Actual aspects of anatomical research of medicinal plant material of *Vinca minor* L

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Abstract. The article presents the results of a microscopic study of the medicinal plant material *Vinca minor* L. (Apocynaceae), used in the pharmaceutical industry to obtain indole alkaloids with antitumor activity. When carrying out anatomical analysis of the leaves and stems of *V. minor*, the main anatomical and diagnostic signs was established, this can be used in the identification and assessment of the authenticity of medicinal plants. Analysis of the anatomical structure showed that the leaves of *V. minor* are dorsoventral; hypostatic sheet plastic. The stomatal apparatus of plants is paracytic. The main vein of the leaf blade is represented by a bicollateral conducting bundle; lateral leaf veins had closed collateral bundles. The cross-sectional shape of the stem is grooved with four ribs. The stem of *V. minor* was characterized by a non-bundle type of anatomical structure. The peculiarity of the stem structure was the presence of internal phloem. The characteristic marker features of the stem and leaf of the plant include the presence of straight, non-segmented, non-anastomosing lactic acidae with an excretory substance of a greenish-yellow color. The information obtained can serve as a basis for the development of the section "Microscopy" when writing pharmacopoeial articles.

1. Introduction

It is known that morphological and anatomical analysis is an important method for identifying medicinal plants of various morphological groups. Currently, not for all types of medicinal plants have been established anatomical and diagnostic signs that allow microscopic analysis of raw materials in order to identify unacceptable impurities. This fully applies to such a medicinal plant as *V. minor* from the Apocynaceae family. Due to the fact that the genus Vinca L. is rather large and polymorphic, the problem of interspecific identification of plants comes to the fore. Analysis of the literature has shown that there are currently no works on the establishment of interspecies differences in Vinca L. species using anatomical methods of analysis [1].

The plants medicinal plant material is its aerial part (*Herba Vincae minoris*), consisting of leafy shoots. Shoots contain more than 60 indole alkaloids (0.3-0.4%), bitterness, ascorbic acid (900 mg%), ursolic acid, carotene (8 mg %), glycidosides, flavonoids [2]. It was found that the highest concentration of indole alkaloids *V. minor* is localized in the leaves of the plant [3]. The alkaloids include vincamine, vincamidine, vinoxine, vincine, vincinesine, vincaminorpine, reserpine, isoainaline, acuamycin, etc. [3-6]. Recently, scientists were the first to isolate two new alkaloids from the aerial part of the *V. minor* plant - vallesiachotamine and isovallesiachotamine. It was found that extracts of alkaloids have a high inhibitory activity against lipid per oxidation and trapping DPPH radicals, which allows them to be used in the therapy of various types of malignant neoplasm [7].
In modern medical and pharmaceutical practice, preparations obtained on the basis of *V. minor* raw materials are widely used. Medicines from *V. minor* have long been recognized in Latin America, the USA and Western Europe. The plant *V. minor* is included in the State Pharmacopoeia of Great Britain, European Pharmacopoeia [1]. Medicines are made from plant raw materials: *Vinblastinum*, *Vincristinum*, *Vincapanum* and *Devincan*, which are prescribed for hypertonia and spasms of cerebral vessels, neurogenic tachycardia and other autonomic neuroses. All of these drugs also have antitumor activity and are used in the complex therapy of acute leukemia, lymphogranulomatosis, lung and breast cancer [8]. Clinical interest in vinca alkaloids was clearly identified as early as 1965 [9]. Currently, *V. minor* alkaloids are among the most widely used chemotherapeutic reagents for the treatment of hematological and lymphatic neoplasm, the second most popular class of anticancer drugs [10-12]. It is important to note that *V. minor* alkaloids are very lipophilic, and reach very high intracellular concentrations, they disrupt the abnormal growth of cancer cells, preventing continuous mitotic division [11]. The plant's indole alkaloids also have a hypoglycemic effect; therefore, they are also used to treat diabetes mellitus [12].

Scientists have established a higher oral bioavailability of vincamine when administered as a standardized dry extract of *V. minor* leaves rather than a pure indole alkaloid. The present data demonstrate that the alkaloid vincamine, administered as a whole plant extract, has a higher bioavailability compared to the pure chemical compound [13].

Recent studies have shown that some of the revealed properties of *V. minor* alkaloids, such as antiangiogenic activity, can expand the therapeutic use of natural and semi synthetic plant alkaloids [9]. Thus, *V. minor* alkaloids remain a family of drugs that remain of interest in future anticancer therapy.

There is no information in the literature on the anatomical structure of *V. minor* shoots, which could serve to identify the medicinal raw material of this plant, therefore, the study and identification of micro diagnostic features of plant stems and leaves is relevant. Data on the microscopic structure of *V. minor* shoots can also be used in the compilation of anatomical atlases of useful plants, the creation of keys for determining the taxonomic affiliation of species by anatomical features, in determining the authenticity of plants, standardization, and also in carrying out complex pharmacognostic studies. Therefore, the aim of the work was to establish the anatomical and diagnostic signs of *V. minor*.

2. Materials and methods

Experimental work was carried out in the botanical garden of the Russian State Agrarian University - Moscow Agricultural Academy named after K.A. Timiryazev in 2020. The collection of annual shoots of *V. minor* was carried out during the mass flowering of plants. Preparation of medicinal raw materials for analysis was carried out by the cold softening method according to the method of Yu. S. Cheryatova [14]. The study of the anatomical features of raw materials was carried out in accordance with the requirements of the Pharmacopoeia Articles of the State Pharmacopoeia of the Russian Federation XI [15]. The study of slides was carried out using a Carl Zeiss Primo Star microscope. Microphotography was used in the work.

3. Results

Leaves are simple, short-petiolate, elliptical, 5-9 cm long, entire. The leaf blade is leathery, glabrous, dark green. Leaves on both sides are covered with a single layer of epidermis. The cell walls of the lower and upper epidermis of the leaf have a sinuous outline, partly clearly thickened (figure 1). Covering unbranched trichomes were located only along the main vein on the upper side of the leaf blade.

The leaf blade is hypostomatic; stomata are found only on the underside of the leaf. The stomatal apparatus of *V. minor* leaves is paracytic, characterized by the fact that the stomata are accompanied on each side by one or more subsidiary cells parallel to the long axis of the guard cell aperture (figure 1 B).
Figure 1. The structure of the epidermis of the leaf blade of *V. minor* (× 400): A - upper epidermis, B - lower epidermis, 1 - main cells of the epidermis, 2 - paracytic stomatal apparatus.

The leaves of *V. minor* were characterized by a dorsoventral anatomical structure (figure 2 A). A two-row columnar mesophyll was located under the upper epidermis; the lower side of the plate was occupied by a multi-row spongy mesophyll. In the mesophyll, straight, non-segmented, non-anastomosing lactera with greenish-yellow excretory substance were found (figure 2 C). The milky juice contained alkaloids. The lactic acid cells are long and by careful maceration they could be removed from the plant over a very long distance (figure 2 B). The cell walls of the lactations retained their cellulose cell walls throughout their entire length. The non-segmented lacticella *V. minor* tended to follow the course of the veins of the leaf blade, often located on the side of the phloem of the vascular bundles.

The central part of the leaf was occupied by a rather large bicollateral conductive bundle of the main vein, associated on both sides of the plate with strands of multilayer angular collenchymas. The lateral veins of the second and subsequent order had closed collateral bundles.

Figure 2. Anatomical structure of the leaf blade of *V. minor*: A - cross section of the leaf in the region of the main vein (× 200); B - surgically removed milkmen (× 300); C - lactarias on a cross-section of a leaf (× 400). 1 - upper epidermis; 2 - closed collateral bundle of the lateral vein; 3 - columnar mesophyll; 4 - trichome; 5 - corner collenchymas; 6 - bicollateral bundle of the main vein; 7 - straight unsegmented milk-lover; 8 - lower epidermis; 9 - spongy mesophyll.
When examining the cross section of the stem of *V. minor*, its characteristic shape was established - grooved with four ribs. In the plant stem, conducting tissues were arranged in a closed ring, which indicated the non-bundle type of its anatomical structure (figure 3).

![Cross section of V. minor stem](image)

**Figure 3.** Cross section of *V. minor* stem (× 200): 1 - epidermis; 2 - lamellar collenchymas; 3 - chlorenchyme; 4 - external phloem; 5 - cambium; 6 - xylem; 7 - internal phloem; 8 - parenchyma of the pith; 9 - milkman.

Outside, the stem is covered with a single layer of epidermis with paracytic stomata. Under the epidermis there is a well-developed primary bark of the stem, composed of a three-row lamellar collenchymas followed by chlorenchyma. It should be noted that the number of collenchymas layers in the ribs of the stem increased to eight. In the parenchyma of the stem cortex, there were a few straight non-segmented lactic acidae associated with phloem elements of the conducting system. In the central part of the stem of *V. minor*, a non-specialized pith parenchyma could be observed.

4. Discussion

An anatomical analysis of the leaves of *V. minor* plants revealed the paracytic type of the stomatal apparatus. It should be emphasized that the morphology of the stomatal apparatus is a constant species trait, and therefore can be considered as a marker when identifying the medicinal raw material of *V. minor* leaves. The work also revealed for the first time the formation of bicollateral vascular bundles in the leaf blade of *V. minor*, the type of which is known to be found only in a limited number of taxa.

It should be especially noted that the presence of internal phloem should be considered a marker feature of the periwinkle stem. Straight unsegmented milkweed with greenish-yellow milky sap found in the bark of the stem and mesophyll of the leaves of the plant will be a reliable distinguishing feature when carrying out species identification. In addition, the morphological type and structure of endogenous secretor structures is of paramount importance in the technology of processing medicinal plant materials. Therefore, the established type of milkmen will play an important role in quality control of *V. minor* medicinal raw materials.

It should be added that at the present time there are not enough systematic comparative studies of milkmen, and the possible phylogenetic significance of variations and the degree of their specialization has not yet been clarified. It is believed that articulated and non-articulated lactarius evolved evolutionarily independently of each other and have a polyphyletic origin in flowering plants. Therefore, the establishment of the morphological type of milkmen in a number of taxa in this regard is of undoubted scientific interest. Straight unsegmented non-anastomosing lactarius found in the course of work in the leaves and stem of *V. minor* can serve as a marker for distinguishing taxa and analyzing evolutionary trends in the *Apocynaceae* family.
5. Conclusion

Thus, as a result of the study, the anatomical and diagnostic signs of *V. minor* medicinal plant material were established, which can be used in the identification and assessment of its authenticity, which can serve as the basis for the development of the "Microscopy" section in the draft normative documentation when writing pharmacopoeial articles. The identified and illustrated marker anatomical and morphological characteristics will enhance the level of standardization, increasing the requirements for the quality of medicinal plant materials *V. minor*. The results of the work can also serve as a basis for compiling keys for determining the taxonomic affiliation of species by anatomical features.

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