

# *Vinca minor* L. Leaf Anatomical Structure

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## Abstract.

*V.m.* (c. Apocynaceae) is a semishrub with extended vegetative and orthotropic vegetative-generative shoots. The former in a crop reaches up to 80 cm, and forms up to 20 to 30 opposed pairs of leaves, and the latter reaches 20-25 cm with 5 to 6 pairs of leaves. The plants are evergreen, the leaves are simple, whole, leathery, have elliptic shape, and have very short petioles. The leaf blade of *V.m.* has the thickness of up to 246  $\mu\text{m}$ , the mesophyll is 210  $\mu\text{m}$  thick, it is composed of 2 layers of columnar cells, and 7 to 8 layers of aerenchyma, the number of the latter increases in places where large veins pass. Cells of mesophyll contain drops of laticifer. The leaf is hypostomatic. The stomata are of the paracytic type, however, they are surrounded by 4 cells, since 2 cells along the stoma are small (compared to the main epithelial cells of the lower epidermis), and are not adjacent to each other. The vascular bundles are of bicollateral type. Fibrous sclerenchyma is well expressed on the abaxile side. The walls of sclerenchyma cells are strongly thickened, their cavities are in the form of narrow slits. In the leaf blade, the latex ducts are not detected, they become clearly visible when the leaf gap enters the lacune in the stele. The leaf trail is single-bunch single-lacune. Despite *Vinca minor*'s confinedness to the conditions of steppes, its leaves maintain a distinct mesomorphic structure.

**Keywords:** aerenchyma, epidermis, hypostomatic leaf, mesophyll, stoma, *Vinca minor*.

## INTRODUCTION

*V.m.* (c. Apocynaceae) is a semishrub with two types of shoots: extended vegetative and orthotropic vegetative shoots. The plant is not only ornamental, but also medicinal. Currently, over 50 alkaloids are discovered in *V. m.*, the biochemical properties of which are being actively studied in many countries. The positive effect of a number of *V. m.* alkaloids has been noted in treatment of certain forms of cancer. Regeneration from callus and root fibrilla is studied. With that, no information about the anatomical structure of *V. m.* has been found. In this work, the authors have determined the anatomical structure of the leaf (mesophyll and both epiderms), the parameters of individual tissues and cells have been noted. The fact that the spongy mesophyll tissue is transformed into real aerenchyma attracts special attention, as well as the single-bunch single-lacune leaf trail.

*Vinca minor* L is a semishrub with creeping and upright stems. As a rule, the former actively branch out, promote vegetative reproduction, and may reach the length of 1 meter and more, and the latter are flowering. The formula of the flower is the

following: \*  $\frac{\text{C}_5}{\text{C}_5} \frac{\text{Co}_{(5)}}{\text{Co}_{(5)}} \frac{\text{A}_5}{\text{A}_5} \frac{\text{G}_2}{\text{G}_2}$ . The flowers are so peculiar that once seen, they are hard to forget: single, rather large (up to 3.0 cm in diameter), axillary flowers on long peduncles. The corolla has a narrow tube (up to 1 cm) and 5 rounded funnel-shaped or rotate-funnel-shaped petals colored from pale blue to dark blue. The stamens are attached to the center of the corolla tube, and alternate with petals. The anther filaments are short, rather broad; at the beginning of blooming, the triangular anthers are closed (but not grown together) under the stigma. The pistil consists of two carpels, but with one long column and a short hairy stigma, under which there is a small circular pubescent thickening. The ovary of the pistil is short; at its base, there are 2 small petaliform nectaries. After pollination and fertilization, the two follicular fruits depart from each other. The fruit is an apocarpous complex follicular fruit of two carpels, each opening up along the ventral suture. The follicular fruits have a lot of seeds. The leaves are simple, whole, of elliptic shape, leathery, slightly pointed at the apex, have very short petioles. The plant is considered evergreen, as it does not discard leaves for the winter. The leaves are opposed to each other.

Since the times of Dioscorides and Pliny, the plant has been used in medicine. It is always described in various manuals on medicinal plants [1-3]. It is steeped in numerous legends and fables, and is used in many ceremonies and rituals. The homeland of the plant is Europe and Asia Minor. Cultivated *Vinca minor* significantly expanded its habitat: North Africa, North America, South America, Austria, the British Isles. It grows in the wild in the Caucasus, and in the South of the European part of Russia [4].

In the chemical composition of *Vinca minor*, over 50 alkaloids have been discovered, which determined its medicinal properties. Currently, biochemical properties of the individual alkaloids [5-8], and methods for their determination [9] are being actively studied in many countries. The possibility of plant regeneration from the root fibrilla and callus [10] of the root fibrilla crops [11] is also being studied. With that, no information about the anatomical structure of *Vinca minor* has been found.

In this paper, the authors attempted to study the anatomical structure of *Vinca minor* leaves in cultivated conditions, and to determine the parameters of individual cells and tissues.

## MATERIALS AND METHODS

The plants were grown at the garden plot of amateur gardener, biologist V.U. Mashaev, and reproduced vegetatively - by fragments of the shoot. The authors express their deep gratitude for the provided material that was collected in August 2017. At the end of the summer, both types of shoots in the conditions of Republic of Kalmykia continued to actively vegetate: at the point of growth, inception and development of leaves rudiments occurred. With that, at the base of creeping shoots, there were many connivent adventitious buds and shoots in various degrees of development. The leaves that finished growing on extended shoots were a little smaller than those on orthotropic shoots. Thus, on the former, leaves of intermediate formation reached the length of 3 to 4 cm with the width of 0.5 cm, on the latter - 5.0 to 5.5 cm with the same width.

Anatomical sections were prepared according to the generally accepted method from fresh unfixed shoots [12], images were made using the Biolam-70 microscope with the use of RA-4; measurements of tissues and cells - with eyepiece micrometer MOV-15. Repeatability was dozens of slices of leaves of intermediate formation.

## RESULTS

*Vinca minor* L is different from other *Vinca* species in evergreen, overwintering, dense to the touch, oppositely arranged leaves. In the conditions of cultivation, creeping vegetative shoots reach the length of 50-80 cm with 20-30 pairs of leaves; the upright generative shoots reach 20-25 cm with 5-6 pairs of leaves and solitary axillary flowers.

The average thickness of the mesophyll is 210  $\mu\text{m}$ , and that of the leaf blade - 246  $\mu\text{m}$ . The cuticle is thin; the upper surface of the leaf blade is shiny. The leaves are dorsoventral with 2 layers of palisade tissue and 7-8 layers of spongy tissue, which has been transformed to aerenchyma. On the cross-section, the average cell size of palisade tissue is 32\*19  $\mu\text{m}$ ; the cells are placed quite tightly to each other. Almost every cell contains a

large drop of laticifer, sometimes there may be more drops. Against the background of smaller oval chloroplasts, they are clearly distinguishable due to their rounded shape and slightly shiny surface. The cells of spongy tissue are so loosely arranged that rather represent aerenchyma. As a rule, aerenchyma is observed only in aqueous and marsh plants, and in eudicots, the dorsoventral mesophylls are divided into palisade tissue and spongy tissue. However, in some cases, the spongy tissue becomes aerenchyma in some terrestrial plants as well, when thin parenchymal cells form single-layer links between large air cavities. A real aerenchyma was observed on the adaxial side of the involucre leaves and in the blossom cluster of most Asteraceae, on the abaxial side of leaves of *Gentiana cruciata* L. The shape and the size of cells of this tissue have relatively high response rate from  $16 \times 12 \mu\text{m}$  to  $50 \times 22 \mu\text{m}$ ,  $32 \times 19 \mu\text{m}$  on the average. Along their length, they are stretched across the leaf blade, and are almost not smaller, and sometimes larger than the height of cells of the palisade tissue (Fig. 1, Table 1). Aerenchyma cells also may contain drops of laticifer. Variability of the shape and size of the cells of this tissue (and the fact that it is rather aerenchyma than spongy tissue) was considered on individual parts of the subepidermal layer of cells (Fig. 2). In one of the cells, a drop of laticifer and small chloroplasts on its background are shown. The vascular bundles are of bicollateral type, the internal phloem is well developed. In timber, 1, 2, (3) rows of radially arranged vessels alternate with 1-2 rows of parenchyma. The walls of both are fairly thickened (Fig. 4). Except for the sieve tubes with satellite cells, and the bast parenchyma typical of phloem, single scattered, large parenchymal cells are observed in

this tissue. Above the soft phloem on the abaxial side, phloem sclerenchyma is well represented, on the adaxial side, there are separate groups of cells that do not form a continuous layer. It should be noted that the walls of phloem sclerenchyma in vascular bundles of *Vinca minor* are so greatly thickened that the opening in them remains in the form of a hardly visible narrow slit. The authors were not able to detect the usually noted non-lomentaceous laticifers in the leaf blade. They become clearly visible when the leaf trace enters the leaf lacune (the phloem sclerenchyma disappears at this moment).

Hypostomatic leaf, i.e. the stomata are only on the abaxial side, like in most mesophytes. The upper epiderm is composed of rather large cells with slightly sinuous walls (Fig. 5, Table 1). Up to 7 to 9 cells of palisade tissue fit under one cell of upper epiderm. Tortuosity of the walls, tight fit of the cells, the absence of stomata on the upper epiderm and the cuticle layer reduce transpiration and protect overwintering leaves from getting infected with bacteria, viruses, and fungi. The shape and size of the cells of the lower epiderm are almost indistinguishable from those in the upper epiderm (Fig. 3). Stomata are much smaller than epithelial cells, and arranged randomly: the stomata slits are arranged in different directions. Their guard cells are surrounded by 4 cells, as cells placed along the stomata are small and are not adjacent. On the narrow side, stomata contact epithelial cells of the epiderm. Thus, despite the clearly expressed paracytic nature, stomata are surrounded by 4 cells, rather than by 2 cells. There are quite many stomata, up to  $350 \text{ per } 1 \text{ mm}^2$ . Parameters of cells and tissues of the leaf blade are summarized in Table 1.

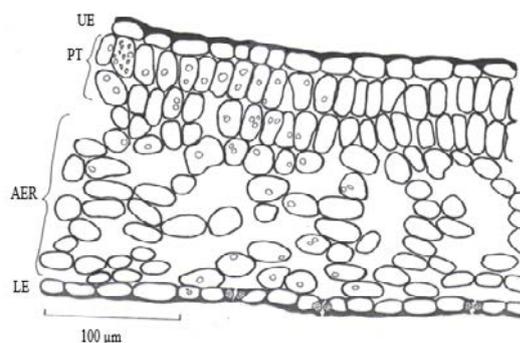
**Table 1 Parameters of tissues and cells in a leaf of *Vinca minor* (all dimensions are in  $\mu\text{m}$ )**

No.	Parameters of cells and tissues (on the average; replication - 5 to 6 slices)	
1	Blade thickness ( $\mu\text{m}$ )	246
2	Thickness of mesophyll ( $\mu\text{m}$ )	210
3	Height of columnar tissue	69
4	Parameters of palisade tissue cells (length*width) ( $\mu\text{m}$ )	$33 \times 13$
5	Height of aerenchyma ( $\mu\text{m}$ )	147
6	The number of aerenchyma layers	7-8
7	Parameters of aerenchyma cells ( $\mu\text{m}$ )	$32 \times 19$
8	The number of cells of the upper epiderm per $1 \text{ mm}^2$	575 cells
9	Parameters of the cells of the upper epiderm (length*width) ( $\mu\text{m}$ )	$59 \times 29$
10	The number of integumental cells + the cells around the stoma on the lower epiderm	$600 + 650 = 1,250$ cells
11	The number of stomata on the lower epiderm per $1 \text{ mm}^2$	350
12	Parameters of stomata (length*width) ( $\mu\text{m}$ )	$22 \times 15$
13	Parameters of the cells around the stomata ( $\mu\text{m}$ )	$32 \times 15$
14	Parameters of integumental cells on lower epiderm ( $\mu\text{m}$ )	$58 \times 28$

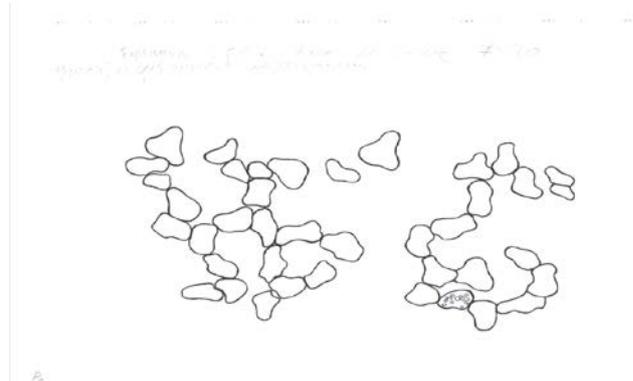
PS: No. 3 (in the table) – sometimes, the 3rd layer of columnar cells is observed; in this case, the tissue thickness increases to 80-86  $\mu\text{m}$ ;

No. 6 – near the midrib, the number of aerenchyma layers increases to 8-11;

No. 10 – 600 (the number of integumental cells) + 650 (the number of cells along the length of the stoma).



**Fig. 1. *Vinca minor* leaf anatomical structure: ue and le are upper and lower epidermis; pt – palisade tissue; aer – aerenchyma; s – stomata. The scale bar is 100  $\mu\text{m}$**



**Fig. 2. Segments of subepidermal layer of aerenchyma. (In a separate cell, a large drop of laticifer and small chloroplasts are marked)**

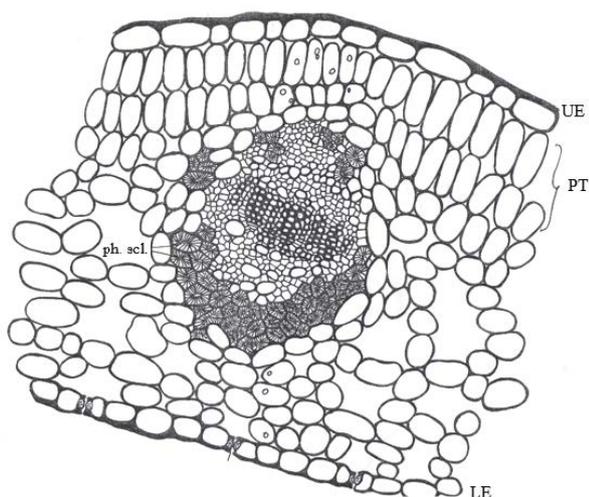


Fig. 3. *Vinca minor* L. leaf anatomical structure in the area of a large vein. ph. scl. in the phloem sclerenchyma; the rest - according to Fig. 1

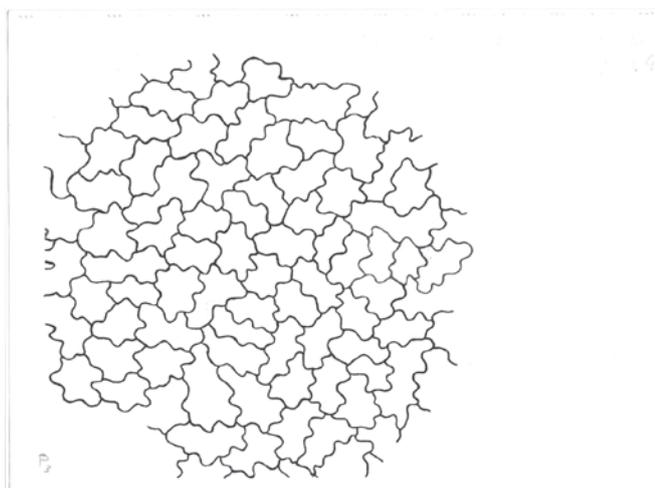


Fig. 4. The structure of upper epidermis

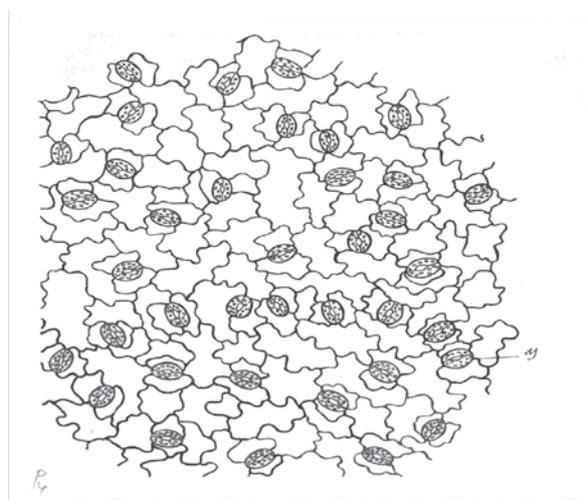


Fig. 5. The structure of lower epidermis

## CONCLUSIONS

Thus, the leaf blade of *Vinca minor* has the thickness of up to 246  $\mu\text{m}$ , the mesophyll is 210  $\mu\text{m}$  thick, it is composed of 2 layers of columnar and 7 to 8 layers of aerenchyma, and the number of the latter increases in places where large veins pass. Cells of mesophyll contain drops of laticifer. The leaf is hypostomatic. Stomata are of the paracytic type, however, they are surrounded by 4 cells, since 2 cells along the stoma are small (compared to the main epithelial cells of the lower epidermis), and are not adjacent. The vascular bundles are of bicollateral type. Fibrous sclerenchyma is well expressed on the abaxile side. The walls of sclerenchyma cells are strongly thickened, their cavity is maintained in the form of a narrow slit. In the leaf blade, the latex ducts are not detected, they become clearly visible when the leaf gap enters the lacune in the stele. The leaf trail is single-bunch single-lacune. Despite its confinedness to the conditions of steppes, the leaf of *Vinca minor* maintains a distinct mesomorphic structure.

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