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Original Article

Leaf venation pattern to recognize austral South American medicinal species of "cow's hoof" (*Bauhinia* L., Fabaceae)

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ABSTRACT

The leaves extracts of some species of *Bauhinia* L. s.l. are consumed to treat diabetes, inflammation, pains and several disorders in traditional medicine in austral South America. Despite its wide use and commercialization, sale is not controlled, and botanical quality of samples is not always adequate because of plant misidentification and adulteration. Here, we characterized leaf vein pattern in nineteen taxa to contribute to the recognition and commercial quality control of plant material commercially available. The vein characters intercostal tertiary and quinternary vein fabric, areole development and shape, free ending veinlet branching and marginal ultimate venation allowed to distinguish the main medicinal species in the region.

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Introduction

The leaves extracts of some species of *Bauhinia* L., Fabaceae, so called cow's hoof, "pata-de–vaca", cow's paw or orchid trees, are consumed to treat diabetes, inflammation, pains and several disorders in traditional medicine in austral South America (Oliveira and Saito, 1989; Bortoluzzi et al., 2006; Barboza et al., 2009; Pizziolo et al., 2011; Fortunato, 2012). With 300 species worldwide, *Bauhinia* s.l. is represented by fifteen species in Argentina, Paraguay, Uruguay and Santa Catarina, Paraná and Rio Grande do Sul states of Brazil (Fortunato, 1986, 1996; Fortunato et al., 2008; Vaz et al., 2010).

Bauhinia bauhinioides (Mart.) J.F.Macbr., *B. microstachya* (Raddi) J.F.Macbr., *B. ungulata* L., *B. holophylla* (Bong.) Steud., *B. forficata* Link, *B. cheilantha* (Bong.) Steud. and *B. rufa* (Bong.) Steud. are the main species for which pharmacological activities have been reported. Hypoglycemic, antioxidant, analgesic, antiinflammatory and larvicidal activities along with antitumor potentiality let them auspicious targets for new bioactive substances researching and phytotherapy (Silva and Cechinel Filho, 2002; Sousa et al., 2004; Gadotti et al., 2005; Luna et al., 2005; Nakahata et al., 2006; Cechinel Filho, 2009; Paula et al., 2014; Rozza et al., 2015; Martins-Olivera et al., 2016).

These taxa are extensively employed and sold in native American, rural and urban communities in the region and surrounding areas (Bortoluzzi et al., 2006; Barboza et al., 2009; Hurrell et al., 2011; Ibarrola and Degen de Arrúa, 2011). Despite its wide use and commercialization, sale is not controlled, and botanical quality of samples is not always adequate because of plant misidentification and adulteration. Commercial samples – crude herbs or industrialized herbal medicines – include entire or broken dried leaves, stems and often pods. In general, plants are harvested from their natural habitats, being popularly recognized by their bilobed or bifoliolate leaves. Species are difficult to identify, especially from vegetative stages or in fragmented material, and sample labeling and trading are mostly accomplished by using common names, generic name or incorrect names (Melo et al., 2004). These often result in adulterated, falsified or substitute samples, which would interfere with the effectiveness and even the safety of the product (Ferreres et al., 2012). Leaf anatomical features are useful for identification and adequate sample botanical quality, in *Bauhinia* s.l., venation pattern has been utilized in taxonomy, phylogenetic analyses and even in palaeobotany (Vaz, 1979; Fortunato, 1986; Zhang, 1994; Lin et al., 2015).

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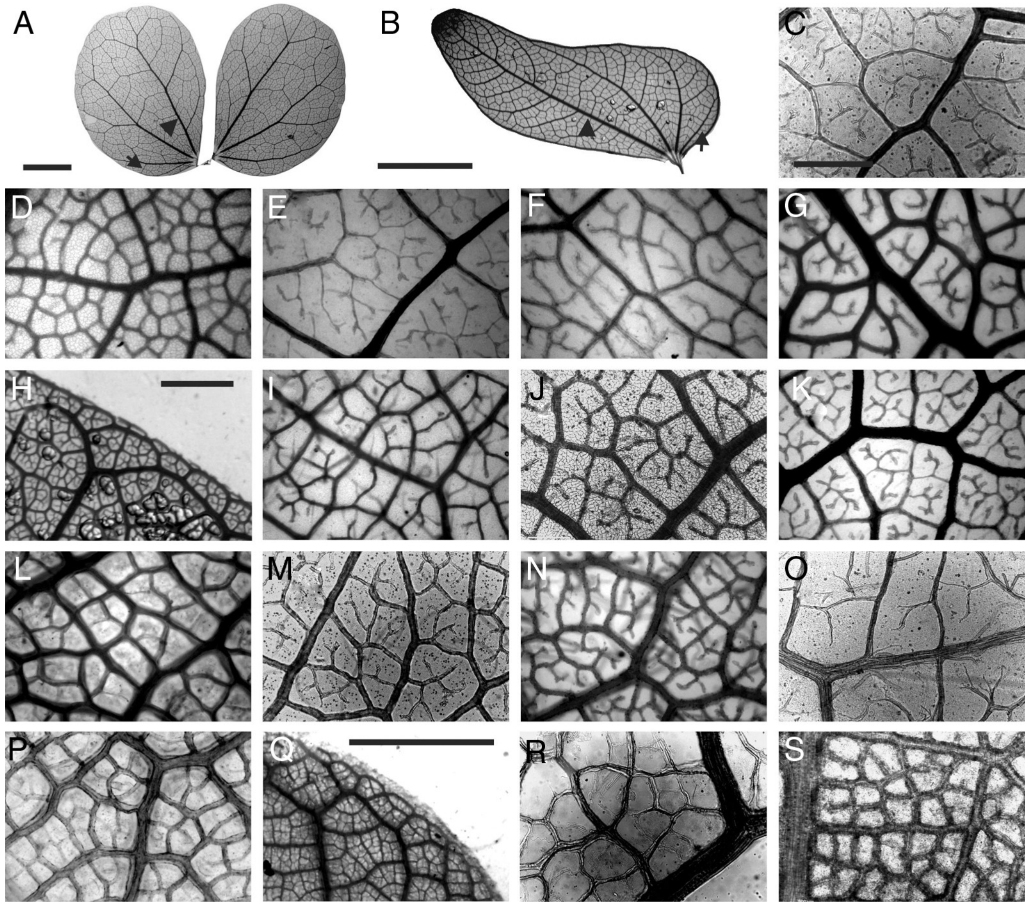

Fig. 1. Leaf venation in southern South American *Bauhinia*. (A and B) General vein pattern. (C–G, I–P, R and S) Areolation. (H and Q) Looped marginal ultimate venation. (A) *B. bauhinioides*. (B) *B. hagenbeckii*. (C) *Bauhinia affinis*. (D) *B. angulosa*. (E) *B. argentinensis*. (F) *B. bauhinioides*. (G and H) *B. campestris*. (I) *B. cheilantha*. (J) *B. forficata* ssp. *pruinosa*. (K) *B. hagenbeckii*. (L) *B. holophylla*. (M) *B. microstachya*. (N) *B. mollis* var. *mollis*. (O) *B. mollis* var. *notophila*. (P and Q) *B. rufa*. (R) *B. ungulata* var. *ungulata*. (S) *B. uruguayensis*. Arrowhead: basal veins. Arrow: agroaphic veins. Scale bar: A and B: 3 mm; C–G, I–P, R and S: 300 μm; H, Q: 1 mm.

In this work, we contribute to the recognition and commercial quality control of austral South American medicinal *Bauhinia* through a study of leaf vein pattern.

Materials and methods

We analyzed 19 austral South American taxa (Appendix 1). The study of leaf venation pattern was carried out following Ellis et al. (2009). For this, fresh or herbarium leaves were cleared according to Dizeo de Strittmatter (1973) and stained in safranin/80% ethanol. Samples were mounted in DPX (Aldrich Chemical Company, Gillingham, UK) and examined using Zeiss Stereo Microscope a Zeiss Axiolab light microscope (Carl Zeiss, Germany) with a trinocular phototube and a color digital imaging camera. Some venation data were extracted from previous work (Fortunato, 1986, 1996).

Results and discussion

All taxa analyzed present basal actinodromous primary vein framework, with no naked basal veins, 3–5 (7) basal veins per lobe or leaflet and agrophic veins simple (Fig. 1A and B). Previously, some of these taxa have been described as acrodromous (Fortunato, 1986, 1996) or campylodromous (Vaz, 1979), and recently both American *Bauhinia* s. s. and *Schnella* are considered actinodromous or acrodromous by Lin et al. (2015). Main vein characters (Fig. 1, Table 1) provide a criterion to identification of medicinal species and detection of falsifications or adulteration. The areole development allows differentiating three main groups, some of them, but not all, taxonomically related.

The first group is characterized by a good areole development that is found in some medicinal members of sect. *Pauletia* ser. Cansenia, in the liana *Bauhinia angulosa* (= *S. angulosa*) and in the spiny tree *B. uruguayensis* (Fig. 1D, L, P–S). The medicinal *B. holophylla* and *B. rufa* both display quadrangular and pentagonal, rarely triangular areoles, with absent and unbranched freely ending veinlets (FEV), the first one can be further recognized since FEV in general cross the areola and marginal ultimate venation and quinternary vein fabric venation are absent (Fig. 1L). *B. rufa*, instead, presents looped marginal ultimate venation and quinternary vein fabric present (Fig. 1P and Q). The medicinal *B. ungulata* has absent, unbranched and one branched FEV and areola shape more variable (Fig. 1R). Some similar features have been described in others taxa from ser. *Cansenia*, and Zhang (1994) named it as the Cansenia venation type. *B. angulosa* is easily distinguished because of the very small areoles lacking FEV and marginal ultimate vein absent (Fig. 1D). *B. uruguayensis* is the only taxon that presents paxillate areole development without FEV, or just very few FEV (Fig. 1S), these features are similar to the Elayuna type described by Zhang (1994).

By the other side, the second group, composed by the prickly trees and shrubs from sect. *Pauletia* ser. Aculeatae and the suffrutices and shrubs from ser. Pentandrae, possesses moderate areole development along with marginal ultimate venation absent and quinternary vein fabric present (Fig. 1B–E, J–K, M–O). Comparatively, areoles are larger and more variable in size than in previous

Table 1
Leaf venation pattern in austral South American *Bauhinia*. FEV, freely ending veinlets; P, pentagonal; PE, percurrent; Q, quadrangular; R, reticulate; T, triangular.

Species	Intercostal tertiary vein fabric	Quinternary vein fabric	Areole development	Free ending veinlet (FEV) branching	Areole shape	Marginal ultimate venation	Fig. 1
B. affinis	PE	Present	Moderate	Unbranched and one or two branched; rarely absent	variable	Absent	C
B. amambayensis	R at apex, PE at base	Present	Moderate	Unbranched and with one or more branches; sometimes absent	variable	Absent	
B. angulosa (= *S. angulosa*)	R at apex, PE at base	Absent	Good	Absent; rarely unbranched	Q and P, rarely T	Absent	D
B. argentinensis	R at apex, PE at base	Present	Moderate	Mostly with one or more branches; sometimes unbranched	Variable	Absent	E
B. bauhinioides	R	Absent, to poor distinct	Moderate to good	Mostly unbranched and one branched; rarely two branched or absent	Variable	Absent	A, F
B. campestris	PE to R	Absent	Moderate, rarely good	Mostly with one or more branches; sometimes unbranched; rarely absent	Variable	Looped	G and H
B. cheilantha	PE	Absent	Good to moderate	Absent, unbranched and one branched; rarely two branched.	Variable	Absent	I
B. forficata	PE, notably at base	Present	Moderate	Mostly with one or more branches; sometimes unbranched	Variable	Absent	J
B. hagenbeckii	R at apex, PE at base	Present	Moderate	Mostly with one and more branches; rarely unbranched	Variable	Absent	B, K
B. holophylla	PE	Absent	Good	Absent and unbranched (FEVs mostly crossing areoles); rarely one branched	Q and P, rarely T	Absent	L
B. microstachya (= *S. microstachya*)	R at apex, PE at base	Absent, to poor distinct	Moderate	Mostly unbranched and one branched; sometimes with more branches	Variable	Looped and absent	M
B. mollis var. *mollis*	PE to R	Present	Moderate	Mostly unbranched and one branched; sometimes with more branches or absent	Variable	Absent	N
B. mollis var. *notophila*	PE	Poor distinct	Moderate	Mostly unbranched and with one or more branches; rarely absent	Variable	Absent	O
B. rufa	PE	Present	Good	Absent and unbranched	Q and P, rarely T	Looped	P and Q
B. ungulata var. *cuiabensis*	PE to R at apex	Absent	Good	Absent, unbranched and one branched	Variable to Q, P, T	Looped and absent	
B. ungulata var. *ungulata*	PE to R at apex	Absent, to poor distinct	Good	Absent, unbranched and one branched	Variable to Q, P, T	Looped	R
B. uruguayensis	R at apex, PE at base	Present	Good/paxillate	Absent; rarely unbranched and one branched	Q and P, rarely T	Looped and in part absent	S

group. The medicinal *B. forficata* (both subspecies), *B. argentinensis* and *B. hagenbeckii* show almost the same pattern, with mostly branching FEV (one or more branches) and less frequently, unbranched FEV (Fig. 1J, E, K). *B. forficata* can be distinguished by the percurrent tertiary vein fabric, while the others are percurrent at the base and reticulate at apex. *B. affinis* (Fig. 1C), *B. amambayensis* (not shown) and *B. mollis* (Fig. 1N, O) have in general both unbranched and branched FEV, in different frequency. The medicinal liana *B. microstachya* (= *S. microstachya*) possesses also moderate areole development, mostly unbranched and one branched FEV, but is recognized because of the presence of both looped and absent marginal ultimate venation (Fig. 1M), this report is similar to the one by Fortunato (1986) but differs from previous (Zhang, 1994).

Finally, the medicinal *B. cheilantha* and *B. campestris* (sect. *Pauletia* ser. Cansenia) present areole development intermediate between good and moderate. *B. cheilantha* alternates between absent, unbranched and one branched FEV and ultimate venation absent while *B. campestris* can be distinguished because FEV are in general branched and ultimate venation is looped (Fig. 1I, G and H). The medicinal *B. bauhinioides* (sect. *Pauletia* ser. Perlebia) also possess areole development moderate to good but areoles are mostly unbranched and one branched (Fig. 1F).

Interestingly, all species present prismatic crystals of calcium oxalate associated to vascular bundles and often druses in mesophyll cells, more notable in *B. affinis*, *B. argentinensis*, *B. mollis* and *B. ungulata* var. *cuiabensis*.

Here we provide a helpful tool for the recognition of the main regional medicinal plants through the analysis of the leaf vein pattern based on the intercostal tertiary and quinternary vein fabric, areole development and shape, free ending veinlet branching and marginal ultimate venation characters.

Authors' contributions

RHF collected and identified the plants, performed the laboratory work and data analysis and participated in the results discussion and the manuscript final writing. MAC participated in the laboratory work and data analysis. BGV helped perform the analysis with constructive discussions. MJN drafted the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2016.10.007.

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