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Leaf Morphoanatomy of *Solanum capsicoides* All. (Solanaceae) from Restinga Area

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***SUMMARY. Solanum capsicoides* All. (Solanaceae) can be found in restinga vegetation of Brazil and its areal part extracts have shown cytotoxicity in *in vitro* models. The aim was to study the leaf morphoanatomy of *S. capsicoides* in order to contribute to safely identify the species. Macroscopic analysis was performed after morphological studies.The anatomical description of the leaves was performed by microscopic analysis of paradermal and transversal sections, after cleared and stained. Microscopic analysis of leaf powder was carried out after cleared. *S. capsicoides* presented prickles on both sides of the leaf, which is oval contour, recessed at base and with lobed margin. The species has as differential anomocytic and anisocytic stomata and trichomes of several types: uniseriate multicellular non-glandular, bicellular non-glandular, elongate uniseriate multicellular glandular, capitate glandular and claviform glandular in both surfaces. The results obtained are important and can be used for botanical identification and differentiation of *S. capsicoides* compared to other *Solanum* species.**

INTRODUCTION

The restinga areas are considered coastal ecosystems, physically determined by soil conditions of the sandy soil and marine influence, where the flora and fauna have evolved mechanisms to withstand salinity, extreme temperatures, direct sunlight, water shortages, unstable soil and strong winds, distributed in mosaic with great ecological diversity [1]. They are permanent preservation areas with high ecological and social importance, especially in landscape protection, geological stability and gene flow of populations [2]. Plants have been important to humans since ancient times, by providing food and protection from the elements of the environment, or as effective remedy for the restoration of health [3].

Solanum capsicoides All. (Solanaceae), popularly known as "mata-cavalo", can be found in shoal habitats in north-central coast of Santa Catarina, Brazil [4,5]. The word *Solanum* is probably originated from Latin *solamen* and means comfort, relief, in reference to the narcotic effects of some species and it is the most representative genus of the family *Solanaceae* [6]. Some examples of bioactive species native of Brazil are: *Solanum americanum* Mill. ("maria-pretinha") with analgesic effect, *Solanum lycocarpum* A. St.-Hil. ("fruta-do-lobo") used to diseases of the urinary tract, and *Solanum paniculatum* L. ("jurubeba") against liver and digestive diseases [7,8].

Solanum capsicoides is a shrub, upright with 30 to 70 cm tall [9], with only a few studies of botanical description. This species is visually similar to other species of the genus, especially *Solanum sisymbriifolium* Lam., commonly found in orchards, annual crops and pastures in the southern region of the country [9,10]. The morphological similarity is causing misidentification, even among researchers in this area, which justifies further analysis of the leaves of *S. capsicoides,* highlighting the features and differential structures.

KEY WORDS: Microscopy, Morphoanatomy, Restinga, *Solanum capsicoides*.

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Cytotoxicity studies of the aerial parts of *S. capsicoides* in a model of *Artemia salina* showed LC50 of 440.1 µg/mL [11], an activity that is probably related to the presence of glycoalkaloids [11-13]. The steroidal alkaloids and their glycosides occur in *Solanum* species and have a variety of biological activity such as antifungal, molluscicidal, teratogenic and embryotoxic [11-13].

This study aimed to describe the leaf morphoanatomy of *S. capsicoides,* contributing to the secure identification of the species, which has a bioactive potential and occurs in remnants of strongly threatened restinga areas.

MATERIAL AND METHODS

Plant material

The leaves of *S. capsicoides* were collected in a restinga area in the Navegantes city, SC (26° 51 '41.2" S and 48° 36' 13.2" W) on April 2011. The herbarium specimens (in triplicate) of the vegetative and reproductive dry parts were prepared with samples of the material studied, indicating the place of collection, collector, habits, size, flower color and popular name [14]. The samples were deposited in the Barbosa Rodrigues Herbarium of Itajaí (code: HBR 52956.

Macroscopic and microscopic leaf analysis

Macroscopic analysis of the leaves were made with naked eye and supplemented with stereomicroscopy. The epidermis of both surfaces were obtained from the lower third of the leaf and the elements vasculature, texture, contour, margin, and pubescence were analyzed, and then compared to the morphological patterns displayed by Oliveira & Akisue [15] and Gonçalves & Lorenzi [16]. The experiment was performed with 3 leaves of 6 subjects, *i.e.* 18 samples. Leaves for anatomical study were fixed in FAA during 48 h and subsequently stored in 70 °GL ethanol [17]. The slides were prepared from cuttings made by hand, bleached in sodium hypochlorite (2 %) for 15 min, and then washed in turn with distilled water, acidified water, and distilled water again. The colouration of some paradermic sections, as transverse leaf blade and petiole (middle part), was performed with Astra blue (1 %) in 50 °GL hydroethanol solution for 10 min [18]. The chemical nature of the crystals was analyzed through its solubility in acids [19].

To observe the adaxial and abaxial surfaces and classification of stomata, was used the technique of epidermic printing with adhesive. The method consists of applying a drop of cyanoacrylate ester on the glass slide, then the region of the fresh leaf is pressed onto the blade for about 10 s, and then removed for further analysis [20].

The microscope slides were previously mounted, observed in anatomic sections and photomicrographed with a Leica CME optical microscope coupled to a digital camera. An ocular was used with a properly calibrated slide containing a micrometer scale, in order to obtain the anatomical measurements.

Microscopic analysis of leaf powder

To analyze the powder of leaves, they were dried in an incubator at 40 °C for 48 h, grounded and the powder was standardized through a sieving process, to have a size less than 300 µm. This material was made transparent for higher anatomical definition, using the solution of chloral hydrate (60 %) through heating [21,22]. Slides were examined under an optical microscope as described above.

RESULTS

The *S. capsicoides* leaves have 10.0-18.0 cm length x 6.5- 14.0 cm width. When extended, the oval contour limb can be observed, the pinnate vascular system (Figure 1a), hairy surface (pubescent), lobed margins, as well as membranous consistency with prickles (trichomes) in the limb and petiole (Figs. 1 and 4g).

On the microscopic analysis of the limb, was observed that the epidermal cells (front view) consist of sinuous anticlinal walls and the stomata located in upper and lower epidermis are of anomocytic and anisocytic type (Fig. 2). In both epidermis (adaxial and abaxial) occur trichomes types glandular: uniseriate, tetra-multicellular elongate, capitate and claviform; non-glandular:

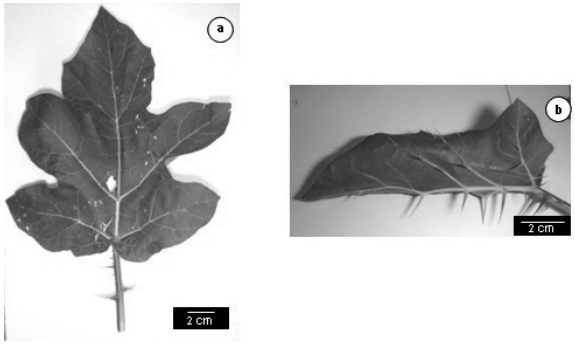

Figure 1. Aspects of *Solanum capsicoides* leaf morphology: **a**) lobed leaf with oval contour and pinnate venation; **b**) leaf surface with put prickles (non trichomes).

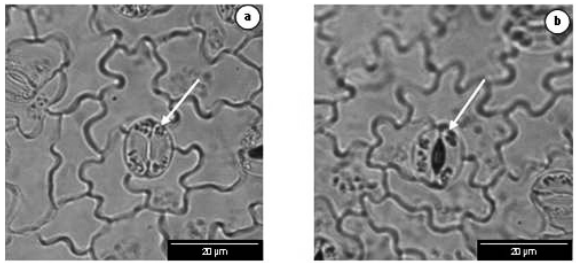

Figure 2. Paradermal view of abaxial surface of *Solanum capsicoides* leaf anatomy. **a**) anomocytic stomata; **b**) anisocytic stomata.

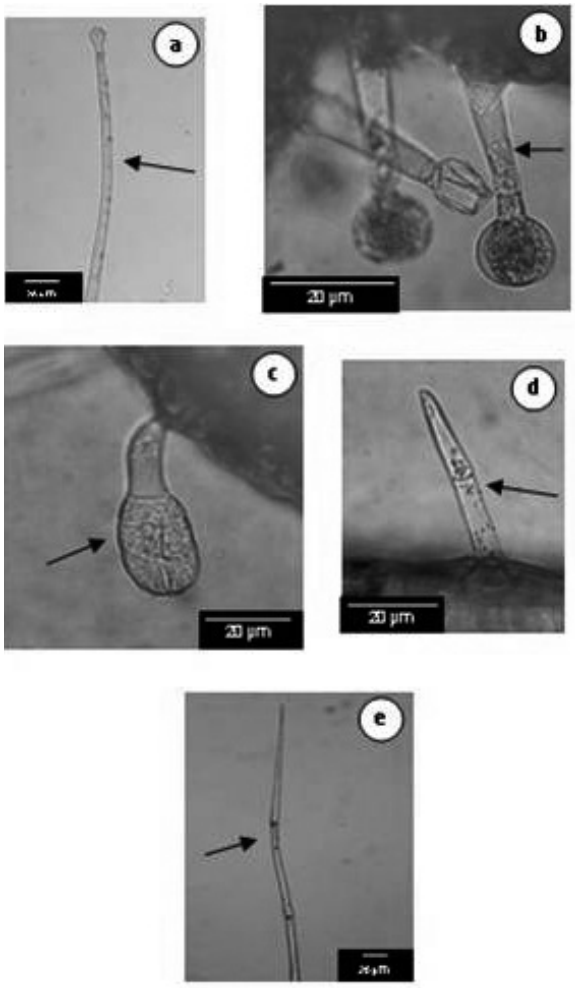

Figure 3. Microscopical description of trichomes observed in the transversal section of *Solanum capsicoides* leaf. **a**) Uniseriate multicellular glandular trichome. **b**) Capitate glandular trichomes. **c**) Claviform glandular trichome. **d**) Bicellular non-glandular trichome. **e**) Uniseriate multicellular non-glandular trichome.

uniseriate, bi-multicellular and hexa-multicellular elongate (Fig. 3). Only one epidermal layer occur on both sides, the mesophyll is an asym-

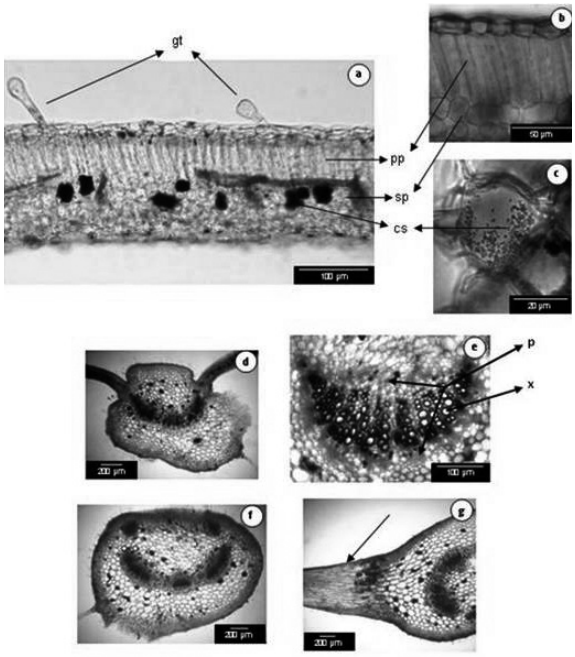

Figure 4. Aspects of *Solanum capsicoides* leaf anatomy. **a**, **b**, **c**) Transversal limb sections. Dorsiventral mesophyll (a). Palisade and spongy parenchyma are seen in detail in b. Parenchyma cell containing crystal sand is seen in detail in c. **d**, **e**) Transversal section of midrib. Detail of bicollateral vascular bundle in e. **f**, **g**) Petiole transversal sections, showing a prickle detail without vascular bundles (g, arrow). gt = glandular trichome; pp = palisade parenchyma; sp = spongy parenchyma; cs = crystal sand; f = phloem; x = xilem.

metrical heterogeneous (dorsiventral), with a layer of cells in the palisade parenchyma, and spongy parenchyma consists of round cells with diminished intercellular spaces (Figs. 4 a and 4b) and by special cells with crystalline sand type of calcium oxalate (Figs. 4a and 4c), confirmed by histochemical test.

The midrib of *S. capsicoides* is biconvex in cross section, has a bicollateral vascular bundle (characteristic of family) in arc surrounded by parenchyma (Fig. 4d, 4e), which is interrupted by cells of subepidermal angular collenchyma on both surfaces. The petiole in cross section has almost a circular shape, covered by a single epidermal layer along with a few layers of angular collenchyma located internally and the rest is filled with parenchyma. The vascular system of the petiole is composed of three bicollateral vascular bundles arranged in an arc shape, located in the central region and two smaller ones located in lower ends of the adaxial surface (Fig. 4f). Glandular and non-glandular trichomes are present on the epidermis of the midrib and petiole, as well as cells with calcium oxalate crystals (crystal sand) appear in the parenchyma.

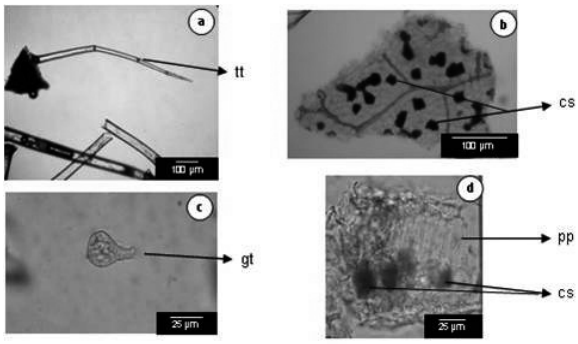

Figure 5. Observed structures of *Solanum capsicoides* leaf powder. **a**) uniseriate multicellular non-glandular trichomes. **b**) epiderm fragment showed crystal sand situated at the mesophyll. **c**) glandular trichome fragment. **d**) mesophyll fragment with palisade parenchyma and crystal sand seen in pieces of the spongy parenchyma layers. tt = uniseriate and multicellular non-glandular trichomes; cs = crystal sand; gt = glandular trichomes; pp = palisade parenchyma.

In the analysis of the powder of the *S. capsicoides* leaves, leaf structures are observed, mainly multicellular and long uniseriate non-glandular, fragments of non-glandular, a few fragments of the glandular trichomes, epidermal fragments showed through crystal sand situated at the mesophyll as was seen in pieces of the spongy parenchyma layers (Fig. 5).

DISCUSSION

In the description of the species *S. capsicoides* by Kissmann & Groth [6] it is reported the occurrence of straight spines and slightly turned downwards. This feature was not observed in this study, but high occurrence of prickles on both sides of the limb and the petiole were observed. Feliciano & Salimena [23] also commented on the existence of spines in the leaves of *S. capsicoides*. The prickles are a process or epidermal attachment, usually sharp, that stand out with relative ease and are often confused with thorns, but differ from these because they do not vascularize. The thorns are a special kind of spike, sharp, rigid with organs always modifying (leaves or stems), therefore they have vascularization [16].

A medicinal plant from the Solanaceae family, with similar leaf morphology to the *S. capsicoides* is the *Solanum paniculatum* L. According to Oliveira *et al.* [22] and Lorenzi & Matos [8], the leaves of *S. paniculatum* have the following similar botanical characteristics: hairy simple leaves, with prickles, petiolate, lobed margins, acute apex and concave base, therefore very similar to *S. capsicoides,* differing in terms of consistency and color. *S. paniculatum* is leathery and has its surface on the abaxial side with a whitish color while the *S. capsicoides* is membranous and green.

Other species of the *Solanum* genus, analyzed by Feliciano & Salimena [23] are also similar to *S. capscoides* mainly on the leaf composition, margin, the presence of pubescence and prickles. Based on morphological analysis of *S. capsicoides,* the following differences were observed: *Solanum lycocarpum* A. St.-Hil. has a lanceolate outline, rounded apex and papery consistency; *Solanum vaillantii* Dunal has truncated and asymmetric leaf base; *Solanum palinacanthum* Dunal has leaves with papery consistency; *Solanum sisymbriifolium* Lam. has leaves with asymmetrical basis.

Solanum viarum Dunal described by Procopio *et al.* [24] and *Atropa belladonna* L. characterized by Oliveira *et al.* [22] present microscopical leaf similarity with *S. capsicoides,* especially in relation to the description of the dorsiventral mesophyll type (heterogeneous asymmetric) with a layer of palisade parenchyma and the occurrence of cells filled with calcium oxalate crystals, very small and triangular, called crystalline sand. The presence of crystals of the crystalline type sand in *S. capsicoides* had not yet been reported by the authors, but it was observed in large numbers during the analysis, occurring in the limb, midrib and petiole regions.

The stomata are epidermal attachments of great importance in identifying plant species, and in *Solanum viarum* [24] and *S. paniculatum* [22], the stomata present in the epidermis are only of the anomocytic type, while in *S. capsicoides* the stomata located in the upper epidermis and lower epidermis are of the anomocytic and anisocytic type. Thus, these species may be differentiated by the types of existing stomata.

Regarding the trichomes (hair) observed in *S. capsicoides,* there is the occurrence of a uniseriate pluricelular non-glandular and capitate glandular trichomes, also found in *S. viarum* [24] and the claviform glandular trichomes also observed in *Atropa belladonna* [22]. One type of non-glandular trichome observed in this study and not yet reported for *S. capsicoides* is the bicellular non-glandular trichome. Feliciano & Salimena [23] report a microscopic presence of stellar trichomes in *S. palinacanthum, S. vaillantii, S. sisymbriifolium* and rare stellar trichomes in *S. capscoides,* which were not identified through

the analysis during the research, nor reported by Kissmann & Grouth [6] or by Lorenzi [10].

Microscopic leaf analysis allows the identification and differentiation of the *S. capsicoides* species and can be performed preferably by the characterization of the types of trichomes (hair). This same feature was also observed in the analysis of the powder of the leaves, with a predominance of the multicellular non-glandular trichomes and long uniseriate, fragments of non-glandular trichomes in large quantities, a few fragments of the glandular trichomes and absence of stellar trichomes.

CONCLUSIONS

With the analysis performed it was possible to describe the leaf morphoanatomy of *S. capsicoides,* obtaining important information about the species and assisting in the differentiation of species belonging to the same genus of the Solanaceae family. The *S. capsicoides* species can be distinguished by characterizing the types of trichomes (hair) and the classification of types of stomata observed in the epidermis.

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REFERENCES

1. Sugiyama, M. (1998) *Bol. Inst. Bot.* **11**: 119-59.
2. Schaffer, W.B. & M.A. Prochnow (2002) *"Mata Atlântica e você: como preservar, recuperar e se beneficiar da mais ameaçada floresta brasileira",* APREMAVI, Brasília.
3. Simões, C.M.O., E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz & P.R. Petrovick (2010) *"Farmacognosia: da planta ao medicamento",* sexta edição, UFSC, Florianópolis.
4. Falkenberg, D.B. (1999) *Insula* **28**: 1-30.
5. Batista-Franklim, C.P.R. & V. Gonçalves-Esteves (2008) *Acta Bot. Bras.* **22**: 782-93.
6. Kissmann, K.G. & D. GROUTH (1995) *"Plantas infestantes e nocivas",* terceira edição, Basf, São Paulo.
7. Souza, V.C. & H. Lorenzi (2005) *"Botânica sistemática",* Instituto Plantarum, Nova Odessa.
8. Lorenzi, H. & F.J.A. Matos (2008) *"Plantas medicinais no Brasil: nativas e exóticas",* segunda edição, Instituto Plantarum, Nova Odessa.
9. Agra, M.F., K. Nurit-Silva & L.R. Berger (2009) *Acta Bot. Bras.* **23**: 823-42.
10. Lorenzi, H. (2000) *"Plantas daninhas do Brasil",* Instituto Plantarum, Nova Odessa.
11. Silva, T.M.S., R.J.B. Nascimento, M.M. Batista, M.F. Agra & C.A. Camara (2007) *Rev. Bras. Farmacog.* **17**: 35-8.
12. Esteves-Souza, A., T.M.S Silva, C.C.F. Alves, M.G. Carvalho, R. Braz-filho & A. Echevarria (2002) *J. Braz. Chem. Soc.* **13**: 838-42.
13. Araújo, M.G.F., W.R. Cunha & R.C.S. Veneziani (2010) *Rev. Cienc. Farm. Básica. Apl.* **31**: 205-9.
14. Ming, C.L. (1996) *"Coleta de plantas medicinais",* in *"Plantas medicinais: arte e ciência. Um guia de estudo indisciplinar"* (L.C. Di Stasi, org.), UNESP, São Paulo, pp. 69-86.
15. Oliveira, F. & G. Akisue (1997) *"Fundamentos de farmacobotânica",* segunda edição, Atheneu, São Paulo.
16. Gonçalves, E.G. & H. Lorenzi (2007) *"Morfologia vegetal",* Instituto Plantarum, Nova Odessa.
17. Johansen, D.A. (1940) *"Plant microtechnique",* McGraw-Hill Book, New York.
18. Roeser, K.R. (1962) *Mikrokosmos* **61**: 33-6.
19. Howarth, W. & L.G.G. Warne (1959) *"Practical botany for the tropics",* University of London Press, London.
20. Segatto, F.B., D.A. Bisognin, M. Benedetti, L.G. da Costa, M.V. Rampelotto & F.T. Nicoloso (2004) *Cienc. Rural* **34**: 1597-601.
21. Biavatti, M.W. & S.N. Leite (2005) *"Práticas de farmacognosia",* UNIVALI, Itajaí.
22. Oliveira, F., G. Akisue & M.K. Akisue (2005) *"Farmacognosia",* Atheneu, São Paulo.
23. Feliciano, E.A. & F.R.G. Salimena (2011) *Rodriguésia* **62**: 55-76.
24. Procópio, S.O., E.A.M. Silva, A.A. Silva & E.A. Ferreira (2003) *"Anatomia foliar de plantas daninhas do Brasil",* UFV, Viçosa.