



Original Article

Foliar anatomy of *Viola maculata* growing in Parque Nacional Los Alerces, Chubut, Patagonia, Argentina



Chana Pilberg^a, Maria V. Ricco^a, Maria A. Alvarez^{a,b,*}

^a Farmacobotánica y Farmacognosia, Farmacia y Bioquímica, Facultad de Ciencias de la Salud, Universidad Maimónides, Ciudad de Buenos Aires, Argentina

^b Consejo Nacional de Investigaciones Científicas y Tecnológicas, Ciudad de Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 6 October 2015

Accepted 17 March 2016

Available online 16 April 2016

Keywords:

Viola maculata

Argentina

Medicinal plant

Traditional medicine

Leaf

ABSTRACT

Viola maculata Cav., Violaceae, grows in the underwood along the Los Andes mountain system from Neuquén to Tierra del Fuego and Islas Malvinas. It is also known as *violeta amarilla* or as *pilundeu*, and is used in popular medicine by the Mapuches as stimulant, in dermatology, and as a lavage in eye diseases. The aim of this work is to examine the morpho-anatomical traits of the leaves and petiole of *V. maculata* (collected at Parque Nacional Los Alerces, Patagonia, Argentina). Cross sections and scanning electron microscopy of leaves blade and petiole showed a simple organization with simple unicellular trichomes and cells containing tannins, and crystals of calcium oxalate mainly in the spongy parenchyma. As *V. maculata* is used in traditional medicine, the specification of the anatomical characters is relevant to describe the species.

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Introduction

Viola is the largest genus of the Violaceae family mostly constituted by shrubs or herbs. Some authors, based in a phylogeny-based revision of the genus, have suggested the worldwide existence of two subgenera and sixteen sections (Yousefi et al., 2012; Mehrvarz and Marcussen, 2013). Numerous anatomical studies have been published on the genus *Viola* (Metcalf and Chalk, 1950; Rubin and Paolillo, 1978; Akarsu, 1989; Dinç and Yildirimli, 2007). In Argentina, there are nineteen species of Violaceae, two of them exotic and naturalized (Mehrvarz and Marcussen, 2013). Among them *Viola maculata* Cav., commonly known as *pilundeu* or *violeta amarilla* or *oreja de ratón*, grows in the underwood along the Los Andes mountain system from Neuquén to Tierra del Fuego and Islas Malvinas. Cusato and Rossow (1991) have cited *V. maculata* as part of the native flora from Parque Nacional Lago Puelo. *V. maculata* is a perennial herb up to 3–25 cm, with rootstock and stolons, pubescent or hairless. Leaves are up to 6–65 × 3–55 mm, ovate or lanceolate, pubescent or hairless, crenate. Petioles are 0.5–20 cm long, in general pubescent. Stipules are serrate, 2–12 mm. Peduncle is 2–25 cm long, pubescent or hairless. Flowers are not fragrant. Sepals are ovate, narrowly triangular or lanceolate, 2–8 mm, pubescent, exceptionally hairless. Petals are yellow, occasionally with reddish or brownish lines, the lowest petal is

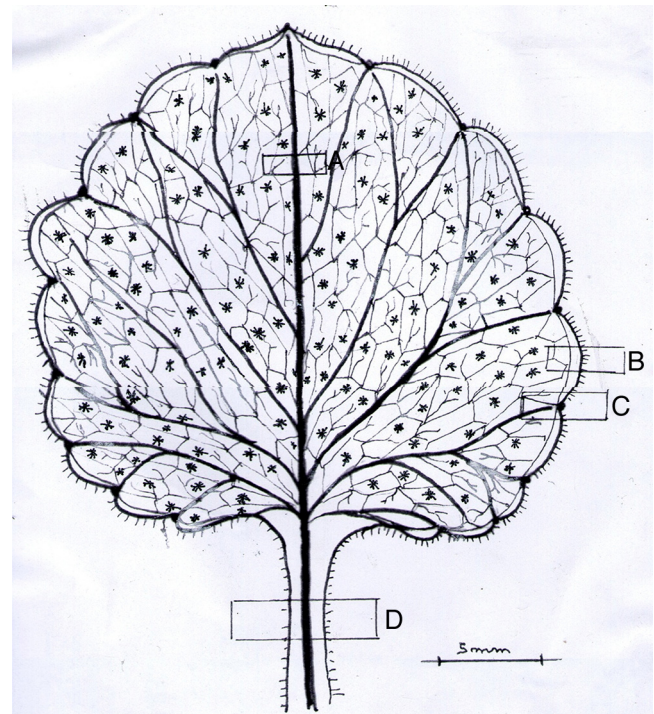


Fig. 1. Clarified leaf, surface view: A: central bundle; B: leaf margin; C: lateral bundle; D: petiole.

* Corresponding author.

E-mail: alvarez.mariaalejandra@maimonides.edu (M.A. Alvarez).

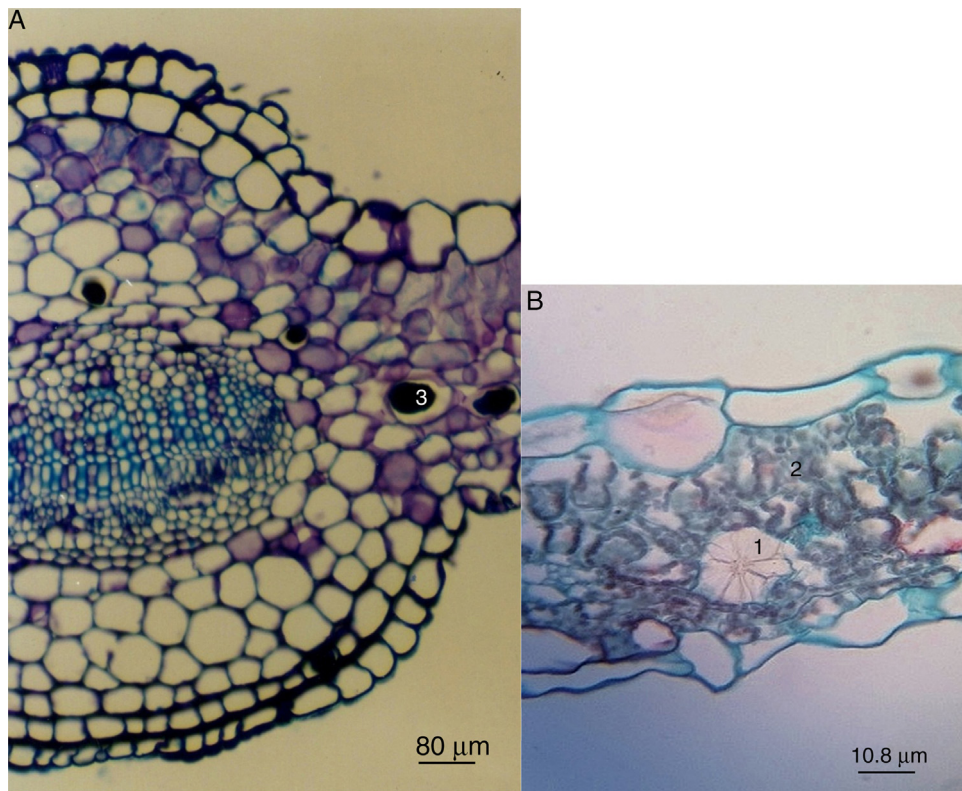


Fig. 2. (A) Cross section of *Viola maculata* Cav. leaf, central bundle with xylem, phloem and a parenchymatic bundle sheath surrounding them. (B) Cross section of leaf margin, detail showing calcium oxalate crystals (1), parenchyma with chloroplasts (2), and cells with tannins (3).

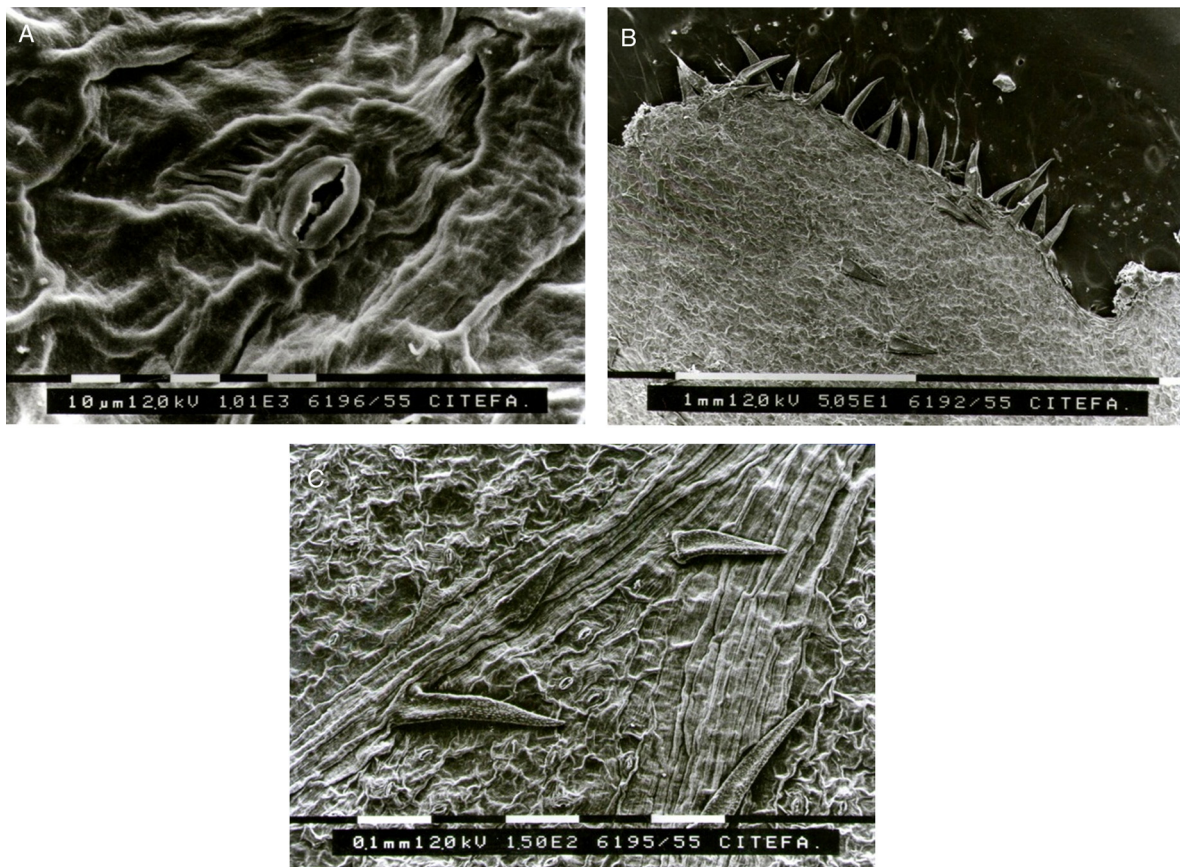


Fig. 3. Electronic scanning microscope: (A) stomata, (B) simple hair and stomata, and (C) simple hair in the leaf margin.

10–25 × 4–10 mm, ovate, slightly or deeply emarginated, the lateral ones have claviform hairs. Spur 1–3 mm. Ovary is conical and hairless. Style is 2 mm, geniculate at base, wider at the apex. Capsule is 5–8 mm long. The samples we studied were collected in Parque Nacional Los Alerces, a National Park established in 1937 to protect the native forest of lahuan or alerce (*Fitzroya cupressoides* Mol Johnst.). It is located in the west sector of Chubut province which is an area of 259,570 hectares in the eco region Bosques Patagónicos (Patagonia Forests) (Cabrera and Willinik, 1980; Rossow, 1988; Toledo and Kutschker, 2012). The area has a complex lacustrine system (Conticello et al., 1997), glaciers (e.g., Torecillas), mountains, and paths, inhabited by rare animal species such as the huemul (*Hippocmelus bisculus* Molina), the pudú (*Pudu mephistophiles*), the paloma araucana (*Patagioenas araucana*), and the gato huiña (*Leopardus guigna*). *V. maculata* is referred to as used in traditional medicine by the Mapuche people, formerly semi-nomadic horticulturists and gatherers inhabitants of the North-Western Patagonia (Argentina and Chile). Their descendants still preserve their ancestral knowledge about the medical uses of some wild native plants, in

spite of the undergoing losses and transformations (Citarella, 1995). Estomba et al. (2006) and Conticello et al. (1997) have gathered part of that knowledge by interviewing some Mapuche communities, describing the use of *V. maculata*, *pilundeu* or *oreja de ratón* by the Churruhinca mapuche community in different situations, e.g., the whole plant is used as an energizer and in dermatology, and the infusion prepared using the leaves is used as a lavage in case of some eye diseases. Tourkarkissian (1980) also mentioned its use as pectoral and diaphoretic, and in gastrointestinal diseases (Molares and Ladio, 2014). In this work we have performed the anatomical analysis from cross sections of leaves and petiole from *V. maculata* collected in Parque Nacional Los Alerces.

Materials and methods

Plant material

Leaves of *Viola maculata* Cav. var. *maculata*, Violaceae, were collected in Parque Nacional Los Alerces (42°48'27"S, 71°53'56"W),

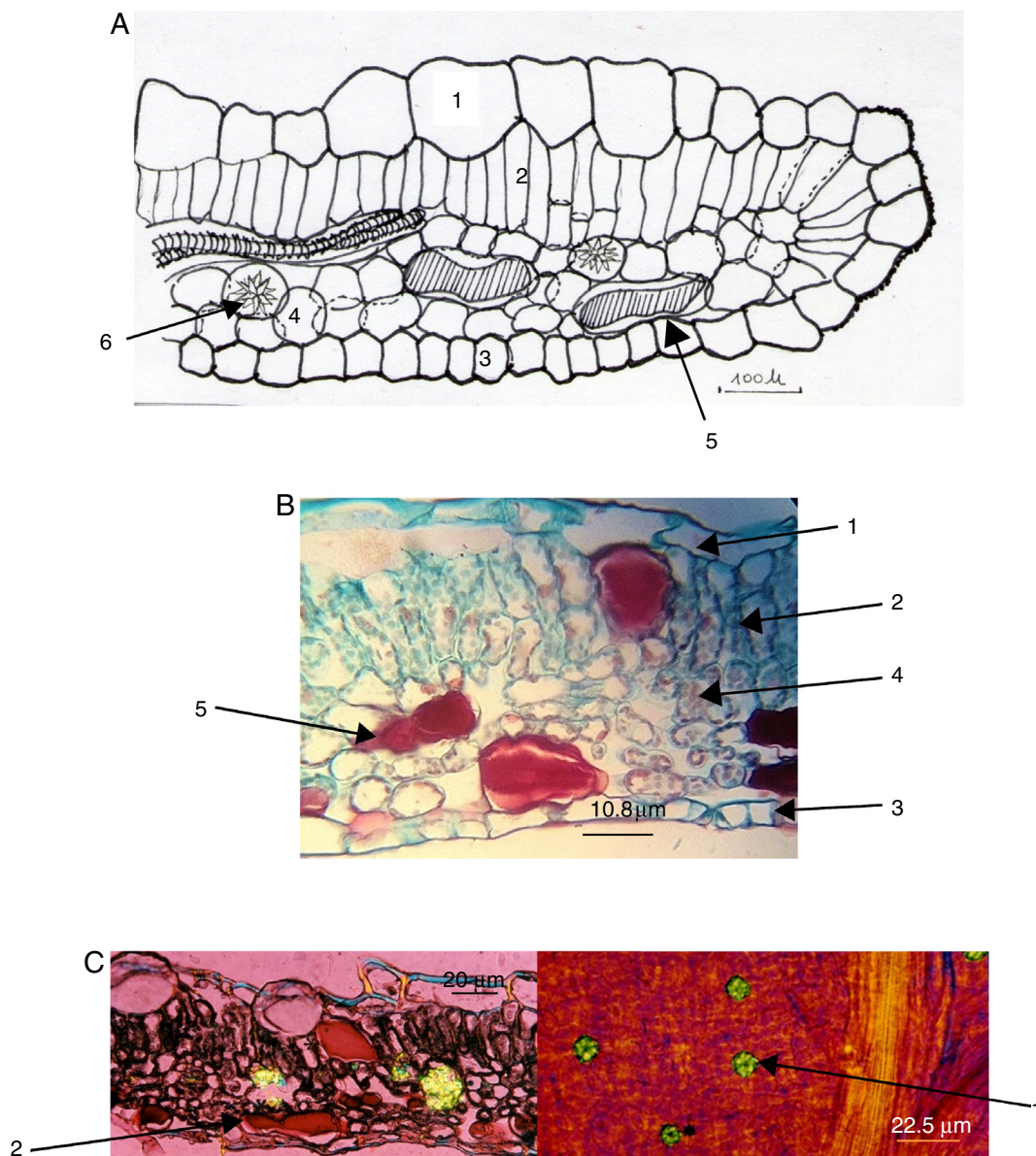


Fig. 4. Cross-section of *Viola maculata* Cav. leaf margin: (A) representative diagram made in a Leiz optic microscope, (B) safranine-fast green dye. 1 – Upper epidermis, 2 – palisade parenchyma, 3 – lower epidermis, 4 – spongy parenchyma with cells containing, 5 – tannin, and 6 – druses, and (C) cross section of leaf with: 1 – druses and 2 – tannins.

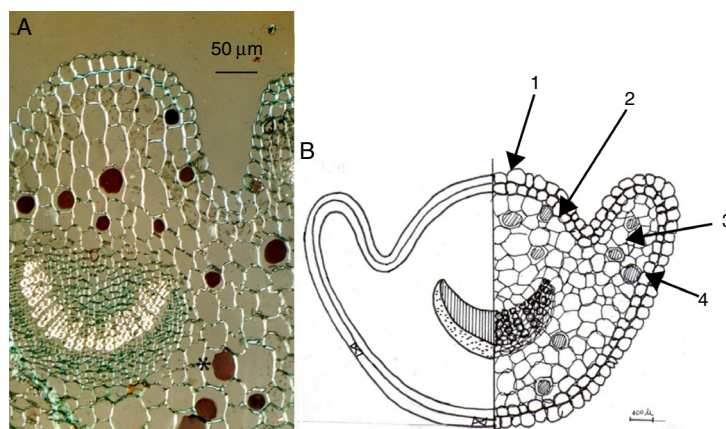


Fig. 5. Cross section of *Viola maculata* Cav. petiole: (A) safranin-fast green dye; and (B) central vascular bundle, 1 – epidermis, 2 – hypodermis, 3 – parenchyma with 4-cells containing tannin.

Argentina, and identified by Cusato, number of deposit 4068 BAF. February 1997.

Assays

Leaf blades and petioles from twenty randomly selected plants were fixed in FAA (formalin, glacial acetic acid and ethanol 80°, 1:1:8) for performing anatomical studies. Five central leaflets from each plant were clarified according to Dizeo de Strittmatter (1984). The parameters analyzed were stomata and hair size and density. The foliar architecture was described following Hickey (1979). Free-hand cross sections and cuts with a Minot microtome from leaf blades and petioles were stained with safranin-fast green and mounted on microscope slides using glycerine-gelly. The following characters were investigated: presence of calcium oxalate crystals, presence of tannins, and presence of hairs. Leaves were fixed in FAA to be analyzed with Scanning Electronic Microscopy (SEM). Also, a Zeiss polarized light microscope was used for the observation of crystals. Drawings were made in a Leiz optic microscope. Three to four measurements and observation replicates were made from several cross-sections.

Results and discussion

Viola maculata leaf blade organization is simple (Fig. 1), the lamina shape is symmetric showing a crenate leaf margin with a chartaceous texture and a normal petiole with a craspedodromous venation (Hickey, 1979). A thin cuticle layer on both surfaces (adaxial and abaxial) covers the leaves. The upper epidermis consists of a single row of square or rectangular cells, 65–85 µm wide (Fig. 2). Stomata are paracytic, located at the same level of the other epidermal cells, with a stomata density of 3 per mm². Cells of the lower epidermis are wavy, 24–42 µm wide, with anomocytic stomata and a stomata density of 80 per mm² (Fig. 3). The analysis with SEM showed that cuticle is thin, folded over the nerves and smooth among them. Also, there are simple and one-cell hairs on both epidermises. Leaf blades are polymorphous, egg-shaped, crenated, and pubescent at the margin. Hairs are simple, unicellular. Mesophyll is dorsiventral, amphistomatic, palisade parenchyma are 1–2 layered below the upper epidermis, with cells containing tannins. Spongy parenchyma cells are 2–3 layered (Fig. 2) with cells containing tannins and calcium oxalate crystals. The vascular bundle in the mid-rib is collateral and the bundle sheath consists of parenchyma cells (Fig. 4). There is sclerenchyma toward the upper epidermis

and collenchyma toward the lower epidermis. The bundle sheath also surrounds the secondary vein.

Petiole (Fig. 5) is covered by epidermal cells with a thick cuticle and few stomata. A 2–3 layered cells collenchyma is situated under the epidermis. Parenchymatous cortex consists of spherical cells, 10–12 layered with intercellular space and abundant cells with tannin. There is a large central vascular bundle, collateral without a bundle sheath. The xylem turns toward upper side of petiole, and the phloem turns toward the lower side of the petiole.

As it was reported for some other species of *Viola* (e.g., *V. odorata*, *V. alba*, *V. sintenisii*, *V. tineorum*, *V. arvensis*), *V. maculata* presents calcium oxalate crystals, isolated or less frequently grouped, between the leaves' palisade and lacunose tissues (Colombo et al., 2007; Watson and Flores, 2009; Toiu et al., 2010). Also, some species (e.g., *V. kizildaghensis*, *V. tineorum*, *V. ucriana*) contain brown mucilaginous bodies in the mesophyll (Colombo et al., 2007; Dinç and Yıldırımli, 2007; Bağcı et al., 2008). As for trichomes, some *Viola* species are glabrous (*V. metajapoica*, *V. tricolor*, *V. micranthella* Wedd) while others have simple and unicellular trichomes (*V. odorata*, *V. kizildaghensis*, *V. arvensis*, *V. hirta*, *V. elatior*, *V. alba* susp *alba*) (Sanso et al., 2003, 2005; Dinç and Yıldırımli, 2007; Bağcı et al., 2008; Toiu et al., 2010; Watson and Flores, 2011; Yousefi et al., 2012; Mehrvarz and Marcussen, 2013; Mareacre et al., 2014). *V. kizildaghensis*, *V. arvensis*, *V. hirta*, *V. elatior*, *V. tineorum*, have anisocytic stomata (Dinç and Yıldırımli, 2007; Bağcı et al., 2008; Mehrvarz and Marcussen, 2013; Mareacre et al., 2014) while *V. maculata* stomata are paracytic in the adaxial leaf surface and anomocytic in the abaxial leaf surface. As the species is used in traditional medicine this study can contribute to the knowledge and characterization of the species.

Authors' contributions

CP contributed in collecting plant sample and identification, designing the study and analysis of data, and in writing the paper. MVR contributed in running the laboratory work and analysis of data. MAA contributed in critical analysis of data, supervised the laboratory work, and in writing the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

M. A. Alvarez is member of CONICET. Universidad Maimonides funded this work. We wish to thank Ms. Isabel Rillo for her advice and careful revision of English.

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