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Stem anatomy of cipó-caboclo (*Davilla rugosa*, Dilleniaceae) in Rio de Janeiro, Brazil

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In Brazilian folk medicine, stems of *Davilla rugosa*, locally known as *cipó-caboclo*, are known for their anti-inflammatory and antiulcer uses, as well as their use as a laxative, stimulant, aphrodisiac, and tonic. The plant is also important in religious rituals. Previous studies showed that the stem contains flavonoids and terpenes. The ethnomedicinal use of *D. rugosa* has been reported all over Brazil. Plants of the genus *Davilla* are used to make safe herbal medicines with only few and insignificant side effects. In Rio de Janeiro, Brazil, six species of *Davilla* occur: *Davilla glaziovii, Davilla grandifolia, Davilla latifolia, Davilla nitida, D. rugosa*, and *D. tintinnabulata*. The stems of all these species have very similar external morphology. We emphasize that *D. glaziovii* is an endangered species and must not be sold. The stems of *cipó-caboclo* are sold in major herb markets in Rio de Janeiro State and open-air street markets in the City of Rio de Janeiro. The present study aimed at assessing the stem anatomy of *cipó-caboclo* sold commercially in these markets to unveil which species are being traded under this name. We conclude that some of the species sold are of the genus *Davilla*, but it is not possible to separate them at species level. We also found species of the families Aristolochiaceae and Bignoniaceae. These results are alarming, as the possibility of selling either *D. glaziovii* or species of other families as *cipó caboclo* can represent a risk to consumer health.

Key words: Anatomy, cipó-caboclo, *Davilla rugosa*, Dilleniaceae, medicinal plants.

INTRODUCTION

Brazilian traditional and folk medicine use plants and plant-derived phytomedicines to treat a wide spectrum of health problems (Biso et al., 2010). *Davilla rugosa* Poir. is a liana, popularly known in Brazil as *cipó-caboclo*, *cipó-capa-homem*, *cipó carijó*, *lixa*, and *lixeirinha* (Azevedo and Silva, 2006; Pires et al., 2009). It is used against elephantiasis (Guedes et al., 1985), as a diuretic, aphrodisiac, stimulant of motor activity, against gastric ulcer (Coimbra, 1942; Corrêa, 1984; Bacchi, 1986; Guaraldo et al., 2000, 2001), orchitis, chronic lymphadenitis, and edema in the lower limbs (Barros and Napoleão, 2009). *D. rugosa* also has religious importance, as it is used in some rituals of Afro-Brazilian religions (Stalcup, 2000; Guedes et al., 1985). The genus *Davilla* is one of the most diverse in the family Dilleniaceae, with approximately 30 species of lianas and shrubs, all occurring in Brazil. The only exception is *Davilla steyermarkii* Kubitzki, which occurs in Venezuela

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Table 1. Reference samples.

Species		Region/State	Collector/number	Habit	RBw
Davilla	*glaziovii*	Campo Grande - Rio de Janeiro	Fraga 2214	Liana	9633
Davilla	*grandifolia*	Cariacica - Espírito Santo	Fraga 2016	Liana	9635
Davilla	*latifolia*	Nova Venécia - Espírito Santo	Fraga 2081	Liana	9651
Davilla	*latifolia*	Apiário - Minas Gerais	Barbosa 2283	Liana	9652
Davilla	*nitida*	Una - Bahia	Fraga 2592	Liana	9635
Davilla	*nitida*	Vilhena - Rond ônia	Fraga 3039	Liana	9646
Davilla	*nitida*	Chapada dos Guimarães - Mato Grosso	Saavedra 833	Liana	9656
Davilla	*rugosa*	Cachoeiras de Macacu - Rio de Janeiro	Saavedra 686	Liana	9664
Davilla	*rugosa*	Santa Maria Madalena - Rio de Janeiro	Saavedra 709	Liana	9665
Davilla	*rugosa*	Campo Grande - Rio de Janeiro	Fraga 2216	Liana	9667
Davilla	*rugosa*	Rio de Janeiro - Rio de Janeiro	Fraga 2841	Liana	9706
Davilla	*rugosa*	Itatiaia - Rio de Janeiro	Fraga 2887	Liana	9188
Davilla	*rugosa*	Itatiaia - Rio de Janeiro	Fraga 2896	Liana	9190
Davilla	*tintinnnabulata*	Itatiaia - Rio de Janeiro	Fraga 2897	Liana	9189

(Fraga and Stehmann, 2010). The part of the plant used commercially is the stem, which has very similar morphology in all species. Stem anatomy can be used to identify the taxa of commercial importance, in particular tree species. Stem anatomy is also a very useful tool for monitoring woody species, as it allows to control the use of vulnerable or endangered species. On the other hand, the identification of liana stems is still under development worldwide, and efforts to identify some families have been made (Tamaio, 2011; Tamaio et al., 2011). In the state of Rio de Janeiro, Brazil, six species of *Davilla* occur: *Davilla glaziovii* Eichler, *Davilla grandifolia* Moric ex Eichler, *Davilla latifolia* Casar, *Davilla nitida* (Vahl) Kubitzki, *D. rugosa* Poir, and *D. tintinnabulata* Schltdl (Fraga, 2014). *D. glaziovii* is critically endangered according to the Red Book of the Flora of Brazil (Fraga et al., 2013).

This study analyses the stem anatomy of six species of *Davilla* that occur in the state of Rio de Janeiro. The results were compared with species commercialized under the common name *cipó-caboclo* to test whether the samples sold in the market were indeed *D. rugosa*. The samples were obtained from four open-air markets and the Mercadão de Madureira, a food distribution center. All markets are located in the city of Rio de Janeiro, Southeastern Brazil.

MATERIALS AND METHODS

Reference samples

Fifteen individuals were collected of the following species: *D glaziovii*, *D. grandifolia*, *D. latifolia*, *D. nitida*, *D. rugosa*, and *D. sellowiana*. The number of individuals, their respective records in the Wood Collection of the Botanical Gardens of Rio de Janeiro (Rbw), as well as the collection site and other information, are presented as shown in Table 1. Samples were fixed in alcohol 70% (Johansen, 1940), hydrated and softened by heating in distilled water until reaching processing consistency, and then embedded in polyethylene glycol (PEG) 1500 (Rupp, 1964). Voucher specimens were collected field and dried and pressed according to Fidalgo & Bononi (1984) and deposited in the herbaria RB.

Samples sold commercially

Samples sold commercially under the common name *cipó-caboclo* were obtained at the Mercadão de Madureira, a food distribution center, and at four open-air markets in the city of Rio de Janeiro (Prefeitura do Rio de Janeiro, 2012), in a total of 12 samples (Table 2). The commercialized samples were hydrated and softened by heating in distilled water and then embedded in polyethylene glycol (PEG) 1500 (Rupp, 1964).

Anatomical analysis

Individual samples were sectioned in a sliding microtome using an adhesive tape with thickness between 10 and 30 µm (Barbosa et al., 2010), stained in Astra blue at 1% and hydroalcoholic safranin at 50% (Kraus and Arduim, 1997), and then mounted on permanent slides with synthetic resin. Anatomical description followed the IAWA Committee (1989). Observations and photographic records were made using an optical microscope (Olympus BX-50) equipped with a digital CoolSnap Pro camera, and macroscopic images were made using a Leica DFC320 camera coupled to a stereoscopic microscope (Leica MZ16).

RESULTS

In the macroscopic analysis (Figure 1), four structural patterns easily observable with the naked eye were observed: (1) reference sample for *cipó-caboclo* (true *D. rugosa*) (1B-C); (2) reference sample for other *Davilla* species (*D. glaziovii*, *D. grandifolia*, *D. latifolia*, *D. nitida*, and *D. tintinnabulata*) (1E); (3) commercialized sample identified as *D. rugosa* (1F); and (4) commercialized sample whose anatomical pattern was incompatible with that of *D. rugosa* (1G-H).

Table 2. Samples sold commercially as *cipó-caboclo*.

Sample	Common name	Collection site
1	Cipó-caboclo	Mercadão de Madureira (Box 28) – Madureira, Rio de Janeiro
2	Cipó-caboclo	Mercadão de Madureira (Box 26) – Madureira, Rio de Janeiro
3	Cipó-caboclo	Maria da Penha (A) – Penha, Rio de Janeiro
4	Cipó-caboclo	Maria da Penha (B) – Penha, Rio de Janeiro
5	Cipó-caboclo	Street Market of Garibaldi street (Seller 1) – Tijuca, Rio de Janeiro
6	Cipó-caboclo	Street Market of Garibaldi street (Seller 2) – Tijuca, Rio de Janeiro
7	Cipó-caboclo	Street Market of Duquesa de Bragança street (Seller 1) – Grajaú, Rio de Janeiro
8	Cipó-caboclo	Street Market of Mearim street– Grajaú, Rio de Janeiro
9	Cipó-caboclo	Mercadão de Madureira (Box 1) – Madureira, Rio de Janeiro
10	Cipó-caboclo	Mercadão de Madureira (Box 17) – Madureira, Rio de Janeiro
11	Cipó-caboclo	Mercadão de Madureira (Box 18) – Madureira, Rio de Janeiro
12	Cipó-caboclo	Street Market of Duquesa de Bragança street (Seller 2) – Vila Isabel, Rio de Janeiro

Not only the reference samples (Type 1 and 2), but also the commercialized samples (type 3) showed a similar structural pattern of “segmented vascular cylinder” (1C), in which broad xylematic rays and a thin bark shed in papyraceous blades could be observed (1E). In Type 4, one sample (number 8) also showed a “segmented vascular cylinder”; the bark was thick and fissured and was shed in large wood plates (1G). This structural pattern is compatible with species of the genus *Aristolochia* (Aristolochiaceae). Furthermore, sample number 12 showed xylem interrupted by wedges of phloem (Pace, 2009), a variation that is typical of the family Bignoniaceae (1H).

In microscopic analysis the reference samples (type 1 and 2) and commercialized samples (type 3) presented common morphoanatomical similarities with the family Dilleniaceae (Figure 2), including indistinct growth ring boundaries (2A, 2B, 2C, 2D), diffuse porosity (2A, 2B, 2C, 2D), solitary vessels (2A, 2B, 2C, 2D) with two distinct diameters (2C), simple and scalariform perforation plates with few bars in vessels of smaller diameters (2E), diffuse apotracheal axial parenchyma tending to form lines (2A), scanty paratracheal parenchyma (2B, 2C, 2D), presence of raphides in parenchyma cells (2F), uniseriate rays (2A), and broad rays, usually with more than 10 cells in width (2G, 2H). In general, all characters described for the secondary xylem of the reference samples were also observed in the commercialized samples (2I, 2J).

DISCUSSION

Studies focused on the anatomy of medicinal plants are of high interest as they contribute to quality control and to the correct identification of phytodrugs (Duarte and Menarim, 2006; Leite et al., 2007; Marques et al., 2007; Scopel et al., 2007; Mauro et al., 2008; Carpano et al., 2009; Gomes et al., 2009). Frequently, only parts of plants are commercialized, hindering their identification through classical taxonomic methods.

Based on our macroscopic analysis, it was concluded that two commercialized samples (Type 4) did not belong to the family Dilleniaceae, and, hence, could not be classified as *cipó-caboclo* (*D. rugosa*). Using this method, it was also observed that all species similar to *D. rugosa* showed similar anatomy. Therefore, these species were also submitted to microscopic analysis.

In general, the characters observed in the stem of the reference and commercialized samples were consistent with previous studies on the family Dilleniaceae (Metcalfe and Chalk, 1950; Dickison, 1967). Some of these characters were crucial for the identification of the commercialized material, such as segmented vascular cylinder, scalariform perforation plate in smaller vessels, and presence of raphides in parenchyma cells. It was also observed that the stems of the reference samples of *Davilla* were very similar, so that it was impossible to separate them based on stem anatomy. This result is very alarming. Among the *Davilla* samples studied, one is an endangered species endemic to Rio de Janeiro State, *D. glaziovii*, and evidence suggests that the *cipó-caboclo* commercialized in Rio de Janeiro is obtained by extractivism in the Atlantic Forest (Azevedo and Silva, 2006).

The stem of *D. glaziovii* is used for medicinal purposes and is commercialized in regions surrounding the area of endemism: Serra do Mendanha Municipal Park, in Rio de Janeiro (Fraga et al., 2013). The Mendanha Mountain Range is covered by atlantic forest. This biome is a high priority area for conservation in Brazil (Myers, 1988). As a result of its high floristic diversity, the atlantic forest is within the five richest ecosystems in the world in the number of species, and it is considered a biodiversity hotspot. According to Brazilian law, the trade of species native to the Atlantic Forest is prohibited (FEPAM, 2000). In spite of these restrictions, *D. glaziovii* may have been sold as cipó-caboclo in the Mercadão de Madureira.

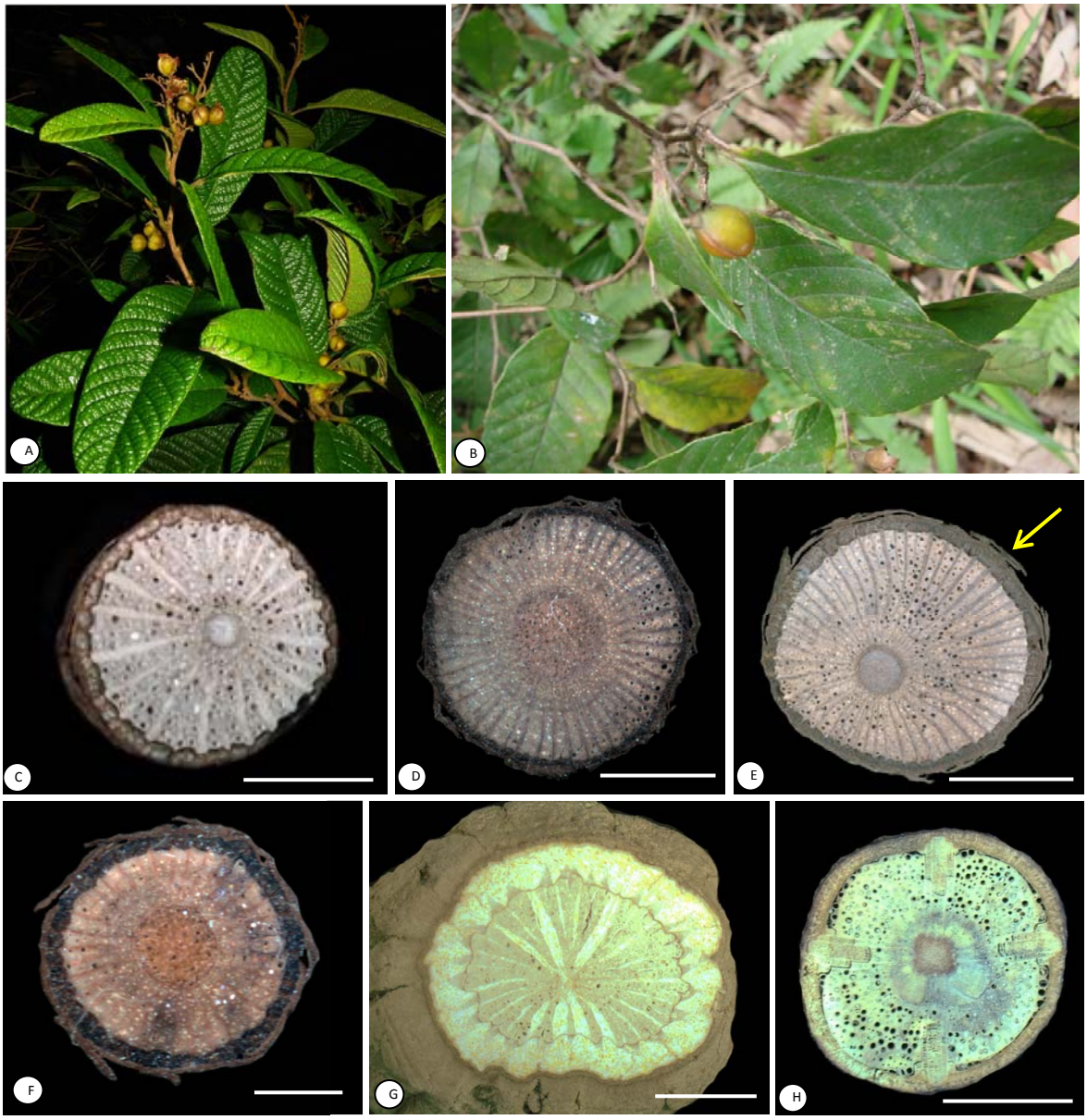

Figure 1. Structural patterns. A: *D. Glaziovii*; B: *D. Rugosa*; C: *D. rugosa* (Reference sample); D: *D. glaziovii* (Reference sample); E: *D. latifolia* (Reference sample). Yellow arrow shows bark shed in papyraceous blades; F: Sample number 9, material very similar to the genus *Davilla*; G: Sample number 8, material can be mistaken for *D. rugosa*, with segmented vascular cylinder and thick and fissured bark; H: Sample number 12, material with no anatomical similarity to *D. rugosa*, which shows xylem interrupted by wedges of phloem. Bar = 0.5 cm.

In Rio de Janeiro, three genus of Dilleniaceae occur: *Davilla*, *Doliocarpus* and *Tetracera*. *Doliocarpus* and *Tetracera* have successive cambia, a cambial variation that facilitates the separation of these genera from *Davilla* (Dickison, 1967; Carlquist, 2001, Horn, 2007). *Davilla* has a stem with a segmented vascular cylinder (Carlquist, 2001). Based on this structural difference, we could separate *Davilla* from *Doliocarpus* and *Tetracera.*

Practitioners of Brazilian traditional and folk medicine use plants and plant-derived phytomedicines to treat a broad variety of health problems (Biso et al., 2010). However, several studies have revealed that many plants used as herbal medicines can be potentially toxic to humans. Therefore, in addition to the illegal trade of endangered species, the intended use should be brought under scrutiny, as no chemical studies have been conducted for *D. glaziovii* so far. Moreover, two commercialized species do not belong to the Dilleniaceae family, and thus, are not *cipó-caboclo*.

We found information on only three *Davilla* species

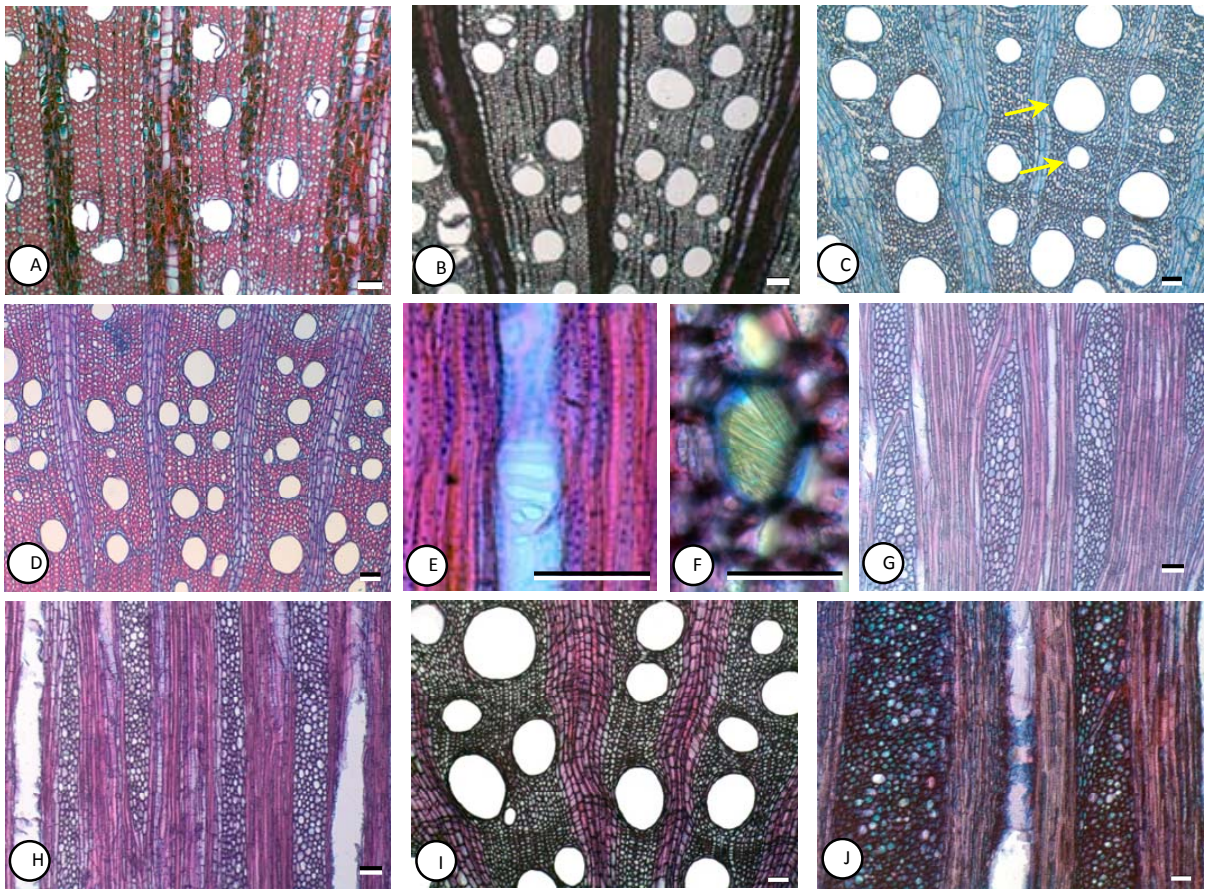

Figure 2 – Secondary xylem in microscopy images. Cross and tangential sections. A. *D. grandifolia*; B, E and G – *D. rugosa*; C and H – *D. tintinnabulata*; D and F – *D. glaziovii;* I and J – Samples of commercially sold species 1.
A, B, C and D – Indistinct growth rings. Diffuse porosity; Solitary vessels; A and B – Unisseriate rays; C – Vessels with two different diameters (yellow arrows); E - Simple and scalariform perforation plates with few bars on the smaller vessels; F - Raphides in the parenchyma cells; G and H - Wide rays with more than 10 cells in width; Bars = 100µm, except E and F where Bars = 50µm.

used for medicinal purposes. *D. rugosa* is used as an anti-inflammatory agent and antiulcer herb, as well as a laxative, motor activity stimulant, tonic, and aphrodisiac (Correa, 1984). *D. elliptica,* endemic of the Brazilian Savana is used as an astringent, tonic, laxative, sedative, and diuretic (Rodrigues and Carvalho, 2011). It is also used in the treatment of hemorrhoids, hernia, and diarrhea, and it is applied topically as an antiseptic to treat wounds (Silva et al., 2001). *D. nitida* a species geographically widespread in the Neotropics is used in gastric treatments (Biso et al., 2010). Furthermore, *D. rugosa* can present toxicity (Guaraldo, 2001), but no conclusive studies have been reported.

Aristolochia species are used against rheumatoid arthritis and several diseases (Lorenzi and Matos, 2002; Gupta, 1995; Duke et al., 2008). The genus *Aristolochia* has mainly terpenoids, lignoids, flavonoids, fatty acids, and nitrogen compounds (alkaloids and nitrophenanthrenes). The trade of medicinal herbs containing the extract of *Aristolochia* species is prohibited in many countries based on its nephrotoxic, carcinogenic, and mutagenic properties, which can lead to progressive nephropathy and urothelial cancer in humans.

Bignoniaceae species are used as a blood cleanser, a stimulant of the nervous and muscular system, as well as an anti-inflammatory agent and contraceptive. It is also used against throat diseases, rheumatism, and bladder calculus (Andrade-Cetto and Heinrich, 2005; Ferreira et al., 2000; Gafner et al., 1996; Gottlieb et al., 1981; Park et al., 2003; Alguacil et al., 2000). Their chemical composition includes terpenoids, flavonoids, alkaloids, iridoids, quinones, and especially, lapachol, which has anticancer properties (Mans et al., 2000). Lapachol is more commonly found in the genus *Tabebuia* (Maganha et al., 2006), but some authors have also found it in lianas (Duarte et al., 2010; Davis, 1983). Previous studies showed that lapachol has strong abortive effects in female rats (Guerra et al., 2001; Morrison et al., 1970).

Considering the potential toxicity of *Aristolochia* and Bignoniaceae, the use of the *cipó-caboclo* commercialized

in Rio de Janeiro could represent a risk to users who use phytotherapic products indiscriminately without medical supervision.

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