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Anatomical structure of the stem and leaf of *Daphne glomerata* Lam.

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Abstract
The present work describes morphological and microstructural characteristics of the stems and leaves of *Daphne glomerata* Lam. studied by light microscopy. This investigation provides referential anatomical information for correct identification of this species. Structural peculiarities of the stem and leaf identified during the study such as the shape and interposition of the leaf's epidermal cells, type and position of stomata, structure of the leaf's parenchyma, structure of the vascular tissue, provide valuable information for the correct identification of *Daphne glomerata* Lam. plant material.

Keywords: *Daphne glomerata*, microscopic, plant anatomy, leaf, stem

1. Introduction
The genus *Daphne* L. contains over 100 species [1] of toxic, deciduous or evergreen shrubs of the mezereum family (Thymelaeaceae), up to 1.5 m tall, native to Asia, Europe and North Africa. Eight species of *Daphne* are found in Georgia: *D. mezereum* L., *D. pontica* L., *D. albowiana* Woronow ex Pobed., *D. glomerata* Lam. (D. imerica C.Koch); D. caucasica Pall., D. axilliflora (Keissl.) Pobed. (*D. caucasica* Pall.var. axilliflora Keissl); *D. transcaucasica* Pobed. (*D. oleoides* auct.) and *D. pseudosericea* Pobed., Among these species *D. axilliflora* (Keissl.) Pobed. and *D. pseudosericea* Pobed are endemics of the Caucasus region. [2].
Daphne species have been shown to contain several groups of biologically active compounds, including di-, sesqui- and tri-terpenes, flavonoids, coumarins, steroids and lignans.
Multiple species of the genus *Daphne* are used in traditional medicine for a variety of different uses, including the treatment of fevers, rheumatisms, stomach-aches, cancers, malaria, poisonings, toothaches, as a laxative, a diuretic etc [3]. Different studies have shown that extracts and compounds isolated from different *Daphne* species possess significant antimicrobial, antioxidant, cytotoxic, immune-stimulating and a number of other effects [4-16].
Preliminary studies have shown that *D. glomerata* extracts process antioxidant and cytotoxic properties [17-18].
One of the most important aspects of pharmacognostic research of plants containing biologically active compounds is the determination of morphological and stable anatomical traits of the plant material that are of diagnostic value.
Thus the objects of our study were vegetative organs of *Daphne glomerata*, a potential source of new biologically active compounds. Considering the variety of *Daphne* species the diagnostic features of the plant's anatomy, identified by the study will allow the correct identification of the plant raw material.

2. Materials and Methods
Daphne glomerata Lam. is found in Anatolia and the Caucasus. It grows in alpine and sub-alpine regions at heights of 1800 to 2500 m. In Georgia the plant is found in every region, where it forms small local groves.
Representatives of this species are evergreen toxic shrubs reaching heights of up to 50 cm. STEMS thick, glabrous with greyish bark, covered with scars or fallen leaves. Lover branches prostrate, elevated at the tips, dichotomously branching. Leaf surface glabrous, coriaceous; adaxial part glossy, abaxial side pale green; blade elongate-lancet late or cuneate, elongate at the base, sessile, 1.5-3.0 cm long, 1.0 cm wide, obtuse or slightly acute at the tip. Leaves situated at the tips of branches. Flowers bisexual, 4- merous, calyx tubular, fragrant, in the axils of apical leaves, 3 to 15 grouped together in a false capitatum inflorescence at the tips of shoots. Bracts very thin, ovoid or elongate, wrapped around the inflorescence, deciduous. Corolla about 15 mm long, with a pink exterior and a white interior, glabrous or glabrescent. Four folded lobes of the corolla lancetolate, pointed, folded back during flowering, twice as short as the corolla tube. Stamins 8, twice as many as calyx lobes, in two series,

filaments short or absent, attached to the base of the perianth tube. Pistil small, 3 mm long. Ovary superior, glabrous, sessile, style absent or capitate. Fruit is a bright red drupe. Flowering - May-July, fruiting July- August.

The plant material of *D. glomerata* used for the research was obtained in 2015 in the Khevsureti region of Georgia on the northern slopes around the village Roshka, during the peak of flowering. The plant material was identified and collected by J. Aneli. A voucher specimen was placed in the I. Kutateladze Institute herbarium (specimen code N14522). Samples for microscopic slides were prepared from living, non-fixed material with a sharp metal razor. Longitudinal, transverse and epidermal sections were prepared from the shoots, the medial part of the leaf blade and the leaf central vein. The samples were died with a safranin solution for 24 hours and were placed on a glass microscope slide in a drop of glycerine. An optical microscope Carl Zeiss, Jeneval was used for micro-structural research. Photo material was obtained by a digital photo-camera (Canon Digital IXVS75). Selected images were digitally processed by the Adobe Photoshop C55 computer program.

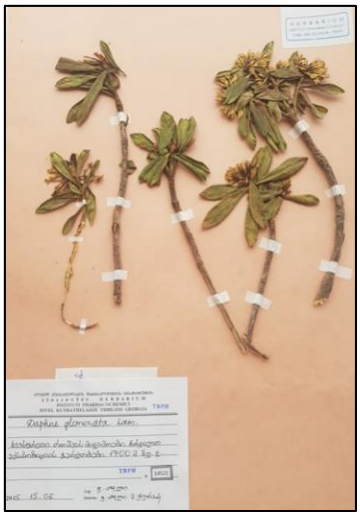

Fig 1: *D. glomerata* voucher specimen

3. Results and discussion

Cells of the adaxial surface of the leaves are oriented chaotically, straight with straight walls. On the abaxial surface also chaotic, curved with curved walls. Leaves are hypostomatic. Stomata anomocytic, chaotically dispersed on the surface of the leaf. The leaf epidermis contains well visible spherical crystals of inulin. Mesophile is dorsoventral. Leaf vascular cylinder is reverse-collateral. On the transverse cut of the stem thick walled, wavy dermal tissue and lamellar collenchyma are noticeable. The latter is bordered by thick walled, densely packed, tangentially twisted parenchyma cells. The vascular system's texture is monocyclic; vascular tissue architecture is bilateral in reference to phloem and xylem interposition. Fibrous cells in perixylary and hypoxylary phloem are positioned in tiers. The vascular cambium ring at the internodes is continuous. Wood is diffuse-porous. Unequal level of growth ring distinction, some are quite distinct, others - less **s**o. Most of the vessel openings are singular, rarely grouped together. Primary xylem vessels with large diameters, form a cycle of 1 to 4 rows, later distancing from each other mostly radially, sometimes obliquely. Thin bands of tracheal elements are noticeable in xylem. Transition zones between primary and secondary xylem are relatively well distinguishable. There is a significant difference in vessel number and a small difference in vessel diameter between early and latewood; however a ring-porous structure is not formed. The vessel openings are angular. Xylem parenchyma is apotracheal and terminal. Thin walled fibres are dispersed in the main texture of xylem. Walls of tracheid and vessel are spirally thickened. Tracheal pits large, closely positioned, parallel. Radial xylem rays distinct, mostly in a single row, heterogeneous, short or long respectively of 2 to 4 or up to 8 cells tall. Approaching vessel openings they do not bend and do not widen through the entire length of the xylem cylinder

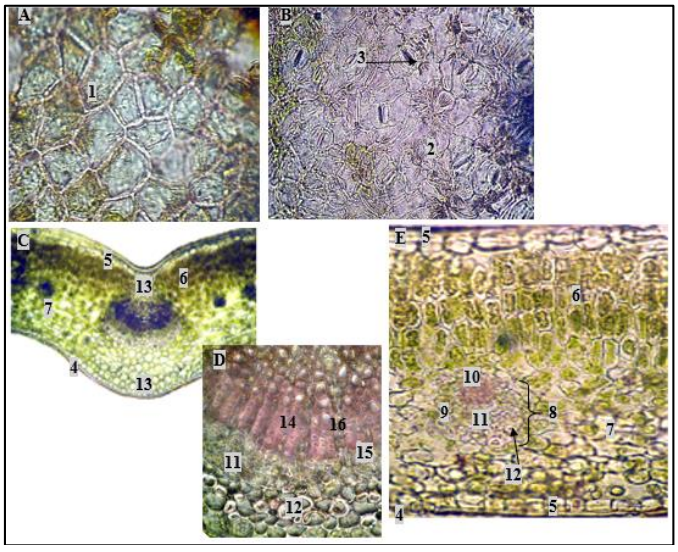

Fig 2: Microstructural traits of *Daphne glomerata*'s leaf

A. Adaxial and B. Abaxial leaf epidermis structure C, D. Leaf vascular system E. Fragment of leaf transverse cut

1) Straight cells, with straight walls; 2) Curved cells, with curved walls; 3) *Anomocytic stomata*; 4) Cuticle; 5) Epidermis; 6) *Pallisade chlorenchyma*; 7) Spongy chlorenchyma; 8) Collateral vascular bundle; 9) Bundle sheath; 10) Xylem; 11) Phloem; 12) Phloem fibres; 13) Collenchyma; 14) Central vein xylem thick-walled parenchyma; 15) Cambium; 16) Radial ray;

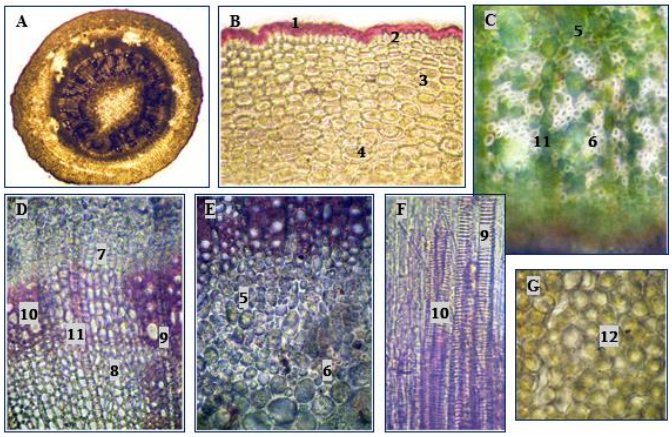

Fig 3: Microstructural peculiarities of *Daphne glomerata*'s stem

A. Panorama of stem texture; B. Transverse cut of the stem from cuticle to vascular cylinder; C. Fragment of the Phloem D. E. and F. Fragments of the vascular system; G. Texture of the pith;
1) Cuticle; 2) Epidermis; 3) Collenchyma; 4) Bark parenchyma; 5) Phloem; 6) Phloem fibres;
7) Cambium; 8) Thick-walled xylary parenchyma; 9) Circular vessels; 10) Spiral vessels; 11) Radial ray; 12) Pith cells;

4. Conclusion

The stable diagnostic traits of a plant species include the form, type and distribution pattern of trichomes; shape and interposition of the leaf's epidermal cells; type and position of stomata; structure of the leaf's parenchyma; details of the vascular tissue's structure and the presence and form of intracellular structures.
The following stable diagnostic anatomical traits of *Daphne glomerata* Lam. were identified:

- Leaf surface glabrous, hypostomatic, dorsoventral;
- Leaf epidermal cells chaotic;
- Adaxial leaf surface cells straight with straight walls; abaxial ones curved with curved walls;
- Stomata distribution chaotic; stomata anomocytic;
- Well visible spherical crystals of inulin in leaf epidermis;
- Leaf vascular cylinder is reverse-collateral;
- Vascular system's texture is monocyclic, collenchyma lamellar;
- The vascular system is monocyclic, bilateral, cambium ring continuous, wood is diffuse-porous, unequal level of growth ring distinction;
- Xylem parenchyma is apotracheal and terminal;
- Most of the vessel openings are singular, rarely grouped together, vessel openings are angular;
- Walls of tracheid and vessel are spirally thickened;
- Radial rays in single rows, heterogeneous, not bending when approaching vessel openings, not expanding in xylem parenchyma;

5. Acknowledgments

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