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FEATURES OF THE ROOT AND RHIZOME ANATOMICAL STRUCTURE OF *POTENTILLA ALBA* L. AS A DIAGNOSTIC SIGN OF THE RAW MATERIALS

Raw materials derived from the species Potentilla alba L. have been widely used in medicine. It has been shown that roots and rhizomes contain compounds with a number of antioxidant properties that may support therapeutic, namely anti-inflammatory and adaptogenic effects. The species is also promising in the complex treatment of thyroid pathology. Analysis of the obtained raw materials for the presence of the required plant species for the manufacture of medicines is essential procedure.

The purpose of the research is to identify the anatomical features of the root and rhizome of Potentilla alba. It will help to identify these plants in the raw material. Comparison of the anatomical structure of the roots and rhizomes of five members of the genus Potentilla L.: P. alba L., P. anserina L., P. argentea L., P. pilosa Willd., P. reptans L. has been held. All species have been grown in the collection of the Fomin Botanical Garden. The cross sections of the root and rhizome with a thickness of 15–20 μm were made by the means of a freezing microtome. The inclusions were identified in the resulting cross sections stained with safranin, KI and sudan. The species differ in the development and location of xylem elements, and by localization of inclusions.

The most effective anatomical markers of P. alba, compared to other species of the genus, are the development and the location of rhizome xylem elements. The vessels of the secondary xylem are small, placed with uneven radial strands. The thicknes of strands is 1–3 vessels, delimited by the rays of the xylem parenchyma. The number of inclusions depends to a greater extent on the conditions of plant growth, so it is a less important factor during the analysis of plant raw materials.

Keywords: Potentilla alba, anatomy, rhizome, root.

Introduction. *Potentilla alba* L. is a valuable medicinal plant widely used in folk and traditional medicine. It is particularly promising in the complex treatment of thyroid gland pathology. Special attention was paid to the research of *P. alba* rhizome extracts. The total content of phenols, flavonoids, flavonols, flavanones and proanthocyanidins was determined in extracts of roots and rhizomes. The antioxidant and pro-oxidant properties of the extracts were described. A number of phenolic compounds in the raw material with strong antioxidant properties were found. It can support the therapeutic, namely anti-inflammatory and adaptogenic effects attributed to the species of this genus [1]. The effect of various microelements contained in these plants on iodine metabolism and thyroid gland function has also been confirmed [2]. Methanolic extracts obtained from the leaves of the genus *Potentilla* were studied for their free radical scavenging activity and polyphenol composition. Many common polyphenols, particularly oligomeric procyanidins, were found in the leaves of *P. alba*. The raw material of *P. alba* has high antioxidant activity, which may be related to the high concentration of procyanidins and other phenols [3]. Thus, the species is a potential source of effective antioxidants. It is promising to investigate the properties of *P. alba* extracts to use them as herbal supplements for the prevention of oxidative stress-related diseases in the future.

The discovery of the contained substances valuable for the pharmacological industry in these medicinal plants contributes to increasing their use. However, the natural resources of this species are insufficient to satisfy the growing needs for modern medicine for this raw material. One of the ways to overcome this problem is to grow plants of this species in vitro. Using this method, the area of P. alba plantations can be quickly and effectively increased. Therefore, Tikhomirova and others developed a method of microclonal reproduction of *P. alba* [4]. However, the most common method of obtaining medicinal raw materials is the gathering of wild plants, which has a debilitating effect on natural biodiversity. In some European countries (in particular, Germany and the Republic of Belarus), these species are already disappearing. On the other hand, impurities of another species of plant material often appear in the raw materials obtained in this way. It is possible to eliminate or reduce the impurities of plant species in the raw materials for the production of *P. alba* extracts with the help of markers that will make it possible easily identify the materials. These diagnostic markers include, first of all, morphological and anatomical signs of vegetative organs. These diagnostic markers include, first of all, morphological and anatomical signs of vegetative organs. There are very little data on the anatomy of the vegetative organs of *P. alba* [5]. Most of the available information concerns the anatomical structure of the leaves of plants of the genus *Potentilla* L. [6, 7]. The result of the research has been found that the diagnostic features of the *P. alba* species include the features of the cells of the upper and lower epidermis of the leaves and the presence of calcium oxalate druses and three types of leaf trichomes [6].

Anatomical features of the structure of underground plant organs can serve as important markers. However, known today markers cannot be a sufficient for diagnosis of raw materials [8].

The research aimed to identify the anatomical features of the root and rhizome of *Potentilla alba*, by which it will be possible to identify these plants in the raw material.

Materials and methods. The research anatomical structure of the root and rhizome (if available) has been carried out for species of the genus *Potentilla* L.: *P. alba* L., *P. anserina* L. (root only), *P. argentea* L., and *P. pilosa* Willd. (synonym of *Potentilla recta* subsp. *pilosa* (Willd.) Rchb.f. ex Rothm.) (root only), *P. reptans* L., which are most common in Ukraine. We used plants that grow in the collection exhibits of the O. V. Fomin botanical garden. Cross-section slices of 15–20 μm thickness were made by freezing microtomes from roots and rhizomes. The samples were stained with safranin (to detect lignified structures), KI-staining of starch grains, and sudan-staining of lipids. Sections were described using a microscope XSP-146TR. Photographs were taken with a Canon Power Shot A630 digital camera.

Results and their discussion. The observed plants of the genus *Potentilla* L. are representatives of Ukrainian flora and extensively are used as medicinal plants. The underground and above-ground parts of *P. alba*, *P. argentea*, *P. reptans*, and *P. anserina* plants are harvested separately and used as medicinal raw materials. Therefore, it is advisable to use specific anatomical features as markers of one or another species. For this, it is necessary not only to have an idea of the anatomical structure of one or another part of the target plant, but also to know the markers by which these plants differ from plants of other species of this genus.

Plants of the genus *Potentilla* L. have a similar anatomical structure to the rhizome: the periderm consists of 5–10 layers of the suberin-impregnated cork, phellogen, and up to 10 layers of phelloderm. Locally scales of suberinized cells are found above the cork, filled with air. Under the periderm, there are 10–15 layers of lamellar collenchyma. In some species, the collenchyma is lamellar-angular. The vascular plant system is of the collateral type, mainly the bundle type. The xylem is more intensively developed than the phloem. The primary phloem is not lignified, and the xylem parenchyma is small-celled and thin-walled. A good development pith consists of thin-walled cells. The pith parenchyma, the cortical collenchyma and xylem parenchyma in varying amounts are filled with starch granules, calcium oxalate druses, and sometimes idioblasts. No lipid inclusions were detected.

The roots of plants of this genus have a slightly thinner periderm, phelloderm, and parenchyma. The latter has less thickened cell walls compared to the rhizome. Radial rays of the xylem, as a rule, are narrow and uneven, separated by parenchymal strands containing many inclusions of starch and calcium oxalate druses.

Along with this, some variances in the structure of the rhizome and root in different species were revealed (Table 1). First of all, species differ in the development and placement of xylem elements and localization of inclusions. However, the number of inclusions is a less important sign, as it can vary depending on the age of the plant, the time of harvesting raw materials, growing conditions, etc.

***Table 1.* Variance in the anatomical structure of the root and rhizome species *Potentilla* L.**

Species	Inclusion	Vascular plant system
P. alba	A large amount of starch is everywhere, except for the periderm of the rhizome and the root (Fig. 1a); Ca^{2+} oxalate druses are everywhere except the periderm, most in the xylem parenchyma of the rhizome and root; there are also idioblasts in the core of the rhizome (Fig. 1b, c, d; 2a)	Rhizome: metaxylem forms an almost continuous circle, narrow radial rows of small vessels, separated by 1–2 cell strands of non-lignified parenchyma. The bundle type remains as the vessels of the secondary xylem are separated by wide rays of the xylem parenchyma. The vessels of the xylem are small. The conducting elements of the secondary xylem are arranged in uneven radial cords, mostly 1–3 vessels thick (Fig. 1 b, c). The xylem in the radial rays of the root is arranged in uneven radial strands with a thickness of 1–3 vessels (Fig. 2 a)
P. anserina	In the cortical parenchyma of the root, there are huge clusters of Ca^{2+} oxalate druses, which are not placed evenly (as in *Potentilla alba*) but form their rays, idioblasts are absent, and there are inclusions of starch; Starch inclusions, many Ca^{2+} oxalate druses, and single idioblasts are found in the root wood parenchyma (Fig. 3 a, b)	In the root, the vascular elements of the secondary xylem are arranged in uneven radial strands with a thickness of 1 to 7 vessels. The phloem is very strongly developed compared to other representatives of the genus and is divided into a third by cortical parenchyma inserts. The phloem of the perennial root also has striations due to the alternation of smaller and larger cells (Fig. 3 a, b)
P. argentea	The pith of the rhizome contains a lot of starch, Ca^{2+} oxalate druses, and idioblasts (Fig. 4 a) Single inclusions of Ca^{2+} oxalates are present in the cortical parenchyma of the root. (Fig. 4 b)	There are powerful vascular bundles with highly lignified small and large vessels in the root and rhizome (Fig. 4 a, b) The secondary xylem forms an almost continuous ring, parenchymocytes highly lignified. The primary xylem is characterized by numerous vessels with a large diameter and nonlignified parenchyma
P. pilosa	Starch is present in all root structures except the periderm. Ca^{2+} oxalates were not detected (Fig. 2 b)	The xylem of the leading bundle of the root forms powerful annual rings, which are torn by thick strands of xylem parenchyma in 3-4 places. The xylem contains vessels with large and small diameters (Fig. 2b)
P. reptans	Rhizome, root: single idioblasts are found in the cortical and pith parenchyma. Starch is abundant in all structures, except for the periderm (Fig. 5 a). Ca^{2+} oxalate druses are present in the pith parenchyma and less in the interfascicular parenchyma (Fig. 5 a, b)	The bundle type, wide strands of the interfascicular parenchyma are separated by radially elongated rays of xylem, 1–6 vessels of small and large diameter (up to 7 µm) thick. The vessels are not separated by parenchymal cells in a separate bundle (Fig. 5 a, b)

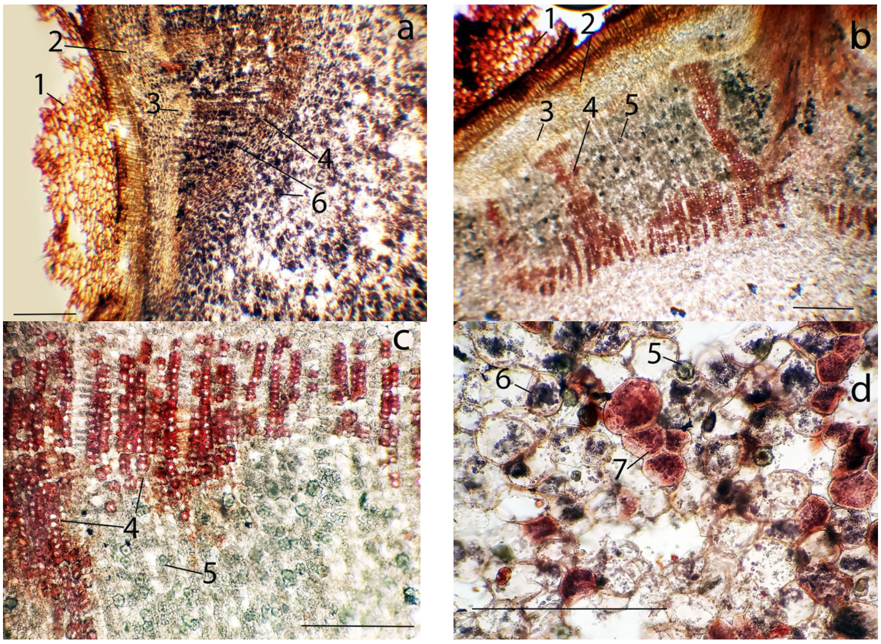

Fig. 1. Cross-section: a) accumulation of starch in the rhizome of *P. alba* (safranin, KI, sudan), b) general structure of the rhizome of *P. alba* (safranin), c) location of xylem elements in the rhizome of *P. alba* (safranin, sudan), d) inclusion in the pith parenchyma of the rhizome of *P. alba* (safranin, KI, sudan)

bar=100 µm, 1 – periderm, 2 – cortical parenchyma, 3 – phloem, 4 – xylem, 5 – inclusion of Ca^{2+} oxalate, 6 – inclusion of starch, 7 – idioblasts

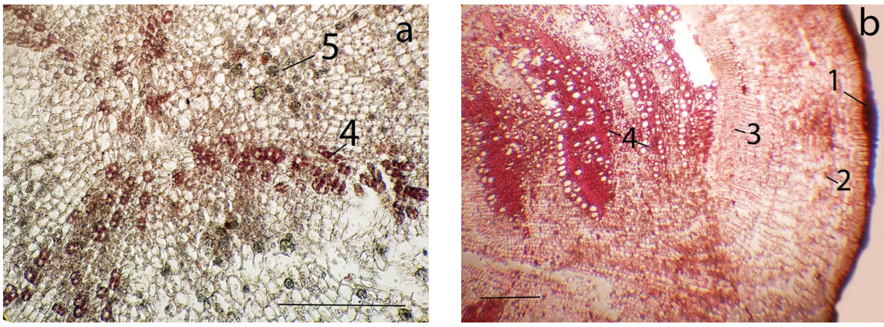

Fig. 2. Cross-section: a) location of xylem elements in the root of *P. alba* (safranin, KI, sudan), b) general structure of the root of *P. pilosa* (safranin, sudan)

bar=100 µm, 1 – periderm, 2 – cortical parenchyma, 3 – phloem, 4 – xylem, 5 – inclusion of Ca^{2+} oxalate

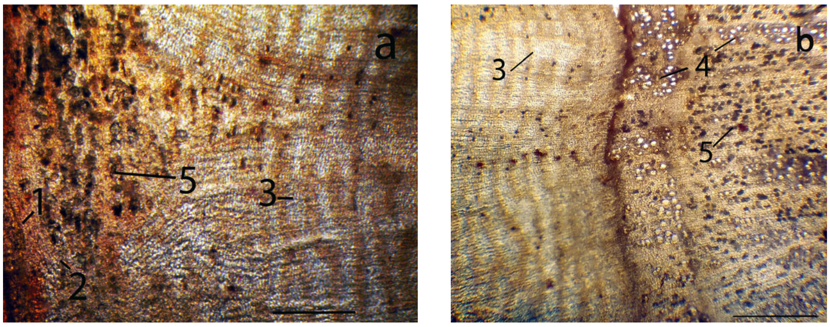

Fig. 3. Cross-section of the root of *P. anserina* (safranin, KI, sudan): a) inclusion in the cortical parenchyma, b) conducting system

bar=100 µm, 1 – periderm, 2 – cortical parenchyma, 3 – phloem, 4 – xylem, 5 – inclusion of Ca^{2+} oxalate

Fig. 4. Cross-section (safranin, sudan): a) general structure of *P. argentea* rhizome, b) general structure of *P. argentea* root

bar=100 µm, 1 – periderm, 2 – cortical parenchyma, 3 – phloem, 4 – xylem, 5 – inclusion of Ca^{2+} oxalate

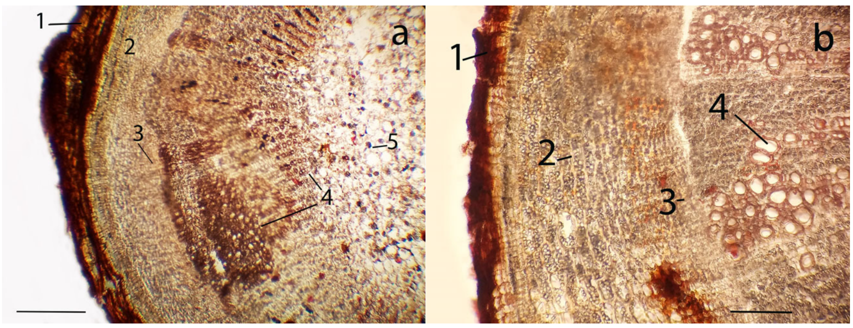

Fig. 5. Cross section: a) general structure of the rhizome of *P. reptans* (safranin, KI, sudan), b) general structure of the root of *P. reptans* (safranin, sudan)

bar=100 µm, 1 – periderma, 2 – cortical parenchima, 3 – floem, 4 – xylem, 5 – inclusion of Ca^{2+} oxalate

Thus, it confirmed basically that the anatomical structure of the roots and rhizomes of representatives of the *Potentilla* genus is similar. Accordingly, some specific features coincide between certain species of the genus. For example, irregular rows of leading xylem elements in the root and rhizome are characteristic of both *P. alba* and the roots of *P. anserina* and *P. reptans*. Also, as in *P. alba*, in the rhizome of *P. reptans*, radial rows of vascular elements one vessel thick are adjacent to the pith parenchyma. However, in *P. anserina* and *P. reptans*, there are large and small vessels in the xylem rays, while in *P. alba* all vessels are small with narrow lumen. Also, the perennial roots of *P. anserina* have a very well-developed phloem, which is an important anatomical difference. The conducting system of the rhizome of *P. argentea* forms a closed ring. The bundle type of the conducting system is preserve in *P. pilosa*, but a cluster of numerous vessels of different sizes is present, they are separated by several transverse layers of non-lignified parenchyma cells, which makes it possible to easily determine the age of the plant.

Among other features, the most distinctive feature was the location and number of Ca^{2+} oxalate inclusions. In particular, for *P. alba* and *P. anserina*, the largest number of inclusions is localized in the xylem parenchyma. The latter characterized by a numerous number of druses in the cortex parenchyma. The most of these inclusions are concentrated in the pith of *P. argentea* and *P. reptans*. But in *P. reptans* they are found in much smaller quantities. Whereas in *P. pilosa* they are completely absent. The lipid inclusions were not detected in any species. While starch reserves are more labile, and their detection varies more intensively depending on the conditions of cultivation, the period of collection of raw materials, etc.

Conclusions. According to the number and location of inclusions in the root and rhizome, some species are similar, some are different, at one time. This indicator is not very stable. It may be slightly varied depending on various factors. The roots have a more similar structure between the species in particular, the anatomical structure of the root of *P. alba* is similar to *P. reptans*. The most effective anatomical marker of *P. alba*, compared to other species of the genus, is the development and location of xylem elements of the rhizome and root. In particular, only in *P. alba* the roots contain small vessels of secondary xylem, arranged in uneven radial strands 1–3 vessels thick, separated by rays of non-lignified xylem parenchyma. In the rhizome, the bundle type of the vascular system remained. But the metaxylem between the bundles, wich bordering the pith parenchyma is represented by short rows of small vessels. These rows are one cell thick and separated by parenchymal cells.

Reference

1. Dorman DHJ, Shikov AN, Pozharitskaya ON [et al.]. Antioxidant and pro-oxidant evaluation of *Potentilla alba* L. rhizome extract. Chem Biodivers. 2011;8:1344-1356. doi.org/10.1002/cbdv.201100043.
2. Kaminskiĭ AV, Kiseleva IA, Teplaia EV. Clinical application of *Potentilla alba* for prevention and treatment of thyroid gland pathologies. Likars'ka Sprava. 2013;8:99-108.
3. Matkowski A, Świąder K, Ślusarczyk S [et al.]. Free radical scavenging activity of extracts obtained from cultivated plants of *Potentilla alba* L. and Waldsteinia geoides L. Herva Pol. 2006;52(4):91-97.
4 Tikhomirova LI, Kechaykin AA, Shmakov AI, Alexandrova OV. An effective way to carry out mass in vitro propagation of *Potentilla alba* L. Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University. 2016;1:433-444.
5. Heo KI, Lee SR, Yoo MH [et al.]. The taxonomic implication of trichome and epicuticular waxes in tribe *Potentilleae* (*Rosaceae*) in Korea. Korean J. Pl. Taxon. 2013;43(2):106-117. http://dx.doi.org/10.11110/kjpt.2013.43.2.106.
6. Faghir B, Attar F, Ertter B. Foliar anatomy of the genus *Potentilla* L. (Rosaceae) in Iran and its taxonomic implication. Iranian Journal of Science & Technology. 2011;35(3):243-256.
7. Samatadze T, Zoshchuk SA, Khomik AS [et al.]. Molecular cytogenetic characterization, leaf anatomy and ultrastructure of the medicinal plant *Potentilla alba* L. Genetic Resources and Crop Evolution. 2018;65:1637-1647. doi: 10.1007/s10722-018-0640-7.
8. Ambrozyuk OB, Matcyk OB. [Anatomical analysis of *Potentilla anserina* roots]. Modern Plant Biology: Theoretical and Applied Aspects. – Abstract Book of IV International Scientific Conference; 2018, October, 09–10; Kharkiv, Ukraine; 2018. P. 16-17. Ukrainian.

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ОСОБЛИВОСТІ АНАТОМІЧНОЇ БУДОВИ КОРЕНЯ ТА КОРЕНЕВИЩА ПЕРСТАЧА БІЛОГО (*POTENTILLA ALBA* L.) ЯК ДІАГНОСТИЧНА ОЗНАКА СИРОВИНИ

Сировина, отримана з рослин виду Potentilla alba L., широко використовується в медицині. Доведено, що запасальні корені та кореневища містять сполуки, які мають низку антиоксидантних властивостей і можуть підтримувати різні терапевтичні ефекти, зокрема протизапальні й адаптогенні. Також вид є перспективним у комплексному лікуванні патології щитовидної залози. Аналіз отриманої сировини на наявність певного виду рослин є необхідною процедурою для виготовлення лікарських засобів.

Метою дослідження було виявити анатомічні особливості кореня та кореневища Potentilla alba, за якими можна ідентифікувати ці рослини в сировині. Дослідження анатомічної будови кореня та кореневища було проведено на п'ятьох представниках роду Potentilla L.: P. alba L., P. anserina L., P. argentea L., P. pilosa Willd., P. reptans L., які зростають у колекційних експозиціях Ботанічного саду імені акад. О. В. Фоміна. Поперечні зрізи кореня та кореневища завтовшки 15–20 мкм виготовляли за допомогою заморожуючого мікротома. Отримані зрізи забарвлювали сафраніном, KI та суданом для виявлення включень. Було встановлено, що корені та кореневища різних видів відрізняються за розвитком і розміщенням елементів ксилеми, а також за кількістю та локалізацією включень. Найбільш ефективним анатомічним маркером P. alba, порівняно з іншими видами роду, є розвиток та розміщення елементів ксилеми кореневища. Лише у цього виду судини вторинної ксилеми дрібні, розміщені нерівномірними радіальними тяжами завтовшки в 1–3 судини, розмежовані променями ксилемної паренхіми. Кількість включень переважно залежать від умов зростання рослин, тому є менш вагомим фактором під час аналізу рослинної сировини.

Ключові слова: Potentilla alba, анатомія, кореневище, корінь.