Pharmacognostic study of *Catharanthus roseus* (L.) G. Don, growing in Uzbekistan

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Abstract. In the article, the morpho-anatomical structure of the leaf and stem of *Catharanthus roseus* species distributed in the local conditions of Uzbekistan was studied, and it was determined that biologically active substances are found in the leaf mesophyll and bark parenchyma of the stem. The following diagnostic characters were determined: in the leaf – dorsiventral type of leaf mesophyll; thick-walled outer walls of the epidermis; amphistomatic leaves; not submerged stomata; chlorophyll-bearing palisade and spongy parenchyma; closed collateral type of conduction bundles; the presence of numerous idioblasts; in the stem there is a more lignified, non-tufted type of structure; libriform extensive; radial rays elongated and short; phloem is extensive, located between the crustal parenchyma and the libriform; the core is not wide, represented by large and small round-oval, thin-walled parenchyma cells containing hydrocyte cells and numerous laticifers. The content of moisture, ash, extractives extracted by heteropolar extractants was also studied, determined by pharmacopoeial methods to establish authenticity and prepare draft regulatory documentation for plant raw materials.

1 Introduction

In medical practice, many medicines of herbal origin are used, since herbal preparations are a reliable tool for the prevention and treatment of a wide variety of diseases, proven by many years of experience in their use. In order to replace imports of highly effective oncological drugs, one of the current areas of pharmaceutical research is still the screening of plant objects. The introduction of plant objects in the Republic of Uzbekistan has been practiced for a long time. One of these objects is the pink periwinkle. A study by V. Soumya, P. Kiranmayi and K. Siva Kumar [1] revealed the effects of combined metal stress on the morpho-anatomy of *Catharanthus roseus*.

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Briefly, the data showed that increased heavy metal content in soil prompted Catharanthus roseus to produce more stomata, enlarge trichomes and glands, increase vessel size, and increase root and shoot diameter. The author demonstrated significant morpho-anatomical differences in the leaves, stems and roots of *Catharanthus roseus* grown on contaminated and uncontaminated soils in the city of Visakhapatnam (India). Pink catharanthus – *Catharanthus roseus* is a species of evergreen perennial subshrub of the genus Catharanthus of the Apocynaceae family. This is a branched evergreen subshrub 30-60 cm tall. The root system is taproot, the root is 25-35 cm long with numerous lateral roots. Young roots without root hairs. The roots are light yellow in color and have a strong specific odor. The bark of pink-flowered plants is anthocyanin-colored, while that of white-flowered plants is green or light green. As the stem ages, it becomes woody and the internodes shorten. The leaves are opposite, lanceolate, short-petiolate, with a narrowed wedge-shaped base. The flowers are about 3 cm in diameter, pink-red; the pharynx of the corolla is purple, pubescent, calloused; a corolla of five petals fused into a tube with separate pink or white limbs, bent in the same plane. The fruit is two sickle-shaped leaflets up to 5 cm long and 3 mm thick with a very short stalk. The seeds are small and black. It grows in the south and east of Madagascar, along the edges of tropical rain forests. In Russia, rose *Catharanthus* is cultivated as an annual plant and is found in Transcaucasia [2].

We studied the morphoanatomical structure of the medicinal plants in the conditions of Uzbekistan and determined diagnostic, taxonomic characteristics, as well as the localization of biological active substances, i.e. pharmacological activity. These identified features are used to determine the taxonomy and raw material of the species [3].

In the scientific literature, there is no description of the external and internal structure of the leaves and stems of *C. roseus* grown under the local conditions of Uzbekistan.

In our research, the study of the morphoanatomical structure of the leaf and stem *C. roseus* grown in local conditions, to determine the main numerical indicators that can subsequently be used to determine the authenticity of raw materials and to prepare draft regulatory documentation for plant raw material will be used.

2 Materials and Methods

Research material: leaves and stems of the medicinal plant *C. roseus* were collected in August-September 2022. To study the morphoanatomical structure of leaves and stems, they were fixed in 70◦ alcohol. The study of the microscopic structure of *C. roseus* species was carried out on the basis of generally accepted anatomical methods [4]. Paradermal and transversal sections were prepared from the leaf and transversal sections from the stem. Anatomical preparations were prepared from the middle part of the leaf and from the basal part of the stem. Preparations made from assimilating and axis organs were stained with methylene blue and safranin dyes and frozen with glycerin-gelatin [5]. Descriptions of the main tissues and cells are given according to R.F. Evert [6], Photomicrographs were made using a *Bioblue* S/N - EC 2209876 trinocular microscope. The content of moisture, ash, and extractive substances extracted by different polar extractants was determined by pharmacopoeial methods [7]. Dried raw materials were used to determine numerical indicators and technological characteristics. Sampling and sampling from batches of packaged "Angro" medicinal plant raw materials was carried out as indicated for "Angro" medicinal plant raw materials (batch), excluding the isolation of a sample to determine the degree of infestation by barn pests; determination of permissible deviations for industrial packaging in accordance with OST 64-492-85. For this purpose, the collected raw materials were dried immediately after collection. The samples collected at the end of August and beginning of September were identified in batches according to the date of collection (No.

1, No. 2, No. 3). The raw materials were dried by artificial drying using dryers to avoid destruction (oxidation or other changes) of indole alkaloids. The optimal drying temperature for raw materials is 50-60°C. For this purpose, samples with an initial degree of grinding were used, which met the requirements of ND on average 4 mm. The finished raw materials were stored in dry, well-ventilated areas.

3 Result and Discussion

The leaves of Catharanthus roseus are opposite, lanceolate, short-petiolate, with a narrowed wedge-shaped base, entire, 2.5-8 cm long, up to 3.5 cm wide, dark green, shiny, pubescent, with pinnate venation and a white midrib on top [2]. Upper and lower epidermal cells in the leaf in the paradermal section, the epidermal cells in the upper part are relatively wavy, and the cells in the lower part are wavy and polygonal. The upper and lower epidermal cells of the leaf have leaf mouths and have an amphistomatic structure. The anamocyte and hemiparacyte leaf mouths are round in the upper epidermis, and oval in the lower epidermis. Leafhoppers were found to fly more in the lower epidermis than in the upper epidermis. Leaf stomata in the upper and lower epidermal cells are not deeply located in the epidermal cell (Fig. 1). In cross-section, the leaf is dorsiventral type due to the arrangement of palisade and spongy cells between the mesophyll upper and lower epidermis. In the leaf, the cells of the upper and lower epidermis are characteristic of mesophotic plants and have a wavy structure. The upper epidermal cells are large, oval-shaped, and the lower epidermal cells are found to have a smaller diameter. The thickness of the cuticle in the epidermal cell was determined in the leaf mesophyll (Fig. 1). Also, the leaf is covered with simple unicellular trichomes on the outside, which serves to protect the plant from various environmental factors and reduce water evaporation. Palisade cells consist of a large, long row of chlorophyll granules in the leaf mesophyll. In the leaf mesophyll, porous cells with chlorophyll granules consist of 6-7 rows of circular, isodermic shape with a small diameter, between which there are intercellular spaces. Between these cells there are main and secondary connections. In the microscopic structure of the leaf, it was found that biologically active substances and drops of essential oil accumulate in palisade and sponge parenchyma cells (Fig. 1).

Because the main vein of the leaf mesophyll is well developed, the lower epidermal cell is bulging. The angular type of collenchyma, consisting of 1-3 rows of living cells, is well developed under the lower epidermis in the main conducting ligament in the rib part of the leaf. Grievous tissue - parenchyma cell occupies the main conducting ligament, its cells are thin, thick-walled, polygonal. In the parenchyma cells of the conducting bundle in the main vessel, there are idioblasts that store black pigment and water storage was found. In a closed, collateral-type conducting bundle, thin-walled xylem tubes are thickened spirally or ring-shaped (Fig. 1).

In cross section, the microscopic structure of the stem is a loose tongue, circular in shape. The periderm covers the stem from the outside, and below it is the primary bark parenchyma and a central cylinder. Under the periderm there are 10-12 rows of bark parenchyma cells, dark red biologically active substances are collected in idioblasts and starch grains. Groups of lignified thick-walled lobular cells are formed at the border of the central cylinder separated by bark parenchyma. These lignified cells represent primary lub fibers. Fibers are composed of secondary phloem or phloem in the interior (Fig. 2).

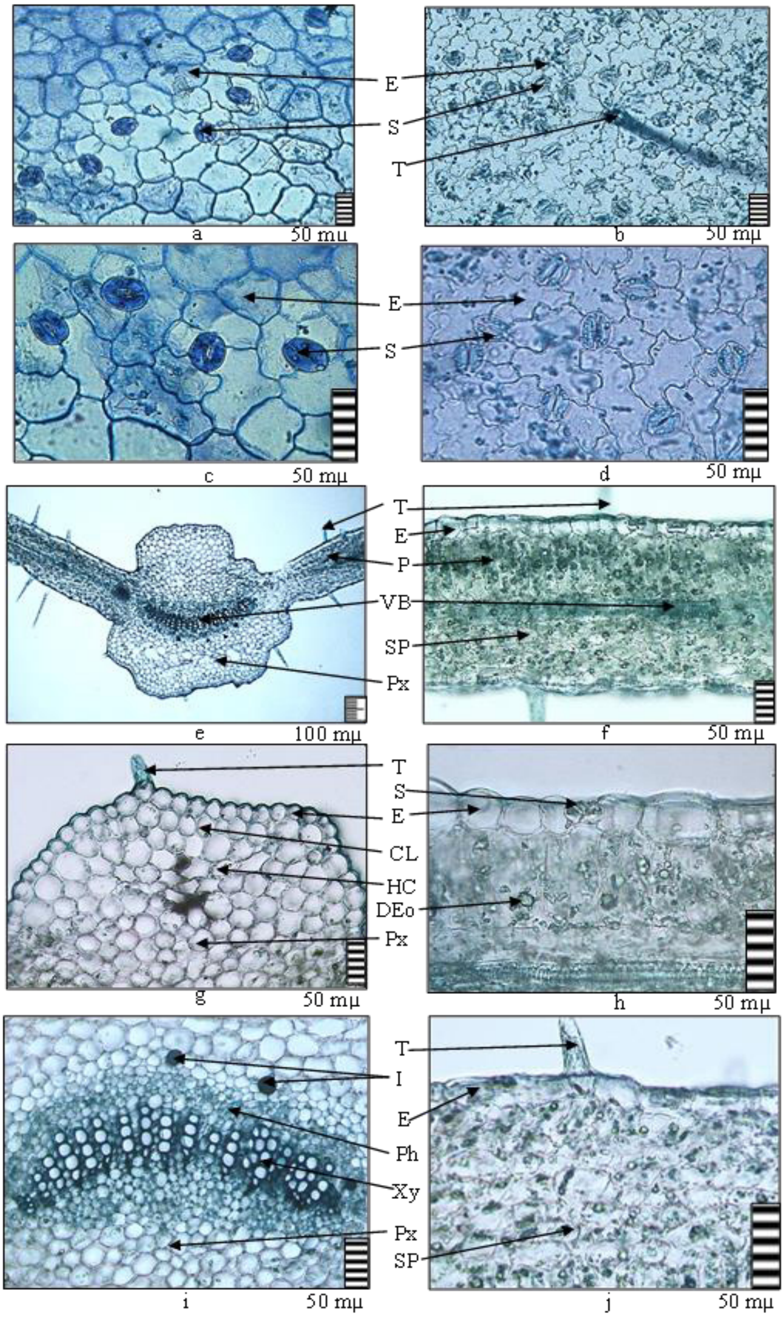

Fig. 1. Microscopic structure of the leaf of *C. roseus* in paradermal and transverse section: a, c - upper epidermis; b, d - lower epidermis; e – the main conductive link; f – detail; g – lower epidermis, angular collenchyma, sad parenchyma and water-retaining hydrocyte cells; h, j – leaf stomata; i - vascular bundles; j – unicellular trichome and chlorophyll sponge parenchyma; a-d - paradermal sections, e-g - transverse sections. Legend: SP - sponge parenchyma, HC - hydrocyte cells, I - idioblasts, CL - collenchyma, Xy - xylem, DEo - essential oil droplets, P - palisade parenchyma, VB - vascular bundles, Px - parenchyma cells, T - trichome, S - stomata, Ph - phloem, E - epidermis.

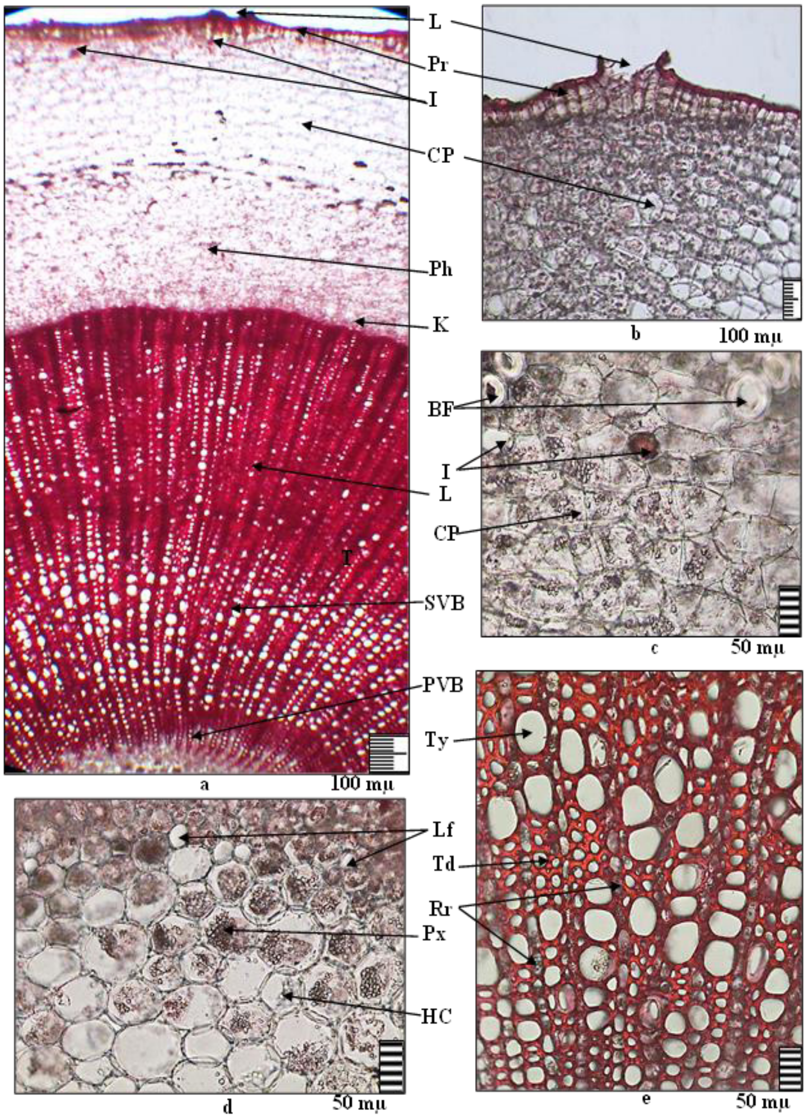

Fig. 2. Microscopic structure of the stem of *C. roseus* in a transverse section**:** a – detail of the stem; b – periderm and lentil; c – bast fibers and idioblasts, d – pith; e – libriform. Legend: SVB - secondary vascular bundles, HC - hydrocyte cells, I - idioblasts, C - cambium, CP - cortex parenchyma, L - libriform, BF - bast fibers, Lf - laticifers, PVB - primary vascular bundles, Pr - periderm, Px - parenchyma, Rr - radial rays, Td - tracheids, Ty - trachea, Ph - phloem, L - lentil.

In the secondary microscopic structure of the stem, the secondary phloem is located between the bark parenchyma and the woody-libriform in a wide annular form (Fig. 2).

A cambium cell in a stem is made up of several rows of cells. Woody parenchyma is well developed in the central cylinder and consists of scattered-tube libriform vascular segments, fibrous elements, woody, ray parenchyma. The main part of the stem is the central cylinder. The primary conducting bundles remain in the core of the stem, and then the secondary xylem tissue forms a cylinder (Fig. 2). Based on the microscopic structure of the stem, as a result of the activity of cambium cells, it was observed that large tracheids and small diameter tracheids were formed in the central cylinder. Secondary xylem -

trachea and tracheids - occupy most of the stem. Among the tracheids and tracheae of the central cylinder, primary and secondary nuclear rays of a heterogeneous type, which are examples of a number of cells, are developed. The core part of the stem - pith occupies 1/4 of the stem and consists of oval, round and isodermic parenchyma with a small diameter. Among the parenchyma cells in the central part of the stem, water-retaining hydrocyte cells are formed, and there are numerous laticifers under the primary vascular bundle and between the parenchyma cells. Biologically active substances and starch grains were also found in the parenchyma cells (Fig. 2).

At the next stage of research, numerical indicators aboveground organs *C. roseus* were studied (Table 1).

Table 1. Results of the study of numerical indicators aerial part of the C. *roseus*.

Batch	Humidity	Total ash, %	Ash is insoluble in HCl, %	Extractive substances, %	
				45 % alcohol	Step gradient - water: methanol with 5% acetic acid
No. 1	5,33±0,37	7,83±0,22	0,3±0,02	48,2±2,31	49,1±1,87
No. 2	6,12±0,24	6,94±0,29	0,4±0,01	47,9±1,94	48,8±2,60
No. 3	4,84±0,39	7,26±0,34	0,2±0,04	48,4±2,31	49,1±1,99

When determining the content of extractive substances, water-alcohol mixtures with different concentrations (45, 70 and 96%) were used, having different polarities and, accordingly, different extracting abilities with respect to alkaloids [7, 8]. It was found that alcohol with a strength of 45% contributes to the highest yield of total alkaloids. The highest yield was recorded when using a step gradient: water-methanol with 5% acetic acid [9, 10]. From the data presented in Table 1, it is clear that the loss in mass during drying of the plant object was within the normal range. The ash content in the analyzed raw material samples ranged from 6.94±0.29 to 7.83±0.22%; ash insoluble in a 10% solution of hydrochloric acid - from 0.2±0.04 to 0.4±0.01%.
Technological indicators were also determined - the degree of swelling of plant raw materials (Table 2).

Table 2. Technological properties of the aerial part of *C. roseus.*

№	Indicators		Batch No. 1	Batch No. 2	Batch No. 3
1	Fractional compound, %	More than 3 mm	2,1±0,06	3,6±0,06	1,8±0,06
		2-3 mm	46,2±2,10	45,2±1,42	49,3±2,12
		1-2 mm	41,7±0,91	41,8±1,62	57,3±2,91
		0,5-1 mm	9,8±0,84	9,3±0,99	6,2±0,56
		Less than 0.5 mm	0,2±0,01	0,3±0,01	0
2	Bulk density, g/cm3		0,75±0,03	0,75±0,03	0,75±0,03
3	Flowability, g/s		2,85±0,23	2,6±0,17	2,90±0,23
4	Angle of repose, degrees		51±0,42	50±0,61	52±0,32

The results obtained contribute to the determination of the extraction method.

This study was carried out with the aim of optimizing the technological process for extracting the number of alkaloids from the aerial part of *Vinca rosea.* Determined that grass has relative flowability and average density. Studies to determine the swelling properties of raw materials have shown that raw materials have a high degree of swelling, which must be taken into account when carrying out the process of obtaining the drug substance [8].

4 Conclusion

The microscopic structure of the leaf and stem of the C. roseus plant growing in the local conditions of Uzbekistan was studied. The anatomical structure of the vegetative organs of the *C. roseus* plant was studied and the following diagnostic characters were determined: in the leaf – dorsiventral type of leaf mesophyll; thick-walled outer walls of the epidermis; amphistomatic leaves; not submerged stomata; chlorophyll-bearing palisade and spongy parenchyma; closed collateral type of conduction bundles; the presence of numerous idioblasts; in the stem there is a more lignified, non-tufted type of structure; libriform extensive; radial rays elongated and short; phloem is extensive, located between the crustal parenchyma and the libriform; the core is not wide, represented by large and small round-oval, thin-walled parenchyma cells containing hydrocyte cells and numerous laticifers. On the basis of the morpho-anatomical structure of the leaf and the stem, morphological structural features typical for the species and the presence of biologically active substances in the cells of the porous, columnar parenchyma and bark parenchyma were determined. These data and numerical indicators can subsequently be used as the basis for regulatory documentation for medicinal plant raw materials. The results of technological indicators of standardized raw materials will serve to optimize the extraction technology of the total alkaloids.

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Authors' contributions

G.M. Duschanova, S. Aripova, and Ya. Nazirova prepared the manuscript, executed, and interpreted the study; G. Duschanova, S. Aripova, Ya. Nazirova, A. Sharipov, N. Jalilova contributed to the concept and study design.

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