal micropropagation and cultivation under hydroponic conditions [7, 16, 17].

**Clonal micropropagation of *P. fragarioides*.** The matured seeds from the collection of the South Siberian Botanical Garden were used as explants. Prior to sterilization, the *P. fragarioides* seeds were washed under running water for 15–25 min. Sterilization was carried out in a laminar flow hood, using a 1% sulfochlorantin solution for 10 min. Then the seeds were washed three times with sterile distilled water. This sterilization method allowed for obtaining 70% of the explants sterile and viable. The nutrient medium for the stage of introduction into the tissue culture was based on the Murasige and Skoog (MS) recipe without phytohormones.

After 20–30 days, the developed shoots were replanted to MS media supplemented with 1.0–0.5 μM kinetin and 0.25 IBA (indole-3-butyric acid) and 0.05 μM GA (gibberellic acid) for the micropropagation. The formed conglomerated microshoots are eas-

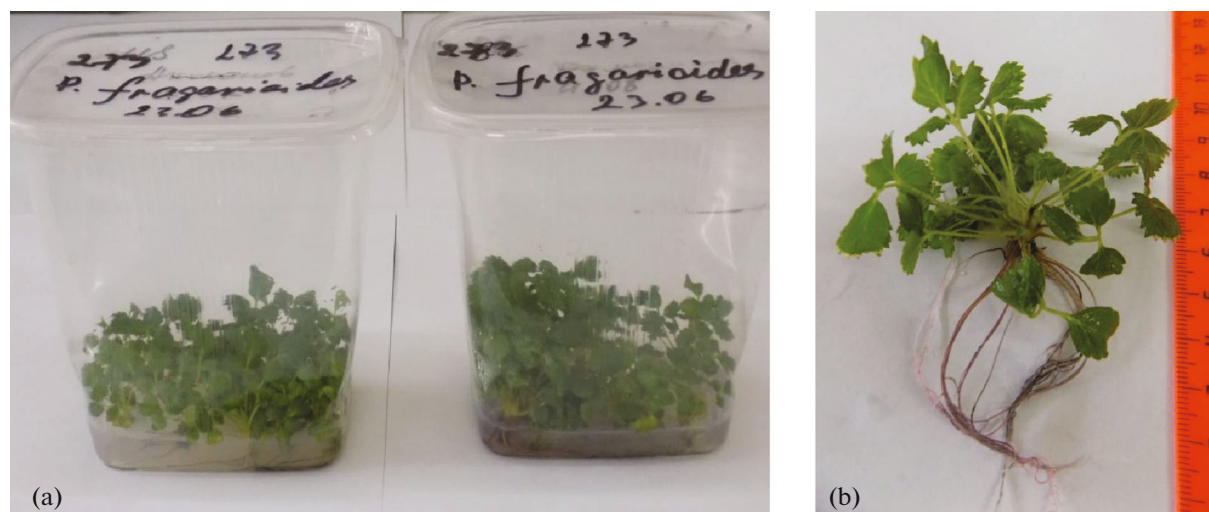


Fig. 1. The micropropagation stage of *P. fragariooides* (a). Regenerant of *P. fragariooides* (b).

ily divided into single ones, which are transferred to fresh media. To obtain an actively proliferating culture for a long time, the nutrient media with high and low BAP content should be alternated every other passage. As a result, a sterile *P. fragariooides* culture with a stable propagation rate of  $5.3 \pm 0.4$  is obtained. The number of shoots per explant for one passage reached from 2 to 15 pcs. The shoots were rooted on MS medium supplemented with  $1.0 \mu\text{M}$  IMC (Fig. 1).

Regenerants were adapted to nonsterile conditions and *P. fragariooides* raw material was cultivated in a hydroponic growth system containing 1/4-strength MS media.

## RESULTS AND DISCUSSION

**Analysis of the elemental composition of in vitro *P. alba* culture.** Bioaccumulation factor (BAF) is a quantitative measure of the intensity of accumulation of chemical elements by plants. BAF reflects the bio-

philicity degree of the elements and the intensity of their involvement in the biological cycle. Based on the results, the elements of vigorous accumulation in in vitro *P. alba* culture are Ca, Mg, Fe, Mn, Zn, Mo, and Cu; as well as a strong accumulation of Co (Table 2).

According to the literature, the content of aluminum, zinc, and manganese in *P. alba* is 1.7, 2.5, and 3.0 times, respectively, higher than that in non-conventional plants. The major elements in *P. alba* are calcium, silicon, boron, iron, and nickel [23].

A distinctive feature in the accumulation of the elements was noted, when evaluating the regenerated plants of *P. alba* as a source of medicinal raw materials. In particular, the content of manganese, phosphorus, and Fe in the roots of the regenerants was 3, 2.5, and 1.8 times, respectively, higher than that for the intact plants.

Plants that concentrate manganese are used to prevent cardiovascular diseases and to maintain the nervous system function and gonadotropic and musculo-

Table 2. Bioaccumulation factors (BAF) of elements in the organs of *P. alba* regenerants

Content, mg/kg	MS medium	Rhizomes with roots		Herb	
		content	BAF	content	BAF
Ca	120.1	—	—	$1573 \pm 472$	13.1
Mg	36.6	$1456 \pm 437$	39.8	$1195 \pm 358$	32.6
Fe	5.6	$939 \pm 62$	167.7	$201 \pm 56$	35.9
Mn	5.5	$122 \pm 36$	22.1	$192 \pm 57$	34.8
Zn	1.9	$243 \pm 47$	128.0	$141 \pm 28$	74.0
Mo	0.1	$40 \pm 16$	396.7	$4 \pm 1$	36.43
Co	0.07	$1.3 \pm 0.5$	18.8	$0.3 \pm 0.1$	4.9
Cu	0.01	$23 \pm 4$	2268.0	$1.6 \pm 0.3$	164.0

“—” No available data.

**Table 3.** The content of trace elements in the underground part of *P. alba* and content normalization, mg/kg of dry matter

Parameter	Fe	Mn	Zn	Cu	Pb	Cd	Sb	Be	Cr	Ni	As
Conventional raw material	504 ± 14	40 ± 12	29 ± 6	8 ± 2	0.3 ± 0.8	0.09 ± 0.04	<0.10	<0.05	<0.10	1.1 ± 0.4	0.3 ± 0.1
Biotechnological raw material	939 ± 27	122 ± 36	243 ± 49	23 ± 4	0.6 ± 0.1	0.02 ± 0.01	0.14 ± 0.07	<0.05	<0.10	0.8 ± 0.3	0.2 ± 0.1
Content normalization [26–31]											
Low	<50	<20	<20	<5	–	–	–	–	–	–	–
Normal	50–250	25–250	25–250	6–15	2–14	0–0.5	–	–	0–0.5	0–8	–
Toxic	–	>500	>400	>20	–	>100	–	–	–	>80	–
Mean	200	205	30	8.0	1.25	0.035	0.06	0.01	1.8	2.0	0.5
MPC (maximum permissible concentration) in biologically active additives	–	–	–	–	6.0	1.0	–	–	–	–	–
MPC (maximum permissible concentration) in tea	–	–	–	100	10.0	1.0	–	–	1.0	–	3.0
State Pharmacopoeia of the Russian Federation, XIII ed.	–	–	–	–	6.0	1.0	–	–	–	–	0.5

“–” No available data.

**Table 4.** The results of analysis of the source medicinal raw materials (*P. alba*, *P. fragarioides*)

Parameter	<i>P. alba</i>		<i>P. fragarioides</i>		
	rhizomes with roots of intact plants	biotechnological raw material	rhizomes with roots of intact plants	herb of intact plants	biotechnological raw material
Moisture, %	4.8 ± 0.2	3.9 ± 0.2	4.8 ± 0.1	5.7 ± 0.4	4.7 ± 0.1
Ash, % per abs. dry matter	7.6 ± 0.4	5.7 ± 0.7	3.8 ± 0.4	7.8 ± 0.2	–

“–” No available data.

skeletal functions. Molybdenum is involved in the processes of fertilization and embryonic development in plants. Molybdenum and iron are constituents of the enzyme nitrate reductase, and molybdenum is involved in the reduction of nitrates and in fixation of molecular nitrogen, as well as in the metabolism of vitamins. Molybdenum retains fluoride in the human body and helps to prevent dental caries [24]. The molybdenum content in plants is 0.0005–0.002%. According to our data, the molybdenum content in roots and herbs of *P. alba* regenerants is 11.1 and 4.2 times, respectively, higher than that for the intact plants (Table 1).

High concentrations of chemical elements in plants can cause toxic effects. The concentrations of trace elements in roots and rhizomes of the regenerated and intact plants of *P. alba* were compared with the permissible levels. The content of biophile microelements studied by us, such as Mn and Zn, was at the level of mean values in continental plants, and the content of Fe was much higher. The content of heavy and toxic metals, such as Pb, Cd, Cr, Be, Ni, and As did not exceed the normal level in plants and the permissible level in biologically active supplements (BAS), herbal tea, and medicinal plant raw materials (Table 3); and more research is required on the Sb content in biotechnological raw materials. According to O.A. Yelchinova [24], the Sb content in medicinal plants in

the environmentally friendly region of Northern Altai was from 0.038 to 6.6 mg/kg of dry matter.

**Comparative phytochemical characteristics of *P. alba* and *P. fragarioides* raw materials.** The content of moisture and ash in plant raw material is one of the quantitative indicators of its quality. The content of moisture in medicinal plant materials shall be not more than the permissible levels. For most types of plant materials, the permissible limit of moisture is usually up to 15% [25]. The values for rhizomes with roots and herb of *P. fragarioides* are within the permissible limits (Table 4).

The content of extractives isolated with the solvents of different nature and using different methods was determined after the solvent was removed on a rotary evaporator under vacuum; the values were calculated taking into account humidity.

The total content of extractives in the *P. alba* roots and rhizomes is 26.2%. Table 5 shows that the maximum amount of extractives was extracted with water. It is known from the literature that flavonoids, polysaccharides, amino acids, and tannins are extracted with water [32].

**Quantification of some groups of biologically active substances.** Flavonoids are present in different plant organs, but more often in the aerial ones, including flowers, leaves, and fruits; and much less in stems and underground organs (licorice, Baikal skullcap, rest

**Table 5.** The content of extractives isolated from the roots and rhizomes of intact and regenerated *P. alba*, using with different solvents

Solvent	Content of extractives in <i>P. alba</i> , % per abs. dry matter ( $\pm 0.5$ )	
	intact plants	regenerants
Hexane	1.8	1.3
Chloroform	1.9	4.3
Ethanol (96%)	4.8	7.3
Water	17.7	6.1
Total content of extractives, %	26.2	19.1

harrow). The flavonoid content in plants is different, on average 0.5–5.0%, sometimes up to 20% (in *Sophora japonica* buds). A high content of flavonoids was observed in the leaves of intact *P. fragarioides*, which reached 19.0%. This flavonoid content in *P. fragarioides* is six and four times higher, respectively, than in the roots and rhizomes of the intact plants and biotechnological material of *P. alba* (Fig. 2). In this regard, *P. fragarioides* should be considered as a valuable flavonoid carrier plant.

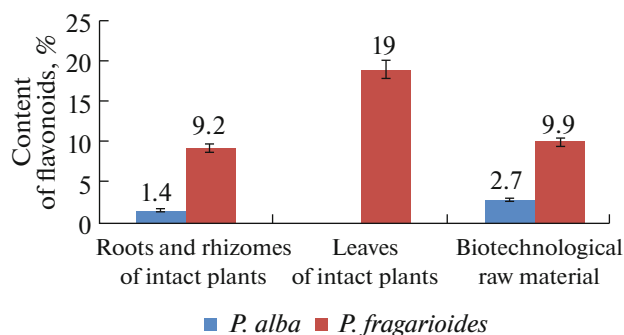
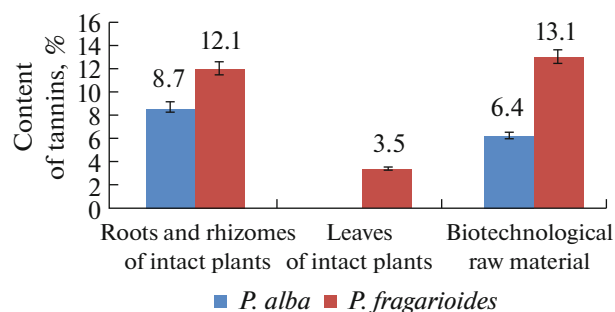
A strong antioxidant D-catechin was isolated from the group of flavonoids contained in *P. fragarioides* [32]. Furthermore, Kosman et al. noted the presence of (+)-catechin in *P. alba* in the composition of phe-

nolic compounds in the fraction of the substances dissolved in water [33].

The results of our research are not contradictory to the data obtained by the scientists from the Central Botanical Garden of the National Academy of Sciences of Belarus, Minsk. Our study revealed that the accumulation of flavonoids occurs unequally at different stages of the plant life cycle. The maximum content of flavonoids in *P. recta* L. and *P. rupestris* L. was observed at the full bud stage ( $2.85 \pm 0.02\%$  and  $4.15 \pm 0.02\%$ , respectively, in the leaves;  $1.81 \pm 0.03\%$  and  $10.1 \pm 0.04\%$ , respectively, in the generative organs). The highest content of flavonoids in *P. alba* was observed at the full blooming stage ( $2.33 \pm 0.01\%$  in the leaves and  $2.69 \pm 0.006\%$  in the generative organs) and it slightly decreased at the secondary blooming stage of the taxon *P. alba*. This might be of interest and serve as recommended practice for optimizing the preparation of medicinal plant materials. In the underground part, the maximum accumulation of flavonoids in the three taxa was at the full blooming stage ( $1.81 \pm 0.03$ ,  $0.26 \pm 0.01$ , and  $2.69 \pm 0.01$ , respectively, in *P. recta*, *P. rupestris*, and *P. alba*) [34].

Most of the pharmacological effects (for example, antiviral and antimicrobial, immunomodulatory, hepatoprotective and anti-inflammatory) in the *Potentilla* species can be due to a high content of condensed and hydrolyzable tannins in the aerial and underground parts [3]. We compared the content of tannins in biotechnological raw materials and intact plants of the two *Potentilla* species (Fig. 3). In the roots and rhizomes of the intact plants of *P. alba* and *P. fragarioides*, the content of tannins was 8.7 and 12.1%, respectively. In the biotechnological raw material of *P. alba* and *P. fragarioides*, the content of tannins was 6.4 and 13.1%, respectively. The results suggest that in terms of the accumulation of tannins, *P. fragarioides* is a more valuable medicinal plant than *P. alba*. As already noted in [9], this feature allows the application of *P. fragarioides* in folk medicine as an astringent tincture.

The investigation of biomass grown under conditions of the central agroclimatic zone of Belarus shows that the maximum uptake of tannins in the aerial part of all the three *Potentilla* L. species occurs at the full

**Fig. 2.** The content of flavonoids in raw materials of *Potentilla alba* L. and *Potentilla fragarioides* L.**Fig. 3.** The content of tannins in raw materials of *Potentilla alba* L. and *Potentilla fragarioides* L.

blooming stage (*P. alba* L.,  $16.4 \pm 0.03\%$ , *P. recta* L.,  $17.8 \pm 0.09\%$ , and *P. rupestris* L.,  $13.3 \pm 0.05\%$ ) and it slightly decreases at the secondary blooming stage (*P. alba* L.,  $13.8 \pm 0.06\%$ , *P. recta* L.,  $14.6 \pm 0.10\%$ ). The minimal uptake of tannins is observed at the end of plant vegetation [34].

## CONCLUSIONS

A biotechnological technique for the production of phytomass of *P. alba* and *P. fragarioides* has been developed. The features of the elemental composition of *P. alba* raw materials, depending on the preparation method have been discussed. The content of heavy and toxic metals (Pb, Cd, Cr, Be, Ni, Pb and As) did not exceed the normal level in plants and the permissible level in biologically active additives, herbal tea, and medicinal plant raw materials.

Phytochemical analysis of raw plant materials of the representatives of the genus *Potentilla* L. (*P. alba* and *P. fragarioides*.) was carried out. The content of flavonoids in the leaves of intact *P. fragarioides* reached 19.0% that is six and four times higher, respectively, than that in the roots and rhizomes of the intact plants and biotechnological raw material of *P. alba*. The content of tannins in roots and rhizomes of the intact plants of *P. alba* and *P. fragarioides* was, respectively, 8.7 and 12.1%. In the biotechnological raw materials of *P. alba* and *P. fragarioides*, the content of tannins was 6.4 and 13.1%, respectively.

*P. alba* and *P. fragarioides* accumulate a significant amount of flavonoids and tannins and are potential sources of these substances for humans. At the same time, the indicators of accumulation of biologically active substances in *P. fragarioides* exceed those determined for *P. alba*.

## COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

### Conflict of Interests

The authors declare that they have no conflict of interests.

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