BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902020000118158

Article

Phytochemical and morpho-anatomical study of the vegetative organs of *Psychotria fractistipula* L.B.Sm., R.M. Klein & Delprete (Rubiaceae)

Camila Freitas de Oliveira*, Vinicius Bednarczuk Oliveira, Vanessa Barbosa Bobek, Katlin Suellen Rech, Fernando Cesar Martins Betim, Josiane de Fatima Gaspari Dias, Sandra Maria Warumby Zanin, Obdulio Gomes Miguel, Marilis Dallarmi Miguel

Pharmacy Department, Federal University of Parana, Curitiba, Brazil

Psychotria fractistipula L.B.Sm, R.M. Klein & Delprete (Rubiaceae) is found in the existing Atlantic Forest in the South of Brazil, in the Paraná and Santa Catarina States. The members of Rubiaceae are characterized chemically by the presence of alkaloids, some pharmacological properties of which include antifungal, antibacterial, analgesic, antioxidant, antimutagenic, and antiviral activities. In this study, we report the results of anatomical, morphological, and histochemical analyses of the leaves and stems of the Rubiaceae family member, *P. fractistipula*. The anatomical analysis involved of light microscopy and scanning electron microscopy. Morpho-anatomical and histochemical characterization were made using standard methodology. The isolated compounds were identified by Nuclear Magnetic Resonance spectroscopic analysis. The following structures are highlighted as the distinctive features of the species: presence of petiolate leaves and a petiole base, leaf blade with smooth cuticle on both surfaces, paracytic stomata, and, dorsiventral mesophyll and presence of trichomes on the leaves and stems. Phytosteroids, identified as β-sitosterol and campesterol, were observed in the hexane fraction of the stem. Future studies should focus on the isolation of other fractions of interest and their characterization through specific biological and cytotoxicity assays.

Keywords: *Psychotria*. Morpho-anatomical. Phytochemistry.

INTRODUCTION

The family Rubiaceae is diverse and represented by small trees and shrubs. It includes approximately 630 genera, and is found all over the world, except in Antarctica. Its identifying features usually include opposite leaves and interpetiolar stipules (Karao *et al*., 2011; Taylor *et al*., 2007). The genus *Psychotria* is considered the largest genus of this botanical family. It is characterized by the presence of alkaloids, although it should be stressed that the members of Rubiaceae also have the following compounds: coumarins, triterpenes, steroids, saponins, iridoids, and anthraquinones (Souza and Lorenzi, 2005). In Brazil, approximately 252 species of *Psychotria* can be found spread across the national territory. *P. fractistipula* is native and endemic to Brazil and is only found in the States of Paraná and Santa Catarina (Taylor *et al*., 2015).

Members of the Rubiaceae family share common anatomical features, including hypoestomatic sheets, dorsiventral mesophyll, paracytic stomata, and vascular collateral bundles (Metcalf and Chalk, 1959). The presence of domains is also a common feature in this family (Barros 1959, Moraes *et al*., 2011). These characteristics are present in *Psychotria*, along with the presence of styloid crystals and alkaloids in the mesophyll cells (Moraes *et al*., 2011), as well as a defined structure of the vascular

***Correspondence:** C. F. de Oliveira, Departamento de farmácia, Universidade Federal do Paraná, Av. Prefeito Lothário Meissner, 632, Curitiba, Brasil. Phone: +5541999742937. E-mail: camilafreoli@gmail.com.

bundle of the petiole, which appears to be diagnostic for the genus (Martínez-Cabrera *et al*., 2009). The presence of palipolytic cells in the epidermis also seems to be an important taxonomic character (Moraes *et al*., 2011, Vieira *et al*., 1992). The anatomical characteristics are important for the identification of species of pharmacological importance, since the commercialized material is scratched and difficult to identify.

In a preliminary phytochemical analysis, thin-layer chromatography (TLC) of this species showed it to be positive for alkaloid, flavonoid, tannins, steroids, and triterpenes, which were present in the leaves and stem (Oliveira *et al*., 2014). As there is an absence of morpho-anatomical studies and a scarcity of phytochemical studies, the objective of the present work was to characterize the morphology and histochemical properties of *Psychotria fractistipula*, and the identification of hitherto unidentified steroids produced by this species.

MATERIAL AND METHODS

Plant Material

The aerial vegetative parts of *Psychotria fractistipula* were collected in the city of Curitiba (49°14'27"W and 25°26'54"S), Paraná, Brazil, in March of 2014. The biologist and curator of the Municipal Botanical Museum, Osmar dos Santos Ribas, carried out the identification of the plant species, and the voucher specimen was deposited in the museum under the number MBM 389153. The research was authorized by IBAMA, case number 02001.001165/2013-47.

Morpho-anatomical study

Adult leaves of 3 plants were collected and fixed in FAA 70 and stocked in a 70% (v/v) ethanol solution (Berlyn and Miksche, 1976). Semi-permanent slides were prepared using transverse and longitudinal. (Oliveira and Akisue, 2000), and subjected to coloring with astra blue and basic fuchsin (O'Brien *et al*., 1964). The slides were mounted with a solution of glycerin (50%) (Berlyn and Miksche, 1976) and colorless enamel was used for the coating. The following dyes were used in the histochemical tests: acid phoroglucine, to observe the lignified structures; Sudan III, to identify lipophilic compounds; ferric chloride, to provide evidence of phenolic compounds; Lugol's iodine, to detect starch; Dragendorff's reagent, to detect alkaloids; and hydrochloric acid (1%), to confirm the chemical nature of the crystals. The images were recorded in an Olympus CX 31 optical microscope attached to an Olympus c-7070 photographic camera. A structural analysis of the surface was conducted via scanning electron microscopy (SEM), with material fixed in FAA 70, dehydrated in an ascending ethanol series, and submitted to metallization in gold (Souza, 2007) by means of the Balzers CPD-010 equipment. The analysis was performed with a Tescan Vega 3 electron microscope.

Obtaining the extract, fractioning, and isolating the chemical compounds

After the plant material was dried at room temperature, sectioning was performed on the cutting mill. The milled material (800 g) was subjected to extraction in a Soxhlet apparatus, with 3 L ethanol (purity 96ºGL). After concentration in the rotary evaporator, the extract was fractionated with hexane in a modified Soxhlet apparatus (Carvalho *et al*., 2006), thus obtaining the hexane fraction. The hexane fraction was subjected to column chromatography using silica gel 60 Merck (0,063–0,200 mm) to isolate the compounds of interest. The mobile phase was a mixture of hexane and ethyl acetate, starting with 100% hexane, followed by the addition of ethyl acetate from 5% to 100%. Approximately 10 mL fraction was collected in each bottle. After evaporation of the collected eluates, bottle number 25 (called HF-25) was chosen owing to the presence of crystals. These crystals were treated with petroleum ether and placed in the freezer for purification, after which the supernatant was removed.

After drying, the crystals were solubilized in $CDCl_3$ containing 0.05% of tetramethylsilane (TMS), and subjected to spectroscopic analysis of ^1H and $^{13}C\{^1H\}$ NMR in an NMR Bruker DPX 200 instrument, equipped with a quadrinuclear 5 mm probe, for direct observation (^1H, ^{13}C, ^{19}F e ^{31}P) at ambient temperature, operating at 4.7 Tesla, and observations of the ^1H and ^{13}C nuclei at 200.13 and 50.62 MHz, respectively. The chemical shifts were expressed in ppm and referenced in relation to the TMS signal, used as an internal reference, at 0.00 ppm.

RESULTS AND DISCUSSION

Macroscopic description

Psychotria fractistipula is an herbaceous plant of 0.5-1.50 m in height (Figure 1A). Their leaves are 5.46 cm long and 2 cm wide (Figure 1B, C); they have an acute apex, a base attenuated petiole, an entire leaf margin, and display opposite phyllotaxis. The fruit is green when it is unripe and has an orange-reddish color when it is ripe (Figure 1A). It has persistent and apical stipules (Figure 1D, 1E).

FIGURE 1 - *Psychotria fractistipula* L.B.Sm. R.M. Klein & Delprete. **AB**: Appearance of aerial reproductive and vegetative organs. **C:** Appearance of leaves ab: abaxial surface, ad: adaxial surface. **D:** apical portion of the leaves ea: stipule apical, no in stem apex. **E**: Appearance of stem *e*p: persistent stipule.

Morpho-anatomical study

The surface of the leaf cuticle of *P. fractistipula* is smooth (Figure 2A). In contrast, *P. viridis* has a striated cuticle on both leaf surfaces, although, the cuticle is thicker on the adaxial surface (Martins *et al*., 2006). On the contrary, *P. stenocalix* has striated cuticle on the leaves subjected to greater sun exposure, and a smooth surface on the leaves growing in the shade, while *P. tenuinervis* also had striation on the shaded leaves (Gomes *et al*., 1995). It appears that cuticular striations in *Psychotria* may be related to solar incidence. As *P. fractistipula* typically occurs in shaded places, the smooth cuticle can be regarded as an important distinguishing characteristic.

The epidermis of the leaf fronds of *P. fractistipula*, in front view, exhibits polygonal cells with straight anticlinal walls on both the faces (Figure 2B, 2C). A similar pattern was found in *P. viridis* (Martins *et al*., 2006). However, this species differs from *P. fractistipula* because of the presence of a drusen in each cell and prismatic crystals. The epidermis of the leaf fronds of *P. fractistipula* also differs from that of *P. hoffmannseggiana* and *P. glaziovii*, which have cells with sinuous walls on the abaxial surface (Morais *et al.* 2011).

The leaf of *P. fractistipula* is hypostomatic, with paracytic stomata located at the same level as the other epidermal cells (Figure 2C, 2D). This kind of stoma and distribution pattern is a characteristic of Rubiaceae (Solereder, 1908, Metcalfe and Chalk 1957).

In *P. fractistipula* the trichomes are simple tectors, unicellular, and present on both surfaces, but particularly on the abaxial surface around the central vein (Figure 3A). According to Todzia and Almeida (2009), the size, shape, and basal difference of the trichomes are useful

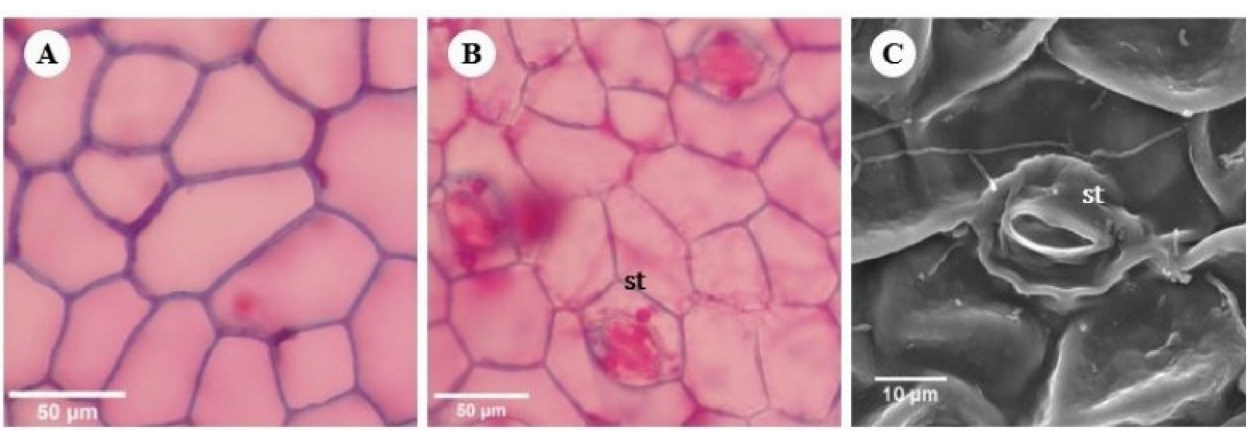

FIGURE 2 - *Psychotria fractistipula* L.B.Sm., R.M. Klein & Delprete – **A:** Adaxial surface of the epidermis, revealing anticlinal epidermal cell wall and smooth surface. **B:** Abaxial surface of the epidermis, revealing anticlinal epidermal cell wall, smooth surface, and stomata (st). **C:** Abaxial surface, showing stomata (st) (SEM).

features for the identification of the taxon. Although the Rubiaceae is described as having simple trichomes, which are unicellular and uniserial (Solereder, 1908, Metcalfe and Chalk 1957), some *Psychotria* are glabrous (*P. glaziovii*, *P. leiocarpa*, and *P. racemosa*) (Morais *et al*., 2011). The type of trichomes is a key factor in determining the quality of herbal drugs, since the latter are marketed in crushed or powdered form.

The mesophilic structure of the leaves of *P. fractistipula* is dorsiventral, since it only consists of one layer of palisade parenchyma, with cells that are slightly elongated, and contains about four layers of spongy parenchyma (Figure 3B). Dorsiventral mesophyll is common for the species of *P. hoffmannseggiana*, *P. carthagenensis*, *P. deflexa*, *P. glaziovii*, *P. leiocarpa*, *P. racemosa*, and *P. vellosiana* (Morais *et al*., 2011).

In transverse section, the central vein of the *P. fractistipula* leaf shows a plane shape on the adaxial surface, which is, however, prominent and rounded on the abaxial surface (Figure 3A, 3C). The terminal cells of the central vein of *P. carthagenensis* touch one another to form a cycle, which distinguishes the two species. The central vein is biconvex for *P. hoffmannseggiana*, *P. deflexa*, *P. glaziovii*, *P. leiocarpa*, *P. racemosa*, and *P. vellosiana* (Morais *et al*., 2011), differing from *P. fractistipula*, which has a convex plane, as in *P. carthagenensis* (Morais *et al*., 2011).

Underlying the epidermis collenchyma, an angular shape can be seen on both the surfaces. On the adaxial surface, the chlorophyll parenchyma is continuous in the nerval system (Figure 3A). The base of the leaf (midrib) has a larger collateral bundle in a horseshoe shape and two smaller bundles at the sides (Figure 3A, C). The collateral vascular bundle is typical of *Psychotria* (Morais *et al*., 2011).

Occurrence of raphides in the palisade parenchyma, as identified in *P. fractistipula*, are characteristic of the genus and have been already confirmed in *P. carthagenensis* (Todzia and Almeida, 1991), *P. hoffmannseggiana*, *P. deflexa*, *P. glaziovii*, *P. leiocarpa*, *P. racemosa*, and *P. vellosiana* (Morais *et al*., 2011).

P. fractistipula presents a small recess in the base of the main vein, formed due to the curvature of the vein next to the limbus (Figure 3A). This recess resembles the illustrated domains near the central rib for *P. deflexa*, which are also present in other species of *Psychotria* (Morais *et al*., 2011). However, further investigation may be necessary to determine the presence of domains.

The stem has an oval shape with some recesses in the side (Figure 4A). The epidermis is uniseriate with stomata, cuticle is striated, and unicellular trichomes are simple tector type (Figure 4B, D). The cortex has several layers of continuous angular collenchyma underlying the epidermis (Figure 4D). The vascular cylinder has xylem and concentric internal phloem, following the stem shape, with internal xylem and external phloem (Figure 4C).

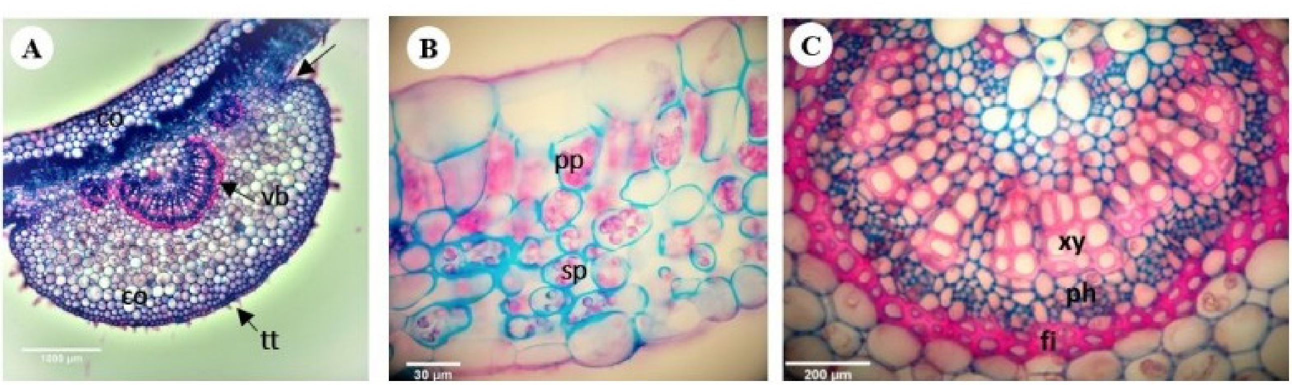

FIGURE 3 - *Psychotria fractistipula* L.B.Sm., R.M. Klein & Delprete **A:** Midrib, showing recesses at the base of the vein (arrow), epidermis with trichomes (tt), **B:** Cross-section of the leaves, revealing dorsiventral mesophyll. **C:** Vascular cylinder showing phloem, xylem, and fibers: tt: Tector Trichomes, vb: vascular bundle, co: collenchyma, sp: spongy parenchyma, pp: palisade parenchyma, fi: fiber, ph: phloem, and xy: xylem.

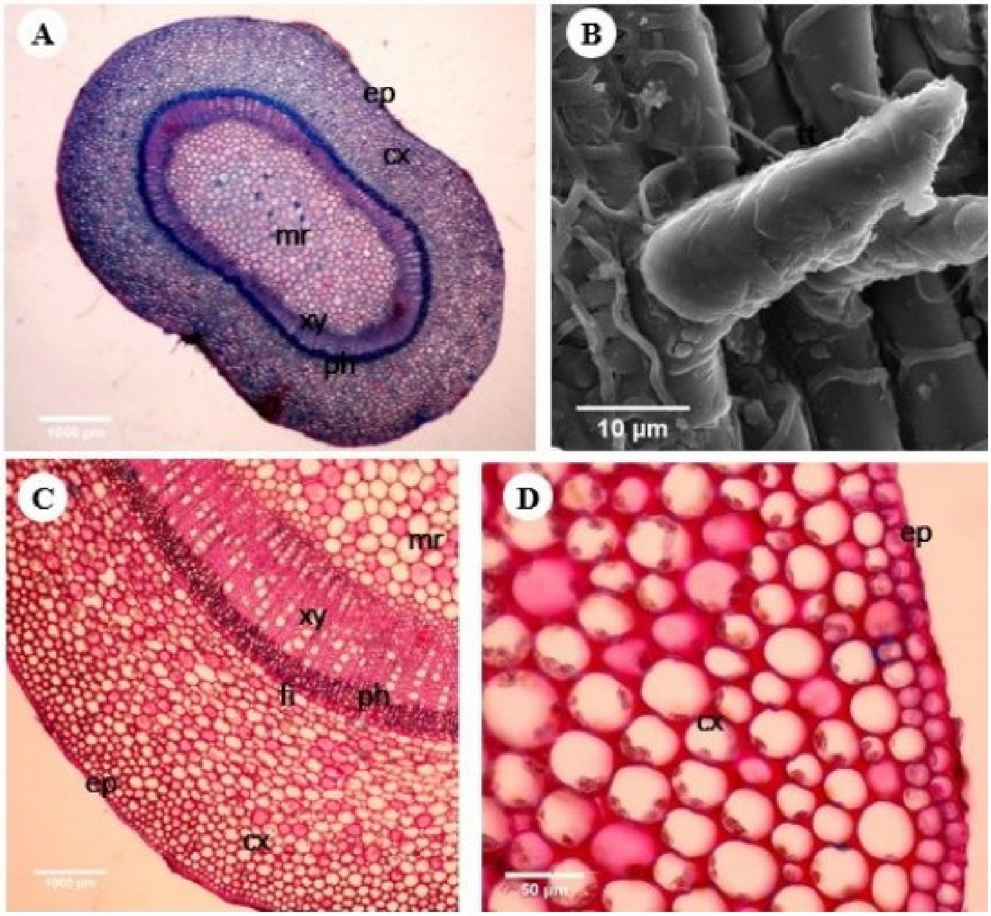

FIGURE 4 - *Psychotria fractistipula* L.B.Sm., R.M. Klein & Delprete stem in cross-section **A:** showing marrow (mr), cortex (cx), phloem (ph), xylem (xy), and epidermis (ep). **B:** Scanning electron microscopy showing trichomes (tt) **C:** Vascular system of the stem showing marrow (mr), cortex (cx), phloem (ph), xylem (xy), fiber (fi), and epidermis (ep). **D:** cortex (cx) and epidermis zoom.

Histochemical analysis

Histochemical tests are a fast and low-cost alternative method for obtaining evidence of a plant's possible chemical composition, as well as identifying the organ containing the substance of interest (Lusa, 2010). The results of the histochemical tests for leaves and stems are described in Table I.

The histochemical tests revealed the presence of lipids in the epidermis of *P. fractistipula*, concentrated mainly in the cuticle of the stem and in the leaves, similar to the distribution found in *P. carthagenensis* (Vitarelli, 2009). Phenolic compounds were observed in the epidermal cells, leaf mesophyll, and stem, and in the parenchymal palisade of the leaves. The stem was positive for starch. Starch and phenolic compounds have been reported in *P. nuda* and *P. nemorosa* (Hering-Rinnert *et al*., 20013), and starch has also been reported in the stem of *P. ipecacuanha* (Gomes *et al*., 2009).

TABLE I - Histochemical tests performed on the leaves and stems of *Psychotria fractistipula*

Reagent	Leaf	Stem
Starch	+	+
Lipophilic compounds	+	+
Lignin	+	+
Phenolic compounds	+	+
Alkaloids	+	+

Phytochemical study

Column chromatography produced 232 fractions. Fraction 25, which had a solvent mixture with 10% ethyl acetate, was chosen for further analysis owing to a precipitation of crystals. The mixture of compounds in this fraction produced needle shaped crystals.

The 1H NMR spectrum showed signals of hydrogen at 3.52 ppm, olefinic hydrogen H-6 at δH 5.35 ppm (J = 5.1 Hz, d), and accumulation of signals in the region from 0.60 ppm to 2.40 ppm assigned to an abundance of methylic hydrogens, methanics, and methylenics, all of which are characteristic signals of the β-sitosterol and campesterol steroids. Analysis of the $^{13}C\{^1H\}$ spectrum revealed signals of unsaturated carbons at 140.77 (C) and 121.72 (CH). Analysis was based on NMR spectral data of ^1H and $^{13}C\{^1H\}$ and was compared with data found in the literature (Andrade, 2003) (Table II). The mixture of compounds was found to comprise two steroids, β-sitosterol and campesterol (Figure 5).

TABLE II - $^{13}C\{^1H\}$ NMR SPECTROSCOPIC DATA OF THE STEROIDS β–SITOSTEROL AND CAMPESTEROL

^{13}C	β -Sitosterol		Campesterol	
	δ_C experimental	δ_C literature[1]	δ_C experimental	δ_C literature[2]
1	37.2	37.2	37.2	36.9
2	31.6	31.8	31.6	34.3
3	71.8	71.7	71.8	72.2
4	42.3	42.2	42.3	42.7
5	140.8	140.5	140.7	141.1
6	121.7	121.5	121.7	122.1
7	31.9	31.8	31.9	28.7
8	31.9	31.6	31.9	32.8
9	50.1	50.0	50.1	50.5
10	36.5	36.4	36.5	32.3
11	21.0	21.0	21.0	21.5
12	39.8	39.7	39.8	37.6
13	42.3	42.2	42.3	40.1
14	56.8	56.7	56.7	57.2
15	24.3	24.3	24.3	23.4

(continuing)

TABLE II - $^{13}C\{^{1}H\}$ NMR SPECTROSCOPIC DATA OF THE STEROIDS β–SITOSTEROL AND CAMPESTEROL

^{13}C	β -Sitosterol		Campesterol	
	δ_C experimental	δ_C literature[1]	δ_C experimental	δ_C literature[2]
16	28.2	28.2	28.2	26.4
17	56.1	55.9	56.1	56.4
18	11.8	11.8	11.8	12.3
19	19.4	19.4	19.4	19.8
20	36.1	36.1	36.1	36.6
21	19.0	18.8	19.4	19.4
22	33.9	33.8	31.9	32.3
23	29.6	26.0	26.2	24.7
24	45.8	45.7	45.8	46.2
25	31.6	29.1	29.2	29.5
26	21.0	19.0	18.7	20.2
27	19.8	19.8	19.4	19.2
28	18.7	23.0	23.1	12.4
29	11.8	11.9	-	-

[1] $^{13}C\{^{1}H\}$ NMR data acquired in NMR 400 MHz ($CDCl_3$) in according to Al-Fadhli and Nasser (2014).
[2] $^{13}C\{^{1}H\}$ NMR data acquired in NMR 300 MHz ($CDCl_3$) in according to Elufioye et al. (2017).

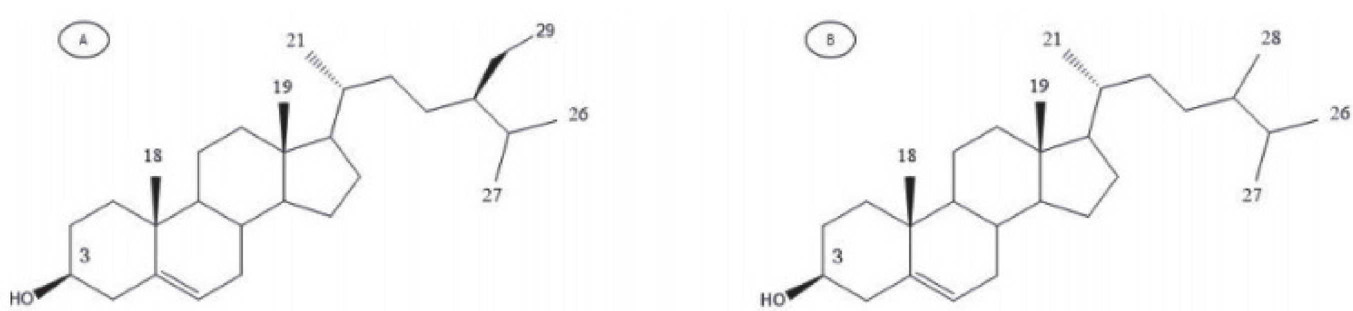

FIGURE 5 - Steroids identified in *Psychotria fractistipula* L.B.Sm., R.M. Klein & Delprete **A:** β–Sitosterol **B:** Campesterol

The β–Sitosterol compound has already been identified in *P. adenophylla*, *P. hainanensis*, *P. malayana* (Calixto *et al*., 2016), *P. vellosiana* (Moreno *et al*., 2014), *P. carthagenensis* (Lopes *et al*., 2000), and *P. nilgiriensis* (Lopes *et al*., 2000). The campesterol steroid has been identified in *P. nilgiriensis* (Lalitha *et al*., 2015). The sterols, campesterol and β–Sitosterol, show several biological activities, including antimicrobial, anti-inflammatory, anticancer, antiarthritic, anti-asthma, and diuretic activities (Lopes *et al*., 2000).

CONCLUSION

The morpho-anatomical characters described for *P. fractistipula* are leaves petiolate, with acute apex and base attenuated in petiole, presence of trichomes with stems and leaves, smooth cuticle on both surfaces, paracytic stomata, dorsiventral mesophyll. By means of ^{1}H and ^{13}C NMR, β-sitosterol and campesterol were identified. The results obtained in this study will help in the identification, standardization, and quality control of cultivated *P. fractistipula*, and encourage further chemical and biological studies on the taxon.

ACKNOWLEDGEMENTS

The authors are grateful to the Federal University of Paraná, Coordination for the Improvement of Higher Education Personnel (CAPES), Financier of Studies and Projects (FINEP), and National Council for Scientific and Technological Development (CNPQ) for financial support.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

REFERENCES

Al-Fadhli AA, Nasser JA. Constituents from the Root of *Lantana camara.* Asian J. Chem. 2014;26(23):8019-8021.

Andrade MR. Alcalóides de Rutaceae: Química e atividade biológica. Tese (Doutorado). UFSCar. São Carlos; 2003.

Barros MAA. Ocorrência das domácias na Família Rubiaceae. An. Esc. Super. Agric. Luiz de Queiroz 1959;16:331-337.

Berlyn GP, Miksche JP. Botanical microtechnique and cytochemistry. Ames: Iowa State University, 1976. p.121-276.

Calixto NO, Pinto MEF, Ramalho SD, Burger MCM, Bobey FA, Young MCM, Bolzani VS, Pinto AC, The genus *Psychotria*: phytochemistry, chemotoxanomy, ethnopharmacology and biological properties. J. Braz. Chem. Soc. 2016;27(8)1-24.

Carvalho JLS, Miguel MD, Miguel OG, Dadalt, RC. PI 0601703-7A Processo de obtenção de extratos hidroalcoólico, extratos secos e derivados do agrião (*Nasturtium officinale*) e espécies medicinais afins, com modificações introduzidas em equipamento Soxhlet para aplicações na indústria, área farmacêutica, cosmética, alimentícia e afins, Brasil. 2006.

Elufioye TO, Obduotor, EM, Agbedahunsi JM, Adesanya AS. Anticholinesterase constituents from the leaves of *Spondias mombin* L. (Anacardiaceae). Biologics: Targets and Therapy 2017;11:107-114.

Gomes RSDL, Oliveira VDC, Jácome RLRP, Pinto JEBP, Lameira OA, Barros AMD. Morphoanatomy comparative study between "poaia" (*Psychotria ipecacuanha* (Brot.) Stokes - Rubiaceae) from the Amazon region (original *habitat)* and from biotechnology process, subjected to different treatments of interception of solar radiation. Rev. Bras. farmacogn. 2009;19(1):276-283.

Hering-Rinnert C, Brüske JJ, Schegoscheski V, Alecrin DA. Caracterização estrutural e histoquímica de duas espécies de *Psychotria* (Rubiaceae) encontradas na mata atlântica. 64 ºCNBot. 2013.

Karao S, Tchacondo T, Ilboudo DP, Simpore J. Sub-Saharan Rubiaceae: A rewiew of their traditional uses, phytochemistry and biological activies. Pakistan J. Biol. Sci. 2011;14(3):149-169.

Lalitha S, Parthipan B, Mohan VR. Determination of bioactive componentes of *Psychotria nilgiriensis* Deb & Gang (Rubiaceae) by CG-MS Analysis. Int.. J. Pharmacogn. and Phytochem. Res. 2015;7(4):802-809.

Lopes SO, Moreno PRH, Henriques AT. Growyh characteristics and chemical analysis of *Psychotria caerhagenensis* cell suspension cultures. Enzyme and Microb Tecnol. 2000;26(2-4):259-264.

Lusa MG. Caracterização morfoanatômica e histoquímica de *Cupea cartaginenses* (Jacq.) J.F. MACBR.(Lythraceae) e avaliação em ambientes hidrofítico e mesofítico. 62f. Dissertação (Mestrado em Botânica). Universidade Federal do Paraná, Curitiba. 2010.

Martins M, Costa DA, Teixeira DC, Garcia J, Silva DA. Anatomia foliar de *Psychotria viridis* Ruiz & Pav. (Rubiaceae). Revi. Univ. Rural. 2006;26(2):30-41.

Gomes DMS, Mantovani A, Vieira RC. Anatomia foliar de *Psychotria tenuinervis* Muell. Arg. e *Psychotria stenocalix* Muell. Arg. (Rubiaceae). Arq. de Biol. e Tecn.. 1995;38(1):15-33.

Martínez-Cabrera HI, Jones CS, Espino S, Schenk HJ. Wood anatomy and wood density in shrubs: Responses to varying aridity along transcontinental transects. Am. J. Bot. 2009;96(8):1388-1398.

Todzia CA, Almeida F. A revision of *Tibouchina* sect. *Lepidotae* (Melastomataceae: Tibouchinae). Proc. Calif. Acad. Sci. 1991;47(6):175-206.

Metcalfe CR, Chalk L. Anatomy of the dicotyledons. Clarendons press. Oxford. 1979.

Moraes TMS, Rabelo GR, Alexandrino CR, Silva SJN, Cunha M, Comparative leaf anatomy and micromorphology of *Psychotria* species (Rubiaceae) from the Atalantic Rain florest. Acta. Bot. Bras. 2011. p. 168-177.

Moreno BP, Fiorucci LLR, Carmo MRB, Sarragiotto MH, Baldoqui DC. Terpenoids and a coumarin from aerial parts of *Psychotria vellosiana* Beth. (Rubiaceae). Bioch. Syst. and Ecol. 2014;56:80-82.

Oliveira F, Akisue G. Fundamentos de farmacobotânica. 2. Ed., São Paulo, 2000. 178 p.

O'Brien TP, Feder N, Mccully ME. Polycromatic Staining of plant cell Walls by toluidine blue O. Protopl. 1964;59(2):368-373.

Oliveira CF, Oliveira VB, Oliveira FF, Miguel OG, Miguel MD. Quality control parameters of *Psychtria fractistipula* L. B. Sm., Klein & Delprete (Rubiaceae): Loss on drying, total ash and phytochemical screening. Vis. Acad. 2014;15(4):17-23.

Souza, W. Técnicas de microscopia eletrônica aplicadas às Ciências Biológicas. Sociedade Brasileira de Microscopia, Rio de Janeiro 2007.

Souza VC, Lorenzi H. Botânica Sistemática: Guia ilustrado para identificação das famílias de angiospermas da flora brasileira, naseado em APG II. Instituto Plantatrum. Nova Odessa, 3. Ed., 2005. 640 p.

Solereder H. Systematic anatomy of the dicotyledons. Vol. II. Clarendon Press, Oxford, 1908. 1182 p.

Taylor CM, Campos MTVA, ZAPPI D. Flora da reserva ducke, Amazonas, Brasil: Rubiaceae. Rodriguésia. 2007;58(3): 549-616.

Taylor C, Gomes M, Zappi D, Rubiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. 2015. Disponível em: http://www.floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB14153. Acesso em: 16 Jan. 2015.

Vieira RC, Gomes DMS, Ferraz CLA. Anatomia foliar de *Psychotria nuda* Wawra e *Psychotria leicarpa* Mart. (Rubiaceae). Hoehnea. 1992;19(1/2):185-195.

Vitarelli NC. Anatomia de estípulas e coléteres de *Psychotria carthagenensis* Jacq. (Rubiaceae). Acta. Bot. Bras. 2009;23(4):923-928.

Received for publication on 05th March 2018
Accepted for publication on 18th April 2019