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Original article

Morphoanatomical and histochemical characterization of *Larrea* species from Northwestern of Argentina

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ABSTRACT

Larrea divaricata Cav., *L. cuneifolia* Cav. and *L. nitida* Cav., Zygophyllaceae, are evergreen xerophytic shrubs occurring in Northwestern Argentina used in traditional medicine. The aim of this work was to characterize the morphology, anatomy and histochemistry of the vegetative organs of three *Larrea* species by light and scanning electron microscopy in order to provide supporting data for their correct identification and to determine the site of synthesis and accumulation of its main active compounds. The shape, number and percentage of coalescence of leaflets, presence or absence of mucrones and rachis and the shape of the stipules represented the main botanical differences between the studied *Larrea* species. Anatomically three species presented amphystomatic leaves, with thick resinous slightly striated cuticle with resinous deposits, polygonal epidermal cells with straight anticlinal walls, ciclocytic, brachy-paracytic and paracytic stomatal types, non-glandular trichomes and isolateral mesophyll. The position and abundance of the sclerenchyma at the mid vein and petiole transection allows the differentiation of the three species, been more abundant in *L. cuneifolia.* Secondary phloem and parenchyma cells presented abundant calcium oxalate druses and solitary rhomboidal crystals. Epidermal cells and cuticle layer of leaflets and stipules of the three species presented amber resin deposits and content which stained positively for polysaccharides, phenolic compounds, flavonoids and tannins, while mesophyll palisade cells showed small refracting droplets stained positively for lipophilic substances.

Introduction

Zygophyllaceae family is represented approximately by 280 species almost restricted to tropical and subtropical areas. In Argentina the family is represented by seven genera (*Bulnesia* Gay, *Kallstroemia* Scop., *Larrea* Cav., *Plectrocarpa* Gill. ex Hook. & Arn., *Porlieria* Ruiz & Pav., *Tribulus* L. and *Zygophyllum* L.), and typically they are often dominant in the landscapea of Chaco and Monte regions (Cabrera and Willink, 1973; Cabrera, 1976; Zuloaga and Morrone, 1999; Flora Argentina, 2018). In Brazil it is represented by three genera (*Kallstroemia*, *Larrea* and *Tribulus*) (Engler, 1827).

The genus *Larrea*, Zygophyllaceae, comprises five species with amphitropical distribution in dry regions of South America (Argentina, Chile, Bolivia, Peru, Brazil) and North America (Mexico to Utah, United Stated of North America) (Engler, 1827; Hunziker et al., 1972, 1977; Flora Argentina, 2018). *L. divaricata* (common names: "jarilla", "jarilla hembra", "chamanilla", "jarilla del cerro", "yarilla"), *L. cuneifolia* (common names: "jarilla", "jarilla macho", "jarilla crespa", "jarilla norte-sur", "jarilla del campo") and *L. nitida* (common names: "jarilla", "jarilla de la montaña", "crespa", "pispa o pispita", "jarilla fina"), are represented in Northwestern Argentina forming shrubby associations called "jarillales". Among these, *L. divaricata* is the only *Larrea* species cited in the Brazilian flora (Engler, 1827).

Larrea species are evergreen, xerophytic, erect aromatic shrubs, 1–4 m with opposite, pubescent, sub-sessile and stipulate compound leaves which show a resinous yellowish appearance. The main botanical difference between *Larrea* species resides in their

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morphology, phenological patterns, and mating systems (Barbour et al., 1977; Simpson et al., 1977; Ezcurra et al., 1991). Ragonese (1960) noted some differences based on anatomical characters mainly the proportion and disposition of sclerenchyma tissues.

Larrea divaricata, *L. cuneifolia* and *L. nitida* are used in Argentinean traditional medicine as anti-inflammatory, antirheumatic, hypotensive, rubefacient, diaphoretic, febrifuge, oxytocic, emenagogo, odontalgic, antitussive and to treat fungal and bacterial infections (Alonso and Desmarchelier, 2006; Alonso, 2007; Barboza et al., 2009).

A wide range of pharmacological activities were previously described for these species indicating their potential usage as alternative or complementary medicine. Aqueous and/or alcoholic extracts from *L. divaricata* showed antibacterial (Stege et al., 2006; Zampini et al., 2007), antitumoral (Anesini et al., 1996a, 2001; Davicino et al., 2010, 2011; Martino et al., 2016), antioxidant (Carabajal et al., 2017) and inmunomodulatory (Anesini et al., 1996b; Davicino et al., 2007) activity. Organic solvent extracts were active against phytopathogenic fungi (Quiroga et al., 2001; Svetaz et al., 2010; Vogt et al., 2013). Whereas *L. cuneifolia* showed larvicidal (Batallán et al., 2013) and antioxidant properties (Torres et al., 2003; Carabajal et al., 2017). A synergistic antifungal effect of *L. nitida* and *Zuccagnia punctata* Cav. was also reported (Butassi et al., 2015).

Martino et al. (2016), Carabajal et al. (2017), Agüero et al. (2011) and Blecja et al. (2007) reported the presence of nordihydroguaiaretic acid, essential oils and flavonoids as main constituents in some *Larrea* species. Several flavonoids including quercetin, apigenin and kaempferol derivatives were identified in organic extracts of *L. cuneifolia* (Valesi et al., 1972).

Bioactive compounds of *Larrea* species are presumably found in the resin that covers their leaves and stems. Ragonese (1960) observed a gradual decline in the resin content of older leaves and stems, and suggested that the resin is synthesize in the stipules from where it spills on nearby organs.

Pointing to the great potential of these species and their traditional use, the aim of this study was to characterize the morphoanatomy and histochemistry of the vegetative organs of *L. divaricata, L. cuneifolia* and *L. nitida* utilized in folk medicine of Northwestern Argentine, to identify anatomical diagnostic characters for their correct identification, and to determine the site of synthesis and accumulation of its main active compounds.

Materials and methods

Plant material

Aerial parts of *Larrea cuneifolia* Cav. and *L. divaricata* Cav., were collected in April 2015 at Amaicha del Valle, Tucumán, Argentina at 2000 m.a.s.l. Samples of *L. nitida* Cav. were collected in April 2015 at Vinchina, La Rioja, Argentina at 3485 m.a.s.l. Voucher specimens of each collection were deposited at the Herbarium of Fundación Miguel Lillo (LIL). Herbarium numbers of specimens are as follows: *L. cuneifolia*: LIL 614829; *L. divaricata*: LIL 614299 and *L. nitida*: LIL 615845.

Light microscopy

Samples of leaves and stems of five plants of each species were fixed in FAA (formalin, acetic acid, 50% ethanol, 5:5:90 v/v/v) and stored during one week after processing. Sections (10–25 μm) were obtained with a rotation microtome, subsequently treated with 50% NaClO solution, washed with distilled water and stained with astra blue-safranin and then mounted in 50% glycerol (Zarlavsky, 2014). Sections were visualized with a Zeiss Axiolab optic microscope equipped with a polarized light filter and a Zeiss Axiocam ERc 5s digital camera.

Measurements were made using AxioVision software version 4.8.2 (Carl Zeiss Ltd, Herts, UK).

Histochemistry

The main classes of chemical compounds of the leaves and stipules were investigated in transverse microtome sections of fresh material. Fresh leaves and stipules were place between dental wax supports and sectioned at 20–25 μm with a rotation microtome.

Vanillin–sulphuric acid (Gaucher et al., 2013) and Neu's reagent (2-aminoethyl-diphenylborinate, Sigma) 10% in absolute methanol (Neu, 1957), were used to visualized flavonoids. Sections stained with Neu's reagent were analyzed under a fluorescence microscope (Nikon Optiphot) with UV light (filter UV-1A: 365 nm excitation filter, 400 nm barrier filter). Under these conditions, flavonoids were detected by a yellowish fluorescence (Mondolot-Cosson et al., 1997). Photographs were taken with a digital Nikon Coolpix 4500 camera. Nadi reagent was used to detect terpenoids, essential oil and oil resins (David and Carde, 1964). Ferric chloride (10%) in methanol (Zarlavsky, 2014) and Vainillin–HCl (Gardner, 1975) were used to visualize phenolic compounds and tannins respectively. Toluidine blue O was used for the detection of polysaccharides (Heslop-Harrison and Heslop-Harrison, 1981).

Some of the sections were treated with 50% sodium hypochlorite and washed with distilled water, prior to dyeing with Sudan IV for the detection of lipids (Zarlavsky, 2014; D'Ambrogio de Argüeso, 1986) and ruthenium red for pectins (Johansen, 1940; Zarlavsky, 2014). Iodine potassium iodide (IKI) (Johansen, 1940) was employed for the detection of starch. Standard control procedures were carried out simultaneously.

Scanning electron microscopy

Samples of leaves were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for 4–6 h at 4 °C. Following rinsing in the same buffer, the material was dehydrated in a graded acetone series and sputter coated with gold. Observations were carried out on a field emission scanning electron microscope (FESEM-ZEISS SUPRA-55 VP). Electronic microscopy observations were performed at the Centro Integral de Microscopía Electrónica (CIME), CONICET, Tucuman, Argentina.

Results

Morphology and anatomy

Larrea species are evergreen xerophytic, erect aromatic shrubs 1–4 m with opposite, composite, sub-sessile, pubescent, and stipulate leaves, with a succulent, resinous yellowish appearance.

Larrea cuneifolia presents leaves (4.5–13.2 × 2.5–16.0 mm) formed by two acute asymmetric oblong-ovate leaflets (2–4 × 1–2 mm) joined along two thirds of their internal edge culminating in a reflex apex with a filiform, vascularized mucro (0.3–0.5 mm) (Fig. 1A and D). Two stipules (squamous, subtriangular, reddish) (1.2–3.1 mm) are inserted at the base of the leaves (Fig. 1A and J).

Larrea divaricata shows divaricated leaves (7.3–17.8 × 2.5–4.5 mm) formed by two oblong-acute divergent leaflets joined at the base in a third of its total length (Fig. 1B), apex reflex with a short and, vascularized mucro (0.3–0.4 mm). The stipules are obtuse and rounded similar to those previously described for *L. cuneifolia* (Fig. 1E and F).

Larrea nitida presents odd pinnately compound leaves (7.2–13.1 × 3.5–5.0 mm) (Fig. 1C), with 11–17 sub-opposite,

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