MICROSTRUCTURAL CHARACERISTICS OF RHODODENDRON PONTICUM L. LEAVES

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The family *Ericaceae* unites more than 4,000 shrubs, lianas and trees species of 150 genera [10]; many of them are used for medicinal or decorative purposes. Noteworthy is the Gen. *Rhododendron* L., which is presented by six species in Georgian flora: *Rh. luteum* Sweet, *Rh. ungernii* Trautv., *Rh. ponticum* L., *Rh. Sokhadze, Charadze et Davlian*., *Rh. caucasicum* Pall., *Rh. smirnowii* Trautv [2,3,8]. In addition to ornamental applications, these species have been used in industry and perfumery, and as well in traditional and modern medicine for treatment of various diseases due to their antioxidant, anti-inflammatory, antimicrobial features [3,4,6].

"Rhodopes" - the anti-herpetic ointment containing the ethylacetate extract from the leaves of *Rh. ungerni* was developed at TSMU Iovel Kutateladze Institute of Pharmacochemistry. The ointment is recommended for the treatment of herpes diseases of different etiology and has a wound healing effect, as well. Moreover, phenolic compounds from the leaves and stems of *Rhododendron ungernii* due specific mechanism of action stimulate hematopoiesis and are used for the treatment of leucopenia, caused by radio- or chemotherapy of cancers [6,7]. On the basis of a positive clinical reports, the Ministry of Labor, Health, and Social Welfare of Georgia allowed the use of "Rhodopes" in medical practice. The medication received a State registration № R - 0001795.

Rh. ungernii Trautv. is forming small massifs only in Adjara (Georgia) and Turkey [3]. The aim of our research was to determine an alternative source of plant raw within the genus Rhododendron and study its chemical and pharmacological properties, for the development of remedy similar to "Rhodopes". Finally, *Rh. ponticum* L., which is widespread in Western Georgia was chosen.

Rh. ponticum L. is an evergreen shrub or low tree (3-6 m) with a brownish bark. The oblong-elliptic leaves have sharp end and are arranged alternatively; leaf size can range from 9 to 27 cm. Cover tissue of the leaves is thick, leathery, green (Fig. 1). Flowers are reddish. Fruits are presented as 1,5 cm cylindrical box, without trichrome. Flowering period lasts from April to May. Multiplies by seeds, root sprouts and twisting branches. The species is typical mesophyte, grown in humid and fertile soil. *Rh. ponticum* L. is spread in western Georgia and it is relict of third flora. The plant grows at altitudes up to 1800 m above sea level in and makes peculiar formations within deciduous forests. All parts of the plant are toxic [3].

The phytochemical study of *Rh. ponticum* L. has shown the main components of the leaves are catechins, anthocyanins, flavonols and flavones. 5 individual compounds were obtained from anethyl acetate fraction of a hydr-alcoholic extract by column chromatography on polyamide and silica gel. Three of these compounds were identified as quercetin, isoquercitrin, catechine using their physical-chemical properties as well as their UV, IR and HPLC-MS spectroscopic data [9].

One of the most important points of pharmacognostic study when determining the content of biologically active compounds in promising medicinal plants is the establishment of stable diagnostic traits of the anatomical structure, along with the morphological characteristics of plants. Since any inaccuracies in the field of pharmacy are unacceptable, the precision of critical taxa should be supported by anatomical data, which, among other parameters, are a reliable method in identification of plants [1].

Hence, the aim of our research was to investigate the microstructural features of the leaves of *Rh. ponticum* - the raw for obtaining bioactive compounds.

Material and methods. The raw materials, leaves of *Rh. ponticum* was gathered from the subalpine mountain slopes of Likhi, (coordinates N 42.06063^0 E043.48363^0) in July, 2019. A voucher specimen is kept in the I.Kutateladze Institute herbarium (specimen code #19560) (Fig. 1). Cross, longitudinal and topical preparatory strips were made from fresh unfixed leaf material by hand using a sharp razor. The samples were stained with light safranin solution for 24 hours and placed on a glass in the drop of glycerin. Light microscope (Carl Zeiss, Jeneval) and stereoscope (MBC-2) were used for microstructural investigations. The images were taken by digital camera (Canon Digital IXUS75). Selected images were processed using Adobe Photoshop CS5 software.

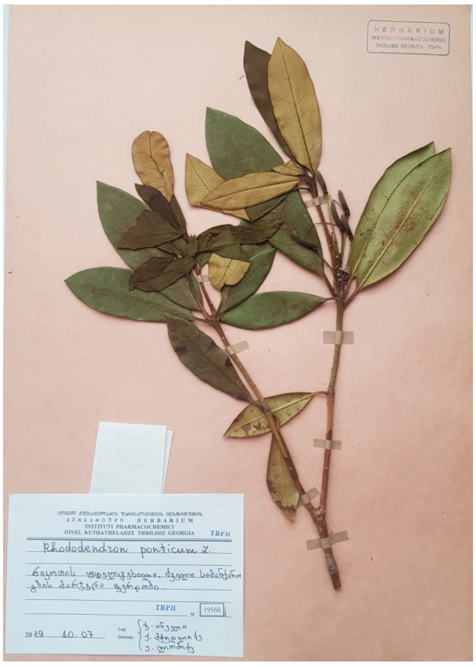

Fig. 1. Reference voucher of the I.Kutateladze Institute of Pharmacochemistry, Rh. Ponticum

Results and discussion. The structure of *Rh. ponticum* leaves is bifacial, hypostomatic and dorsoventral. Guard tissue contains cutin; the cells of the upper and lower epidermis are single-layered. The leaf contains single-layer palisade parenchyma with short, radially arranged cells and some elements of spongy parenchyma. Crystals of calcium oxalate are present in the mesophyll of the leaves. Conductive beams are back-collateral and differentiated (Fig. 2).

Cells of the upper and lower epidermis of the leaf are uneven, oblong and curved; the baggy apparatus is a simple, anomocytic type [5]; taking into account the main leaf litter and intercostal spaces are directed towards each other, the architecture of the ventilation system is chaotic (Fig. 2).

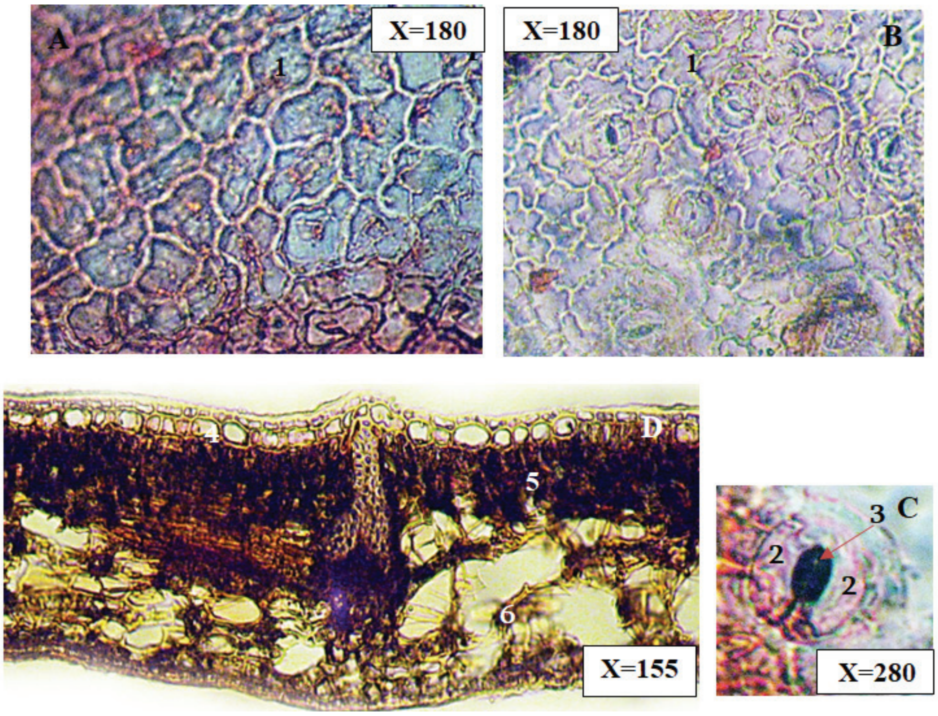

Fig. 2. Microstructural characteristics of the leaf of Rh. ponticum
A-B - Basal cells of upper and lower epidermis of the leaf; C - Anomocytic stoma; D - Dorsoventral mesophyll
1. Crooked epidermal cells; 2. guard cells of stoma; 3. Lumen of stoma; 4. Cutinized epidermis ; 5. Palisade and; 6. Sponge parenchyma

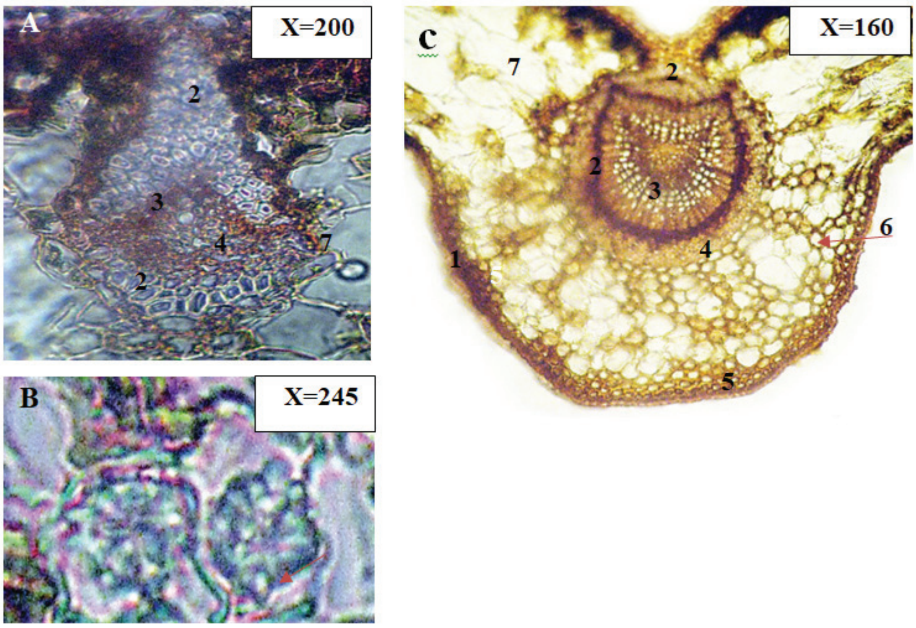

Fig. 3. Microstructural characteristics of the leaf of Rh. ponticum
A - Back-collateral conductive beam; B - Crystals of calcium oxalate; C - Panoramic view of main vascular bundle texture
1. Cutinized epidermis; 2. Mechanical tissue; 3. Xylem; 4. Phloem; 5. Lamellar collenchyma;
6. Polygonal cells; 7. Secretory channels

The protecting guard tissue of main vascular bundle of the leaves of *Rh. ponticum* is cutinized. The structure of the vascular bundle is more or less fascicular, with polygonal cells and multiple secretory areas; Ventral and dorsoventral parts of the main vascular bundle contain lamellar collenchyma; Conductive beams are almost completely surrounded by a massive tissue of mechanical cells. Phloem is finely extracellular and filled with secretory channels, in timber - rounded lumber fibers and spirally coiled conductive vessels are present. The radial rays in the timber are short, single-layered; the structural elements of the timber parenchyma are unevenly thickened (Fig. 3).

Conclusion. Sustainable microstructural characteristics of the leaves of *Rh. ponticum* are: type of villus; shape and location of basal cells of epidermal tissue; Type and location of stomata; Types of structural elements of the mesophyll; Balance of the elements in the cell.

On the base of researches it is revealed that:
- Leaves are naked and bifacial;
- Stomata – Hypo-stomatic;
- Dorsoventral structure of the leaf mesophyll;
- Conductive beams are back-collateral;
- Presence of calcium oxalate crystals in the leaf mesophyll;
- Abaxial and adaxial cells of the epidermis of leaves are crooked, and have rounded or curved shape;
- The robustness of the main vascular bundle is determined by increased amount of mechanical cells – collenchyma, layer of sclerenchyma cells and xylem fibers.
- Presence of secretory channels in the phloem;
- The conductive vessels' lumens in the timber are rounded and have spirally coiled shell;
- Short single-layered radial beams.

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SUMMARY

MICROSTRUCTURAL CHARACERISTICS OF RHODODENDRON PONTICUM L. LEAVES

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The microstructural characteristics of the leaf of *Rhododendron ponticum* L., an alternative source of biologically active compounds - constituents of the antiherpetic ointment Rhodopes, were studied.

The study resulted in the establishment of the sustainable diagnostic characteristics of *Rh. ponticum* L. leaf. The leaf is naked, double-sided, hypostomatic, with dorsoventral structure of mesophyll; the conductive system represents a vascular fibrous, reverse collateral structure. Druze crystals of calcium oxalate are found in the pulp of the leaf. The underlying cells of the adiaxial and abaxial leaf epidermis are uneven, oblong and curved. The robustness of the main vascular bundle is determined by increased amount of mechanical cells – collenchyma, layer of sclerenchyma cells and xylem fibers. In the phloem, differentiation of secretory channels is visible; the lumen of the wood conducting vessels is rounded, with a spirally thickened inner shell; radial rays are short, single-layered.

Keywords: Rhododendron ponticum, Plant anatomy, Leaf, Phytochemistry.

РЕЗЮМЕ

МИКРОСТРУКТУРНЫЕ ХАРАКТЕРИСТИКИ ЛИСТА РОДОДЕНДРОНА ПОНТИЙСКОГО RHODODENDRON PONTICUM L.

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Изучены микроструктурные особенности листа *Rhododendron ponticum* L. – как альтернативного источника биологически активных соединений, входящих в состав противогерпетической мази «Родопес».

В результате исследования установлены характерные диагностические признаки листа *Rh.ponticum* L. Лист голый, двухсторонний, гипостоматический, мезофилл листа имеет дорсовентральную структуру; проводящая система представляет собой сосудоволокнистую, обратно-коллатеральную структуру. В мякоти листа обнаруживаются друзы кристаллов оксалата кальция. Подлежащие клетки адаксиального и абаксиального эпидермиса листа неровные, продолговатые и изогнутые. Прочность центральной жилки листа обусловлена обилием в ней механических клеток - коленхимы, пояса склеренхимных клеток и древесных волокон. Во флоэме видна дифференциация секреторных каналов; просвет проводящих сосудов древесины закругленный, со спирально утолщённой внутренней оболочкой; радиальные лучи короткие, однорядные.

რეზიუმე

Rhododendron ponticum L., პონტური შქერის ფოთლის მიკროსტრუქტურული თავისებურებანი

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ი. ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, საქართველო

განხილულია ჰერპესის ვირუსის სამკურნალო პრეპარატ "როდოპესი"-ს მალამოს, როგორც ბიოლოგიურად აქტიურ შენაერთთა ალტერნატიული წყაროს კვლევის ობიექტის, *Rhododendron ponticum* L.-ის ფოთლის მიკროსტრუქტურული თავისებურებანი.

კვლევის შედეგად დადგენილია *Rh. ponticum*-ის ფოთ-

ლის შინაგანი აგებულების მდგრადი სადიაგნოსტიკო მახასიათებლები: ფოთოლი შიშველი, ბიფაციალური, ჰიპოსტომატურია, ხოლო ფოთლის მეზოფილი დორზოვენტრალური სტრუქტურის; გამტარი კონა ჭურჭელ-ბოჭკოვანი, უკუ-კოლატერალური აღნაგობისაა. ფოთლის რბილობში განლაგებულია მჟაუნმჟავა დრუზა კრისტალები. ფოთლის ადაქსიალური და აბაქსიალური ეპიდერმისის ფუძემდებარე უჯრედები არადაგვირისტებულია, მრუდხაზოვანი და მრუდკედლიანი. ფოთლის მთავარი ძარღვის სიმტკიცეს განაპირობებს მასში არსებული მექანიკური უჯრედების სიჭარბით – კოლენქიმა, სკლერენქიმული უჯრედების სარტყელი და მერქანში არსებული ბოჭკოები; ლაფანში აისახება სეკრეტორული არხების დიფერენცირება; მერქანში გამტარ ჭურჭელთა სანათურები მომრგვალო მოხაზულობისაა, ხოლო მათი შიდა გარსი სპირალურად გარსგასქელებული; რადიალური სხივები კი მოკლე, ერთრიგიანია.

MORPHOLOGICAL DIAGNOSIS OF PANCREATIC NEUROENDOCRINE TUMORS (REVIEW AND CASE REPORT)

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Neuroendocrine tumors of the digestive system are a relatively rare but extremely heterogeneous pathology. The frequency of detection of gastrointestinal neuroendocrine tumors does not exceed 1-2% of the total oncological pathology of this localization. Pancreatic neuroendocrine tumors account for up to 2% of all pancreatic neoplasms. At the same time, in targeted search during autopsies, the frequency increases up to 3-3.5%, which indicates that a significant number of tumors are asymptomatic. The increase in NET incidence in 30 years by more than 5 times is probably associated with improved diagnostic capabilities due to the use of immunohistochemical studies and improved imaging techniques. Pancreatic NETs are a heterogeneous group of epithelial malignancies that develop from neuroendocrine cells of the pancreas (islets of Langerhans).

Tumors characterized by neuroendocrine properties were first described in the XIX century; Langhans (1867) described a dense, fungal, submucosal tumor, with clear boundaries, protruding into the lumen of the intestine; histologically it was similar to poorly differentiated glandular tissue with well-developed fibrous stroma. Later, Lubarsch (1988) described multiple tumors of the ileum with hyperplasia of the serous-muscular membrane [3,5,6]. In 1890, Ransom described similar tumors and concomitant clinical symptoms, including diarrhea and shortness of breath. In 1907, Oberndorfer coined the term "carcinoid" to describe these tumors. Later in 1914, Gosset and P. Masson described the nature of carcinoid tumors in more detail [1]. In 1938, an Austrian pathologist F. Feyter proposed a theory of the origin of neuroendocrine tumors (NETs) from the diffuse neuroendocrine system, which he described not only as localized in epithelial organs, but also as single cells distributed throughout the body or localized together with ductal cells of the pancreas [7,8]. However, due to the importance of this problem, scientists around the world were not ready to rest, and as early as in 1968, A.G. Pearse suggested a concept of the APUD (amine precursor uptake and decarboxylation) system, which allowed histochemical separation of the components of the previously suggested diffuse neuroendocrine system. The classification was based on the isolation of more than 40 different cell types that can secrete polypeptide hormones; their cytochemical and ultrastructural composition was determined experimentally, which allowed their combination into one biochemical group. And in 1969, Hungarian endocrinologists I. Szijj and K. Kovacs used the term "Apudoma" to describe a patient with medullary thyroid carcinoma producing ACTH [1,8,9].

In 1963, Williams and Sander suggested a classification of all neuroendocrine tumors by embryological origin, dividing all tumors into 3 groups: tumors arising from the proximal gastrointestinal tube (foregut), and localized in the lungs, stomach, upper duodenum, and pancreas, the second group originates from the middle tube (midgut), and localized in the distal part of the duodenum, small intestine, right half of the colon and appendicular process, and the third group originating from the posterior or distal gastric tube (hindgut), tumors of this group are located in the transverse, descending colon, and rectum.

The historical development of neuroendocrine tumors in its beginning included only hormonally active neuroendocrine tumors (HANET), those that manifested as a specific clinical picture. In 1902, E. Nicholls described a tumor arising from Langerhans islet cells, but the corresponding endocrine syndrome was described only after the discovery of insulin [4]. In 1927, W.J. Mayo for the first time performed surgery to remove a malignant insulinoma, and by 1990, there were more than 2,000 insulin cases in the literature. Much later, only in 1995, American surgeons R.M. Zollinger.

Ellison made a detailed description of islet cell tumors of the pancreas in patients with extremely aggressive peptic ulcers [11]. Over the years, other forms of neuroendocrine tumors have been described.

Neuroendocrine tumors are a rather rare oncological pathology, but according to the US Registry of Surveillance Epidemiology and End Results (SEER), the incidence of neuroendocrine tumors has increased by 500% over the past 30 years [2,12].

For a long time, there were rather simplified classification schemes for pancreatic neuroendocrine tumors, which included the division into benign tumors, adenomas, and malignant tumors, carcinomas, which did not allow adequate division of the