



Morphoanatomical and Phytochemical Study of *Buddleja thyrsoides* Lam. (Scrophulariaceae) Leaves

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SUMMARY. This work deals with foliar morphology, anatomy and phytochemical profile of *Buddleja thyrsoides* Lam. (Scrophulariaceae) leaves. The leaves present a lanceolate-linear shape, straight adaxial face; the margin is serrate to sub-entire and little revolute close to the basis. The epidermis is uniseriate, with the epidermal cells of the adaxial face bigger than those of abaxial face, stomata and trichomes in abaxial face. The mesophyll is heterogeneous, bifacial and presents branched cells and crystals in druse form. The vascular system is of the open collateral type. These characters are significant in the botanical quality control of this species. Phytochemical screening revealed the presence of alkaloids, flavonoids, cumarins, anthocyanic heterosides, and saponins.

RESUMEN. “Morfoanatomía y Estudio Fitoquímico de las Hojas de *Buddleja thyrsoides* Lam. (Scrophulariaceae)”. Se estudió la morfoanatomía y se determinó el perfil fitoquímico de las hojas de *Buddleja thyrsoides* Lam. Las hojas presentan forma linear-lanceolada y la cara adaxial plana con el margen aserrado o más o menos entero ligeramente revuelto hacia la base. La epidermis es uniseriada con las células epidérmicas de la cara adaxial más grandes que las de la cara abaxial. Los estomas y tricomas se encuentran en la cara abaxial. El mesófilo es heterogéneo, con disposición dorsiventral y con la presencia de células ramificadas formando el parénquima esponjoso y cristales en la forma de drusas. El sistema vascular está formado por haces colaterales abiertos. Estos caracteres son significativos en el control botánico de esta especie. El estudio fitoquímico reveló la presencia de alcaloides, flavonoides, cumarinas, heterósidos antocíánicos y saponinas.

INTRODUCTION

The genus *Buddleja* L. was proposed by Houston¹ in 1737 and published by Linnaeus² in 1753. Jussieu was the first to consider *Buddleja* as a member from Scrophulariaceae³. Since 1790s to 1999s, *Buddleja* passed through several orders and families according to its morphological, anatomical, genetic and phytochemical characteristics. Bentham & Hooker⁴ and Solereder⁵ removed *Buddleja* from Scrophulariaceae family and integrated it to Loganiaceae family based on the morphological characteristics of flower and the nature of the fruits⁶.

Dahlgren⁷ published the genus *Buddleja* in the family Buddlejaceae, contained in the order Gentianales. As well as Cronquist⁸, other authors recognized Buddlejaceae within the order Scrophulariales⁹⁻¹¹. Nowadays, the Angiosperm Phylogeny Group (APG)¹² integrated the Buddlejaceae family into the Scrophulariaceae, in Lamiales order.

Buddleja is a genus of pantropical distribution, present in Southern Asia, Africa and America. It comprises nearly 100 species, and a large number of these species occurs in Americas¹³. In Brazil¹⁴ native species and one exotic (*Bud-*

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dleja davidii Franch.) are known with distribution in Southern and Southeastern regions, with the exception of *Buddleja brasiliensis* Jacq. ex Spr. which is distributed in Central-Western and Northeastern regions of Brazil⁶. Several species of this genus have been utilized in traditional medicine in many parts of the world. Roots, barks, flowers and leaves of the plants of this genus are used in different diseases as dysentery, eye and skin inflammation, as diuretic agents, antiseptics, and antirheumatic and in asthma, bronchitis and cough^{14,15}. Researches of some species of the genus *Buddleja* resulted in the isolation of terpenoids¹⁶, flavonoids¹⁷, iridoids¹⁸, phenylethanoids¹⁹, sesquiterpenes²⁰⁻²², lignans²³ and saponins²³⁻²⁵. Several of these chemical compounds have shown inhibitor effect on the generation of eicosanoids of leucocytes²⁶, antihepatotoxic²⁷, antifungal²⁸, antibacterial¹⁵, and antiprotozoan²⁵ activities.

The species *Buddleja thyrsoides* Lam., object of this study, present in these countries -Argentina, Brazil, Uruguay and Paraguay²⁹- is popularly known as barbasco and cambarazinho-do-campo. This plant is a shrub that reaches one meter tall, but it can grow up to four meters high. It blossoms between the months of August and November and fructifies in October²⁹. Due to the importance of cambarazinho-do-campo as a medicinal plant of wide traditional use, the purpose of this work was to determine the morphoanatomical characteristics of the leaves, useful in the quality botanical control, and to obtain a qualitative chemical profile of the constituents present in this vegetal organ through an preliminary phytochemical screening.

MATERIALS AND METHODS

Plant material

The leaves of *Buddleja thyrsoides* Lam. were collected in the countryside of the municipality of São Luiz Gonzaga, state of Rio Grande do Sul, Brazil, and a sample of the flowering vegetal material was identified by Dr. Thais Scotti do Canto-Dorow and stored in the Herbarium of Biology Department of the Federal University of Santa Maria, according to exsiccate SMDB 10125. The anatomical analysis was carried out with samples of 2 x 2 mm of leaves, fixed in glutaraldehyde at 2%, and kept at 4 °C. For the qualitative phytochemical analysis the material was dehydrated in a circulating-air greenhouse at 36 °C and pulverized in a knife-mill, obtaining 50 g of a powder. This material was stored in amber vial for posterior extraction.

Determination of macro and microscopical features

The macroscopically characteristic determination was accomplished through naked-eye observations and with help of a stereomicroscope (amplifying 10x)³⁰. For the study of venation the vegetal material was cleared and dyed with safranin at 0.1 % in ethanol at 50%, dehydrated and mounted in glycerine³¹⁻³³. In the microscopically characterization, the apical-median region of adult-leaf blades³⁴⁻³⁶ was analyzed. The foliar epidermis study, in the abaxial and adaxial faces, was carried out in semi permanent slides, with paradermal sections obtained by freehand from fresh material, dyed with methylene blue at 0.1%³⁷. For the analysis of the transversal sections the preserved material was dehydrated in ascendant ethylic range, at the graduations of 50°, 60°, 70° and 96° GL, for one hour in each solution, and included in hydroxide ethyl methacrilate blocks^{38,39}. Sections of 4 µm were obtained in rotation microtome, taken to histological bath, repassed to the blade, dyed with toluidine blue at 0.1%^{40,41} and assembled in synthetic balsam. The permanent blades were stored in the Laboratório de Farmacognosia do Departamento de Farmácia Industrial da Universidade Federal de Santa Maria. The determination of organoleptic, and macroscopic and microscopic characters of fractures, as well as the granulometry of the pulverized leaves were carried out for sieving and in semipermanent blades, utilizing as a dye, methylene blue at 0.1%^{42,43}. Fifty grams of the ground drug were submitted to a forced passage by vibration, through a range of sieves with opening of the nets that vary from 71 to 2.500 µm, utilizing a vibratory device. The fractions taken away in each sieve, were weighed and the fraction percentages of passage (P) and of retention (R) were calculated. The values were represented in percentage (% p/p)⁴⁴. The medium diameter of powder particles was obtained by probits.

Phytochemical screening

The qualitative chemical analysis was accomplished in ethanolic extract at 70% obtained in cold, according to Moreira⁴⁵. After this analysis, the extract was dried and resuspended in water for subsequent partition in organic solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, and butanol, 3x 15 mL each fraction). The chromatographic profile of the crude extract and the fractions was obtained by thin layer chromatography (TLC) over chromate

sheets of silica gel G60 F₂₅₄, utilizing dichloromethane, ethyl acetate, methanol and water in the proportions of 6:3:2:0,5 v/v/v/v, respectively. Sulphuric anisaldehyde (110 °C, 10 min) was used as a chromogenic reagent.

RESULTS AND DISCUSSION

A great phenotypical plasticity was found in *Buddleja thyrsoides* leaves. The margin varies from serrate to sub-entire, being little revolute near the base, with a lanceolate-linear shape. In the transversal section, the middle part of the foliar blade is slightly concave in the adaxial face and convex on the abaxial one. The leaves of the genus *Buddleja* presents significant morphological variation among the species, with serrate, crenate or dentate margin, seldom lobate and the linear to obovate limb form⁶. The found data are similar to those obtained by Smith *et al.*²⁹ and differ from *B. oblonga* Benth and *B. brasiliensis* Jacq.⁴⁶ that present crenate margin. The lanceolate-linear limb form found in *B. thyrsoides* is a relevant feature, since the limb form in *B. vetula* and *B. brasiliensis* is obo-

vate, elliptical-strict in *B. cestriflora* Cham. and oblong to lanceolate in *B. campestris* (Vell.) Walp.²⁹ (Figure 1 and Table 1).

The epidermis is uniseriate. The epidermal cells, in a paradermal view, are polygonal in shape in both faces, the adaxial and the abaxial one (Fig. 2).

In the transversal section, the adaxial face epidermal cells present a nucleus dislocated from central region, near the plasmatic membrane that is opposite to the cuticle. The anticlinal wall size is larger than the periclinal wall (15 µm x 10 µm) and the cellular wall opposite to upper face is lightly curved (Fig. 3A). These results are the same as those observed by Arambarri *et al.* (2006) in the epidermal cells of *B. thyrsoides* leaves⁴⁷.

The epidermal cells of the abaxial face present an ovoid (3 µm x 2 µm) or rectangular (4 µm x 5 µm) shape, and in the regions of vascular bundles of second order and central present a rounded form (3 µm x 3 µm) (Figure 3). In the adaxial face the cuticle is straight and thick without epicuticular formations. In the abaxial face the cuticle is plicate and less thick than in the adaxial face (Figure 3A and 3B). The presence of glandular and tector trichomes (Figure 2C and 3C) and hygrophyte stomata type were verified only in the abaxial face (Figure 3D).

Metcalf & Chalk³⁵ reported the genus *Buddleja* in the Loganiaceae family evidencing the presence of stomata just in the abaxial face, which is in agreement with our *B. thyrsoides* analysis. These authors³⁵ also pointed out the lack of trichomes, being discordant to the abaxial face of the analyzed material. According to Metcalf & Chalk³⁵, in the Scrophulariaceae family the stomata are present in both surfaces, which wasn't evidenced in *B. thyrsoides*, and the tector trichomes are often numerous. Oliveira *et al.*⁴⁸ pointed out that the trichomes are a feature of great value in the diagnosis of

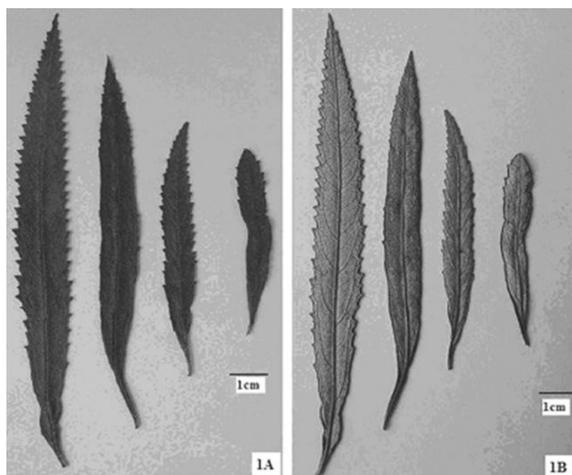


Figure 1. General aspect of the *Buddleja thyrsoides* Lam. leaves. **1A.** Adaxial face. **1B.** Abaxial face.

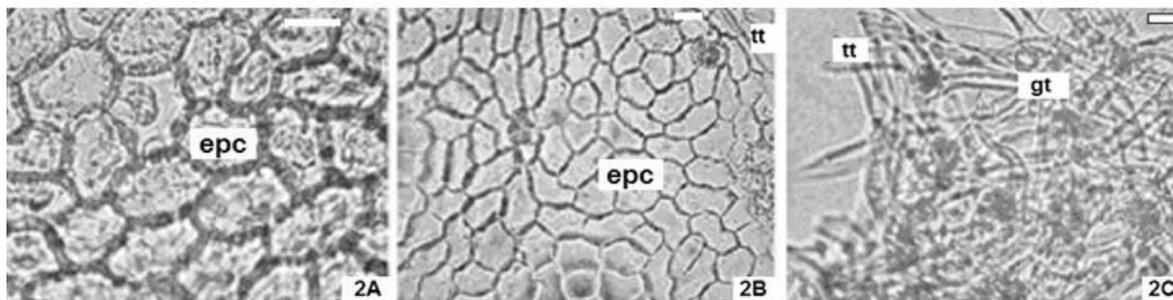


Figure 2. Paradermic section of the *Buddleja thyrsoides* Lam. leaves. **2A.** Adaxial face. **2B** and **2C.** Abaxial face. Legend: epc: epidermal cells; tt: tector trichome; gt: glandular trichome. Scale: 10 µm (represented in the Figure by a white trace).

	Characters	Characterization
	<i>Petiole</i>	Absent
	Type	Simple and sessile
	Shape	Lanceolate-linear
	Size	11.05 x 0.9 cm (9.0 x 0.7 - 13.0 x 1.1)
	Margin	Serrate to sub-entire Slightly revolute near the basis
	Apex	Acuminate
	Base/Basis	Cuneate/Atenuate
<i>Limb</i>	Colour	The adaxial face dark green and a little bright The abaxial face whitish-gray
	Texture	Smooth at the adaxial face Lightly velvety on the abaxial face
	Consistence	Membranaceous
	Venation	Pinnate, camptodrome of eucamptodrome type
	Stipules	Reduced linear
	Phyllotaxy (leaf arrangement)	Crossed-opposite

Table 1. Macroscopic Features of *Buddleja thyrsoides* Lam. Leaf.

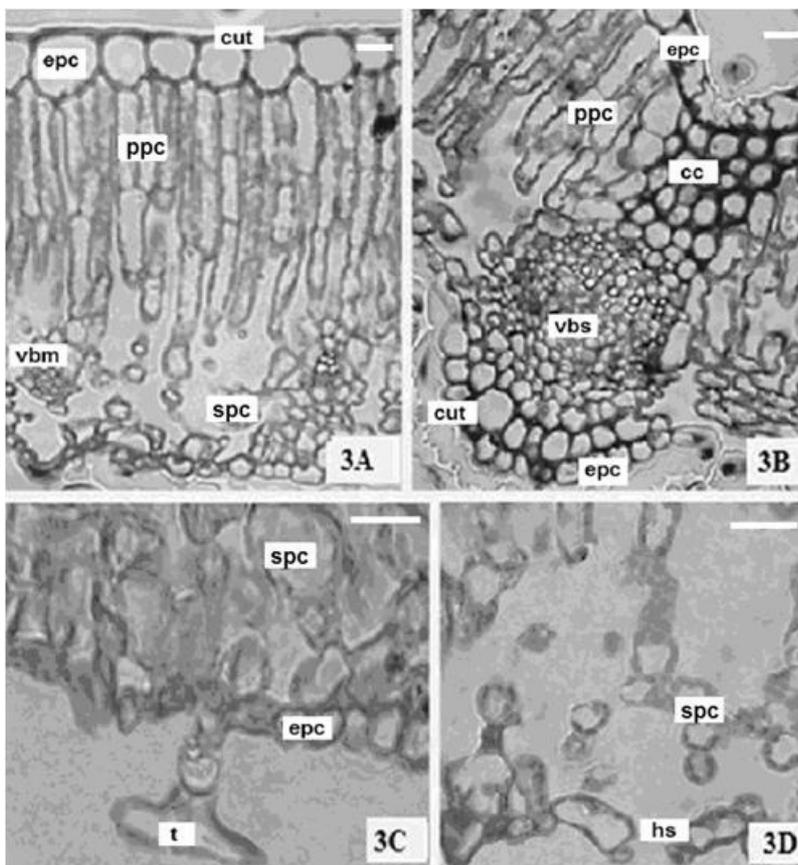


Figure 3. Transversal sections of the *Buddleja thyrsoides* Lam. leaves. **3A.** Side region of the mesophyll. **3B.** Region of a vascular bundle of second order. **3C.** Detail of the side region evidencing a trichome on the abaxial epidermis. **3D.** Detail of the side region showing stoma in the abaxial epidermis. Legend: **epc**: epidermal cells; **cut**: cuticle; **ppc**: cells of the palisade parenchyma; **spc**: cells of the spongy parenchyma; **vbm**: vascular bundle of minor order; **vbs**: vascular bundle of second order. **cc**: collenchymatic cells; **t**: trichome; **hs**: an hygrophyte stoma. Scale: 10 μm (represented in the Figure by a white trace).

vegetal drugs. On the other hand, Metcalfe & Chalk ⁴⁹ mentioned the trichome type classification as a consistent feature in the determination of a given vegetal species.

The mesophyll is heterogeneous, assuming a bifacial disposition (Figure 3A), thus in agree-

ment with the characteristics reported by Arambarri *et al.* ⁴⁷. According to Metcalfe & Chalk ³⁵ these features are a common characteristic for the Scrophulariaceae. The cells that constitute *Buddleja thyrsoides* mesophyll possess chloroplasts close to plasmatic membrane, as was re-

ported for other species e.g., *Syzygium cumini* L. leaves by Alberton *et al.*⁴⁴, and prominent nucleus, generally displaced from the central region (Fig. 3A and 3B). The palisade parenchyma located adjacently to adaxial face epidermis, possesses from two to three layers of juxtaposed cells, the first layer presenting bigger cells than the others (Fig. 3A). This kind of palisade parenchyma (formed by one or two and also, but rarely, for more layers) was considered typical for *Buddleja* species by Arambarri *et al.*⁴⁷, and also can be observed in the *Digitalis lanata* (Scrophulariaceae) leaves⁴³. In this work we established a palisade index of 6.12.

The spongy parenchyma possesses about 5 layers of cells. The branched cells occur thereupon the palisade parenchyma and close to the abaxial epidermis, the cells are ovoid to spherical (Fig. 4A). The presence of branched cells in the spongy parenchyma, were reported by Metcalfe & Chalk³⁵ for Scrophulariaceae. It was verified in this microscopical analysis of *Buddleja thyrsooides* leaves. The intracellular space in the spongy parenchyma is of the chamber and lacuna type (Figs. 3C, 3D and 4A). Both, the palisade parenchyma median layer and some spongy parenchyma cells, possess little and fragile crystals, in druse form (Fig. 4B).

The vascular system is composed by bundles of open collateral type. The xylem presents itself forming a radial system with interxylematic parenchymatic cells. The phloem is well developed (Fig. 5). The vascular bundle has numerous characteristics that help in the medicinal plant diagnosis. In addition to be very constant among the individuals of same species, one verifies a range of variations in the anatomical patterns.

A parenchymatic formation with about 10 layers of cells fills the central region. These cells assume minor dimensions over the central vascular bundle (Fig. 5). There is a lamellar collenchyma formation subjacent the epidermis of the adaxial face. This formation is composed by

nearly 5 cell layers (Fig. 5A). There is also a lamellar collenchyma formation, with up to 3 cell layers, in the region subjacent to the abaxial face (Fig. 5B). The vascular bundles of minor order are situated thereupon the cell layers in palisade and are surrounded by parenchymatic cells, which tend to form an irregular and little prominent sheath vascular (Fig. 3A).

Metcalfe & Chalk³⁵ characterized the mesophyll of the Loganiaceae leaves as simple dorsiventral, mentioning the presence of mucilaginous cells, features that were not observed in *Buddleja thyrsooides*. Yet, the same authors pointed out a great anatomical variety in the mesophyll of Scrophulariaceae, as well as the presence of little crystals and a revolute margin³⁵, characteristics which also approximate *Buddleja thyrsooides* to this family.

Drug Characterization and Phytochemical Screening

Drug characterization is necessary to establish the technological parameters of a vegetal drug. For this, the granulometric evaluation is an indispensable parameter to be established, because it reflects directly the extraction optimization. When the powder has the medium diameter of a very little particle (smaller than 200 μm) it may compromise the filtration and consequently, the active(s) substance(s) content(s). In the same way, if the medium diameter is very large (over than 800 μm) it can prejudice the extraction because it difficult the penetration of extractor liquid in all cells⁵⁰. Both situations can lead to the compromising of the quality of the extract obtained, which won't present the chemical content uniformity. The evaluation of the leaf granulometric behavior allowed to detect a medium diameter of particle equal to 440 μm . The powder obtained from *B. thyrsooides* leaves fits in the heterogeneous and moderately rough, once the most fractures 84.5% remained in sieve with net 297-500 μm . The vegetal powdered material presented predominantly whitish-green

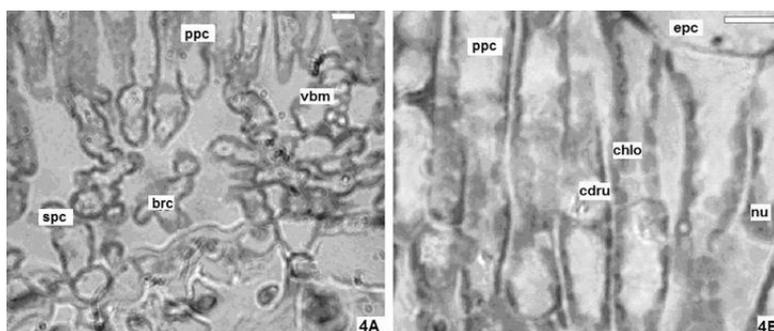


Figure 4 (A-B). Transversal section of the *Buddleja thyrsooides* Lam. leaves evidencing the mesophyll in detail. Legend: **ppc**. palisade parenchyma; **spc**. spongy parenchyma cells; **brc**. branched cells; **vbm**. vascular bundle of minor order; **cdru**. crystal in druse form; **chlo**. chloroplasts; **epc**. Epidermal cells; **nu**. nucleus. Scale: 4 μm (represented in the Figure by a white trace).

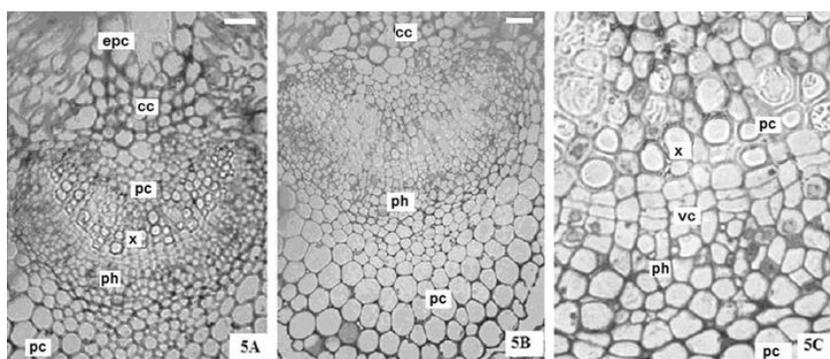


Figure 5. (A-C) transversal section of the central vascular bundle region of the *Buddleja thyrsoides* Lam. leaves. Legend: **epc.** epidermal cells; **cc.** collenchymatic cells; **pc.** parenchymatic cells; **x.** xylem; **ph.** phloem; **vc.** vascular cambium. Scale: 10 μm (represented in the Figure by a white trace).

coloration, *sui generis* odor and sweetened taste.

The extractive content determination is another important parameter for the extractive capacity evaluation of the active substances present in the drug. For *B. thyrsoides* the yield of the dry extract was of 16.28% to the ethanolic extract at 70% and to the hexane, dichloromethane, ethyl acetate and butanolic fractions were 1.34%, 3.11%, 4.86 % and 6.93%, respectively.

Phytochemical screening of the ethanolic extract at 70% showed positive reactions to alkaloids, flavonoids, coumarins, anthocyanins, saponins and volatile oils. The TLC profile established for the crude extract and fractions allowed to perceive the presence of the principal groups of substances⁵¹ revealed in the phytochemical screening (data not shown). These results are in agreement with to the chemical groups of isolated compounds reported in literature¹⁴. Tannins and cardio active substances were not detected.

CONCLUSIONS

The form, texture and margin of *Buddleja thyrsoides* leaf which possesses the peculiarity of being serrate and revolute near the base, are morphological characteristics that contribute for the morphodiagnosis of this species. The anatomical characters, like the epidermal cells of the adaxial face larger than the abaxial face, the presence of hygrophyte stomata and tector and glandular trichomes in the abaxial face, bifacial mesophyll, vascular bundles of the open collateral type with a xylem radially arranged, when analyzed together, help in the authenticity of the leaves as pharmaceutical drug and approximate *Buddleja thyrsoides* to the Scrophulariaceae family. The high yield of the ethanolic extract at 70% could be attributed to the great concentration of high polarity constituents, confirmed by the qualitative phytochemical screening.

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