Toxicological and anatomical study of vegetative organs of *Anthurium maricense* Nadruz and Mayo (Araceae)

André Luis de Alcantara Guimarães[1]
Patrique Riepe do Tanque[2]
Alexandre dos Santos Pyrrho[3]
Ana Cláudia de Macêdo Vieira[4]

Abstract

Among other taxa of Araceae, the genus *Anthurium* is the largest of the family and one of the most important taxa. Some species of this genus have been used as a condiment, and its plants as ornaments and medicines for over a century. *Anthurium maricense* is an endemic species from "restingas" of Rio de Janeiro, Brazil, and it is used as ornamental and medicinal plant. This paper aimed an anatomical and toxicological study of its vegetative organs. *A. maricense* has subterraneous rhizome, adventitious roots and complete leaves. The anatomical features of vegetative organs are similar to other species of Araceae and genus *Anthurium*, especially the velamen in roots and calcium oxalate crystals in all organs. Under scanning electron microscopy (SEM), longitudinal grooves were observed in the raphides. For toxicological analyses, whole (with crystals) and centrifuged (free from crystals) fractions of the juices of each organ were administered via mouthwash and plantar inoculation to groups of five mice. The formation of edema was observed at 1, 3, 6, 24 and 96 hours and 7, 14, 21 and 28 days. The oral inoculations presented negative results for all groups, without edema. The plantar inoculations showed differing results: with centrifuged juices, mild edema formed but regressed; with whole juices, some animals presented severe edema. Our results indicated distribution and morphology of raphides and diverse chemical substances were related to the edematogenic process. *A. maricense* may potentially induce more chronic edema if compared to other species of Araceae.

Keywords: Anatomy. Crystals. Restinga. Toxicity.

Introduction

The use of ornamental plants in urban areas is based on aesthetic and habit characteristics and their adaptation, availability and costs to obtain. Nevertheless, toxicological aspects of these plants are seldom studied or even considered before their use as ornamental plants. Additionally, most of the human victims are children under ten years old, which represent more than 63 % of all registered

1 Universidade Federal do Rio de Janeiro, Laboratório de Farmacobotânica, Faculdade de Farmácia. andreguimaraes@pharma.ufrj.br.
2 Universidade Federal do Rio de Janeiro, Laboratório de Farmacobotânica, Faculdade de Farmácia. patriqueriepe@gmail.com.
3 Universidade Federal do Rio de Janeiro, Laboratório de Imunoparasitologia e Análises Toxicológicas, Faculdade de Farmácia. pyrrho@pharma.ufrj.br.
4 Universidade Federal do Rio de Janeiro, Laboratório de Farmacobotânica, Faculdade de Farmácia. acmvieira@pharma.ufrj.br. Av. Carlos Chagas Filho, 373, CCS, Bloco A, 2º andar, sala 22, Cidade Universitária, Rio de Janeiro, Brasil, CEP: 21.941-902.

poisoning cases in Brazil (BRASIL, 2016). The lack of knowledge regarding toxicological aspects of plants in private or public gardens is the main factor for accidental poisonings (SCHVARTSMAN, 1992; MATOS et al., 2011; BOCHNER et al., 2013). Furthermore, the indiscriminate use of ornamental plants as a therapeutic resource in folk medicine, with no concern regarding their possible toxicity, reinforces the importance of studying the toxicological traits of these plants (LORENZI; MATOS, 2008; MATOS et al., 2011; BOCHNER et al., 2012).

Araceae is a family with 109 genera and 2.830 species distributed worldwide (JUDD et al., 2009). Approximately 35 genera and 400 species occur in Brazil (SOUZA; LORENZI, 2005). Growth forms and habitat variety are greater than in any other monocot family, including vines or large herbs in tropical habitats (KEATING, 2002). Species of *Anthurium, Alocasia, Caladium, Colocasia, Dieffenbachia, Monstera, Philodendron* and *Spathiphyllum* are often used as ornamental and mystic plants in public and home gardens in Brazil, despite being toxic for humans and pets (SILVA; ANDRADE, 2005; PASA et al., 2005; FELIPPE, 2009). Some original studies identified the mechanism of intoxication of two common ornamental plants, *Dieffenbachia picta* and *D. seguine*, due to a large number of poisoning cases (RIZZINI; OCCHIONI, 1957; DRACH; MALONEY, 1963). Since then, there have been reports in the scientific literature of accidental poisonings with Araceae species involving, in most cases, children and pets, which highlights the importance of proper identification of toxicological properties of such species.

Among other taxa of Araceae, the genus *Anthurium* is the largest of the family and one of the most important taxa, with approximately 900 species (JUDD et al., 2009), of which several new species were registered in Brazil (COELHO; MAYO, 2000; COELHO, 2006; COELHO; CATHARINO, 2008; COELHO, 2010; TEMPONI; COELHO, 2011; GONÇALVES, 2012). The species of this genus are distributed in the American tropics, from northern Argentina to Mexico, and it consists of terrestrial or epiphytic rain forest plants (DAHLGREN et al., 1985). Many aspects of the biology of *Anthurium* species have been investigated, including taxonomy, chemistry (AQUINO et al., 2001), ecology and anatomy (LORENZO et al., 2010; POLI et al., 2012). Nevertheless, to the best of our knowledge, toxicological aspects of *Anthurium* remain mostly unknown (TWARDOWSCHY et al., 2007).

Some species of this genus have been used in traditional medicine for over a century in the tropical parts of the Americas and the West Indies for the treatment of different diseases (JOLY et al., 1987; ZAMORA-MARTÍNEZ; POLA, 1992), some of them associated with immunostimulant activity. In the northeastern Brazil, *Anthurium affine* Schott. has been used in folk medicine for cardiac problems, circulatory diseases and diabetes (AGRA et al., 2008). Native tribes in northwestern South America also used parts of some species of *Anthurium* and other genera as female contraceptives (DUKE; VASQUEZ, 1994). In Brazil, Corrêa (1984) registered the traditional use of several species of this genus as a condiment and as ornamental and medicinal plants for over a century. The author also highlighted that all species of the genus were commonly misdiagnosed as "*Anthurium"* in folk medicine and other traditional uses (CORRÊA, 1984). This confusing popular designation to all species of the genus is dangerous, especially in folk medicine, since different species may be erroneously used as medicinal plants.

Anthurium maricense Nadruz and Mayo is a psammophyte, which grows on sandy substrates in restingas of Rio de Janeiro State (COELHO; MAYO, 2000). This species exhibit leaves and reproductive organs with extensive ornamental value. However, to the best of our knowledge, there is no record of any study investigating the anatomy and toxicity of this species or even other species of *Anthurium*. Thus, to bridge this gap, the aim of our study is to evaluate the toxicity of vegetative organs of *A. maricense*. Additionally, we also studied the anatomy of these organs to characterize

the structures related to the mechanism of intoxication and provide additional characters to aid the identification of this species.

Material and methods

Plant material

The botanical material and field data were obtained in Barra de Maricá Environmental Protection Area, in Rio de Janeiro State, Brazil. Specimens of *A. maricense* with roots, stems and leaves were collected in January 2013. Vouchers were properly cataloged and deposited in the Herbarium of the Institute of Biology (RFA) at the Federal University of Rio de Janeiro (UFRJ), under registrations RFA 34605 and RFA 34606.

Morphology and anatomy

Samples of vegetative organs were fixed in formalin (LILLIE, 1948 apud CLARK, 1981) or FAA_{50} (BERLYN; MIKSCHE, 1976). For anatomical studies, we used plant material fixed in FAA_{50} (KRAUS; ARDUIN, 1997). Transversal sections were obtained from samples of roots, stems and leaves. The fixed samples were embedded in polyethylene glycol 1500 (ISOFAR, Rio de Janeiro, Brazil) in triplicates, and then cut into 20 μm thick sections with a rotative microtome (KRAUS; ARDUIN, 1997) and stained with 1% astra blue and 1% safranin (9:1, v/v) (BUKATSCH, 1972). The root fragments did not resist the embedding process. For this reason, transversal sections were obtained from samples located about 3 cm from root apex with a Ranvier microtome. Additionally, paradermal sections were also obtained from samples of leaves with a razor blade. The terminology used in the descriptions follows the proposals of Radford et al. (1974) and Keating (2002). All anatomical analyses and imaging studies were performed using optical microscopes Olympus CH30RF100 connected to photographic equipment Olympus PM-PBK-3 with Kodak Gold ASA 100 film and Zeiss Axio Scope A1 with polarized light.

The histochemical analysis was performed in sections of fresh material with the aid of Ranvier microtome for the detection of lipids, starch and evaluation of the chemical nature of the crystals. For lipid detection, the sections were immersed in a saturated solution of Sudan IV (CI 26100) in 70° GL ethanol (SASS, 1951). For detection of phenolic compounds, the sections were immersed in a solution of ferric chloride (JOHANSEN, 1940). For starch detection, the sections were immersed in Lugol reagent (iodine potassium iodide) for 5 min (JOHANSEN, 1940). For detection of proteins, the sections were immersed in a solution of mercury chloride 1% and Bromophenol blue 0, 1% (MAZIA et al., 1953). For the demonstration of calcium oxalate crystals, we used the Pizzolato method ($AgNO_3$-H_2O_2) (SILVER; PRICE, 1969). For the controls, lipids were extracted with methanol: chloroform (1:1, v/v), starch with salivary amylase and phenolic compounds with 5% potassium dichromate for 24 h (REEVE, 1959). Blank sections were used for comparative analysis.

Scanning Electron Microscopy (SEM) images were made with samples previously fixed in paraformaldehyde and glutaraldehyde in phosphate buffer (KARNOVSKY, 1965), dehydrated in ethanolic series and critical point dried with carbon dioxide at 73 atm and 35 °C. The samples were mounted on metal stubs with double-sided adhesive tape and sputter coated with 30-35 nm of gold at 6.10^{-2} atm (SILVEIRA, 1989). Specimens were observed in a Jeol-5310 microscope.

For the observation of the crystals in SEM, fragments of each organ were submitted to a maceration process (FRANKLIN, 1945). The macerate was centrifuged at 2600G for 5 minutes (CARNEIRO et al., 1989), and the precipitate was kept in Ethanol 100 % for later kiln drying without airflow at 50 ºC. The samples were mounted for observation as previously described.

Toxicological analysis

In the pharmacological tests for toxicity in animals, we used juices from the fresh vegetative organs. Parts of each organ were triturated in a food processor and then pressed and cloth filtered. The juices were used in two fractions, whole and centrifuged at 3.800G for 5 minutes to remove the crystals. Each fraction of the juices from the 3 organs (root, stem and leaf) was administered once to a group of 5 animals (female Swiss mice, 8 weeks old and weighing 20±2g) using an insulin syringe, in two ways: mouthwash (100μL, without needle) and plantar inoculation (50μL, with needle). The control was made with sterile 0,85 % NaCl solution. After the mouth washings, the animals received water and food ad libitum only one hour after the procedure. We observed the formation of edema to evaluate the toxicity of each juice. All administrations were made on the same day of the preparation of the juices.

For classification of the edema, a level chart was created, due to the expressivity of results: level I (light swelling, discrete color change); level II (bigger swelling than level I, with reddening); and level III (great swelling, with darker bruising and significant increase in paw size). The follow-up was documented at 1, 3, 6, 24 and 96 hours and 7, 14, 21 and 28 days after the inoculations, in order to register the acute and chronic toxicity (BRITO, 1994). The experiments with animals were conducted according to the ethical protocol by the Ethics Committee for Animal Use of the Health Sciences Center (CCS-UFRJ - protocol number DBFCICB032).

Results and discussion

Results

A. maricense grows in “restingas”, in open areas and within shrub vegetation. It exhibits a thick rhizome with adventitious roots and dark green leaves with long leaf blade, petiole and sheath. The leaves present lanceolate shape, obtuse base, entire edge and an acute apex. The leaf veins are reticulated, and the midrib is very distinct. It has spathe inflorescences with bisexual flowers, and its fruits are reddish-orange berries, typical of the species. Figure 1 shows a fertile specimen in its natural habitat.

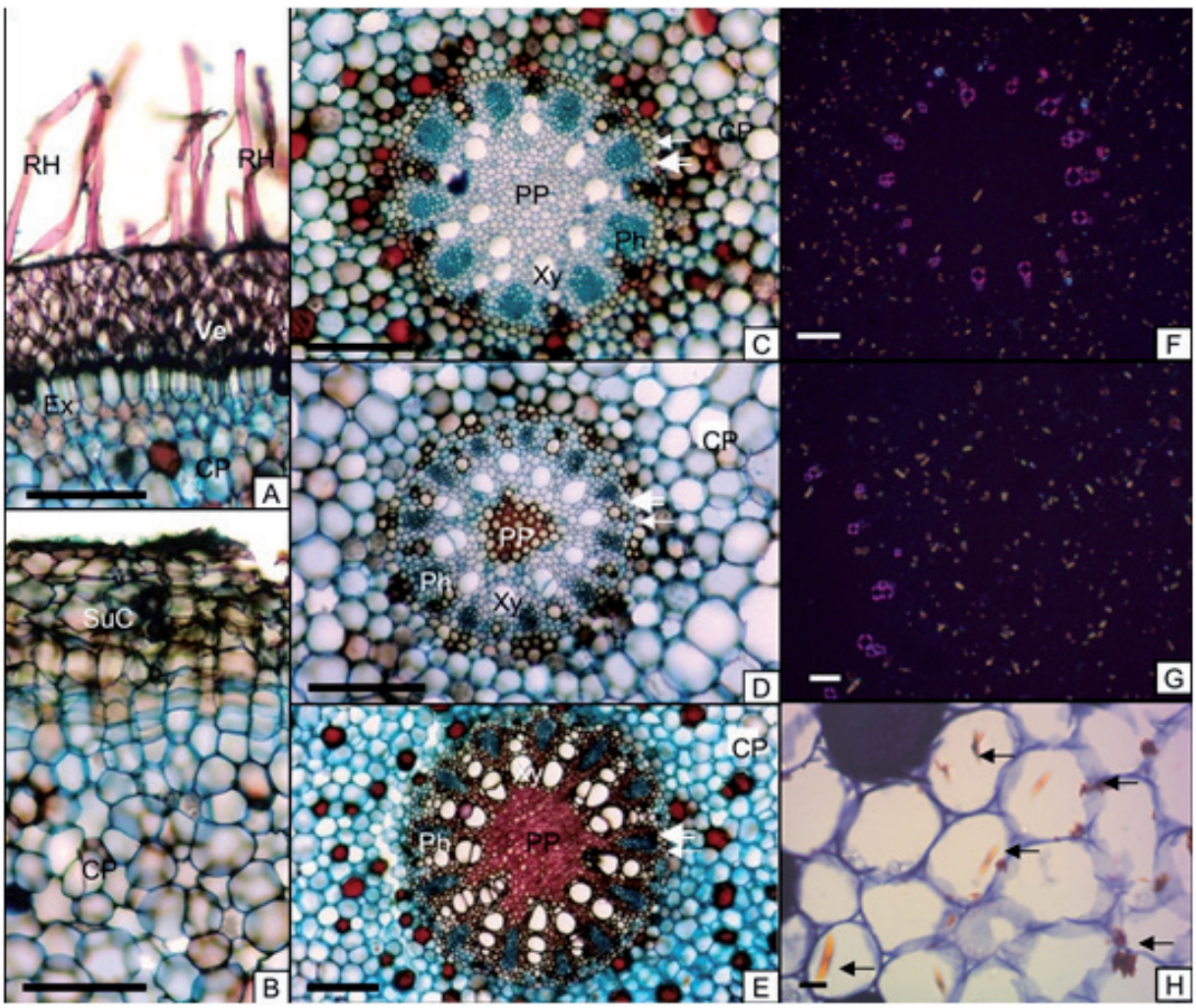
Figure 1. *Anthurium maricense* in its natural environment, the environmental protection area of restinga of Barra de Maricá, Rio de Janeiro State, Brazil.

Source: Vieira (2013).

Anatomical description

The species has adventitious roots originated from the rhizome. They are long and light brown colored. In the portions closer to the apex, the coating tissue is the velamen (FIGURE 2A), with three to five layers of cells with helical thickening of their walls. Unicellular root hairs were found, originated from the outermost layer of the velamen (FIGURE 2A). Exodermis is found beneath velamen. It consists of oblong to columnar cells, thin-walled with Casparian strips (FIGURE 2A). The cortex consists of ground parenchyma cells with an elliptical shape and variable size (FIGURES 2A-E). Idioblasts with phenolic compounds and starch are common, especially in the innermost cell layers. Isolated raphides are abundant in almost all parenchyma cells (FIGURES 2F-H), and drusa are scarce. Proteins and lipids are scattered throughout the cortex. Endodermis consists of elliptical to oblong cells with thick walls (FIGURES 2C, D, E). We noticed passage cells opposite xylem poles. Pericycle is formed by one layer of elliptical thin-walled cells. The stele consists of nine to thirteen xylem and phloem poles (FIGURES 2C, D, E). The center of stele exhibits several layers of small parenchyma cells with thin-walls in the apical portion (FIGURES 2C). We observed sclerification of parenchyma cells towards the rhizome (FIGURES 2D, E). In the portion closer to stem, the velamen is replaced by suberized cork, originated from the parenchyma cells adjacent to the exodermis (FIGURE 2B).

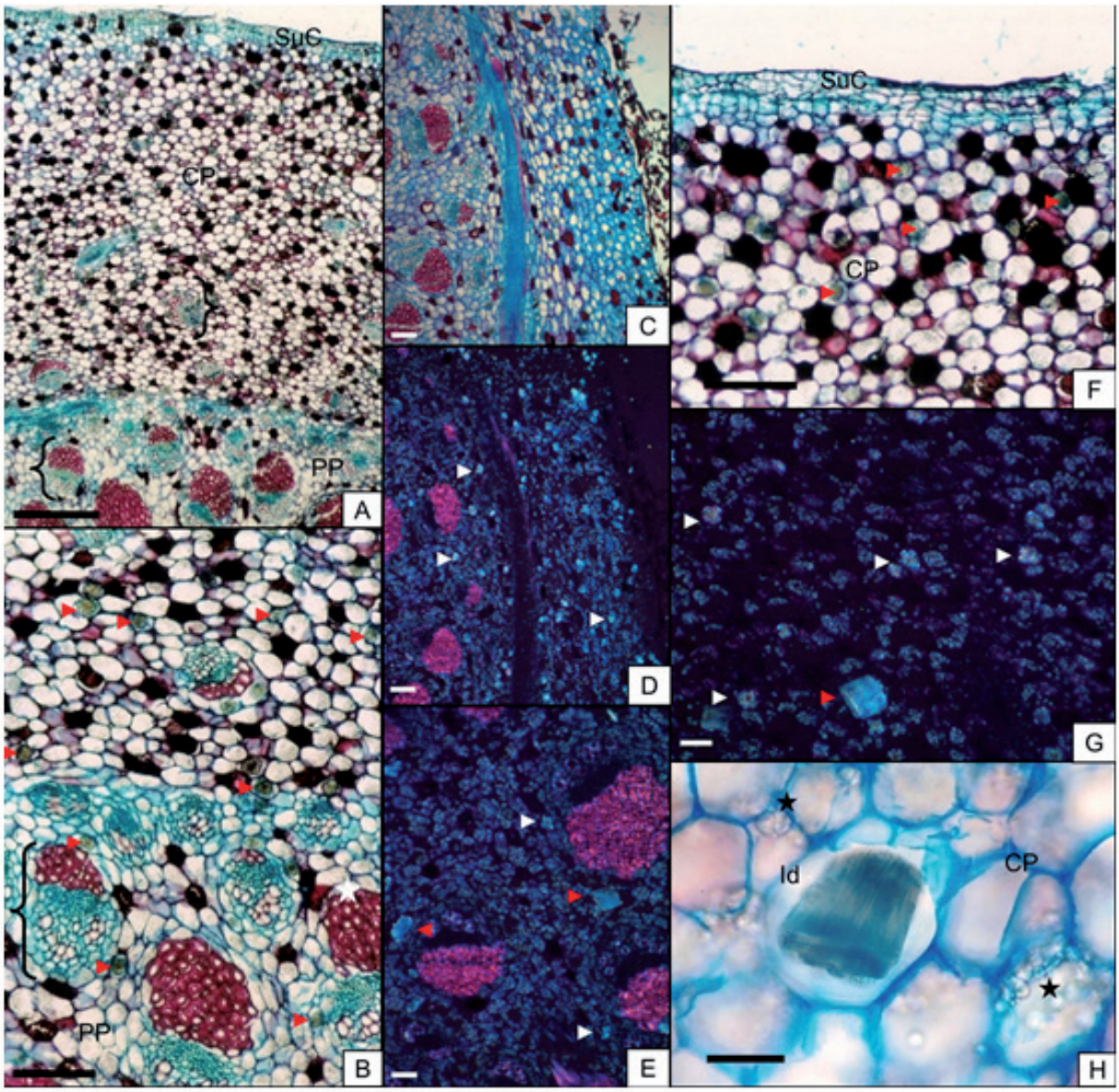
Figure 2. *Anthurium maricense.* Light micrographs of anatomy of roots. (A) Transverse section (TS) of the portion close to the apex, showing velamen with root hairs and the exodermis beneath. (B) TS of a section close to the stem (rhizome). Note the velamen is replaced with suberized cork. (C) to (E) TS of the center of root from portions close to the apex (C), middle (D), and close to the rhizome (E). Note the development of estele in different portions, showing sclerification of parenchyma towards the rhizome. (F-G) Tranverse section under polarized light, showing raphides scattered throughout the cortex. (H) Details of isolated raphides in parenchyma cells from the cortex. Key: RH= Root hairs; Ve= Velamen; Ex= Exodermis; CP= Cortical Parenchyma; Ph= Phloem; Xy= Xylem; PP= Pith Parenchyma; SuC = Suberized Cork; Single Arrow = Endodermis; Double Arrow = Pericycle. Dark Arrow = Raphides. Bars: 200 μm (A-G), 50 μm (H).



Source: Guimarães (2017)

The stem of *A. maricense* is a subterraneous rhizome, with dark brown color, and it is covered with fibrous cataphylls. The nodes are distinct, and the internodes are extremely short. The transverse sections of the internode region showed suberized cork with three to five layers covering the entire surface (FIGURES 3A, C, F). The cortex consists of ground parenchyma with sparse vascular bundles (FIGURE 3A), and several collateral vascular bundles with groups of sclerenchyma cells are scattered throughout ground tissue (FIGURES 3B-E). Starch and idioblasts containing druses, raphides and phenolic compounds are abundant in the whole organ (FIGURES 3B-H). Proteins and lipids are scattered throughout parenchyma tissues.

Figure 3. *Anthurium maricense.* Light micrographs of anatomy of rhizomes. (A) and (B) Transverse section (TS) of the rhizome, showing the dermal tissue, cortex and pith. Note the vascular bundles with fibers capping phloem in the center and the presence of idioblasts with crystals among parenchyma cells from the cortex. (C) TS of rhizome showing the cortex and inner region. (D-E) TS under polarized light showing the abundance of starch and idioblasts with drusa and raphides scattered throughout the organ. (F) TS of the dermal tissue (suberized cork) and cortex with idioblasts with crystals. (G) Details of the same region under polarized light revealing the abundance of starch and idioblasts with crystals. (H) TS of cortex, showing idioblast with calcium oxalate raphides and cells containing starch grains. Key: SuC= Suberized Cork; CP= Cortical Parenchyma; Braces = Vascular Bundle; PP= Pith Parenchyma; Sc= Schlerenchyma; Ph= Phloem; Xy= Xylem, Id= Idioblast; White Arrow heads = Druses; Red Arrow heads = Raphides Stars = Starch Grains. Bars: (A, C, D, E): 500μm; (B, F, G): 200μm; (H): 50μm.



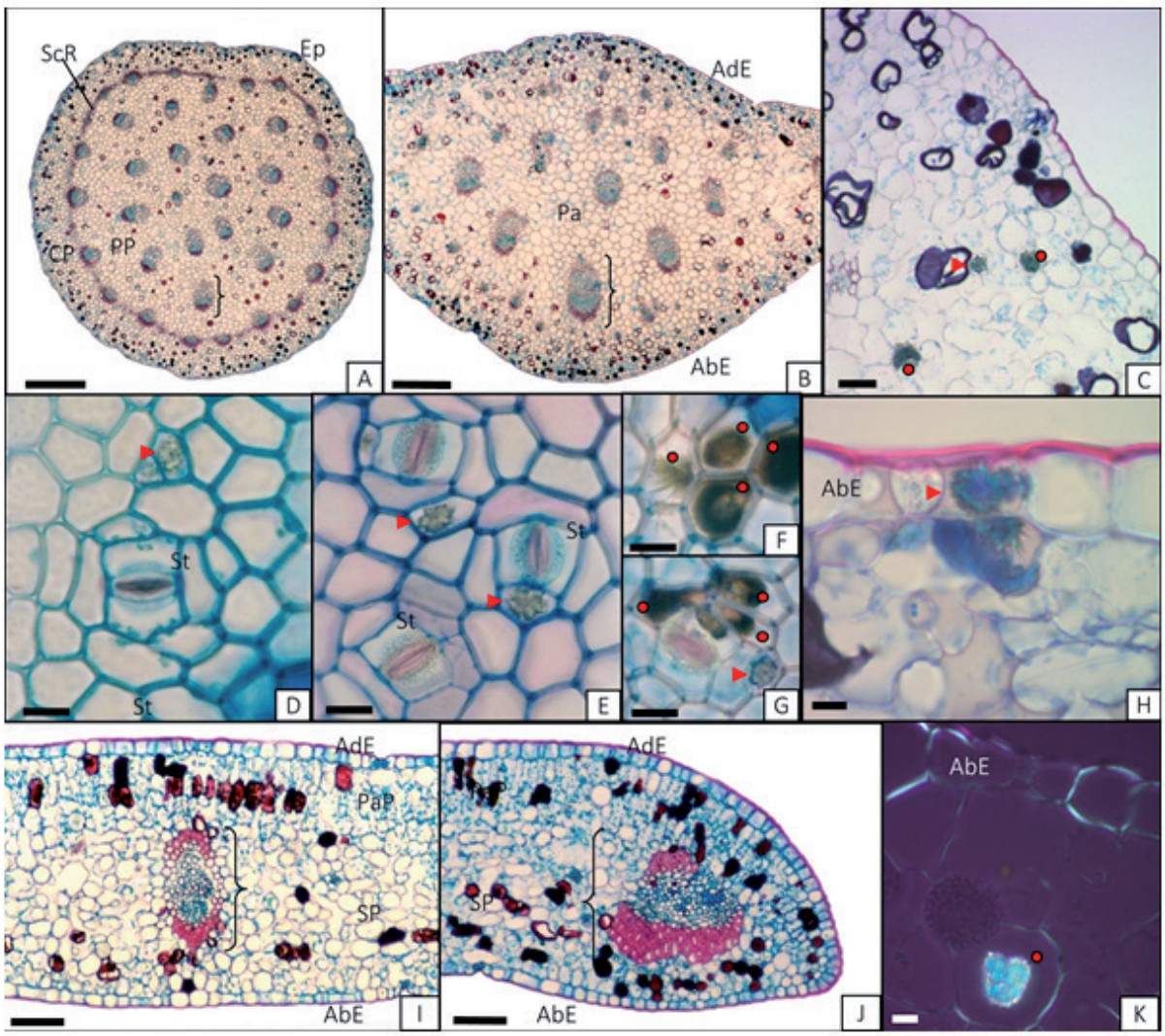
Source: Guimarães (2017)

In the middle portion of the petiole (FIGURE 4A), we observed one layer of epidermal cells coated with conspicuous cuticle. The cortex consists of ground parenchyma, with idioblasts containing druses and raphides in the outermost layers. The central cylinder is mostly compact with large and thin-walled parenchymatous cells. Collateral vascular bundles with fiber strands capping phloem are scattered throughout the ground tissue. We observed an interfascicular ring of fibers connecting the outer circle of bundles. Idioblasts containing druses are abundant, especially in the cortex.

In the midrib region (FIGURES 4B, C, H), we observed uniseriate epidermis in both surfaces, which periclinal cell walls are coated with thick cuticle. The mesophyll consists of parenchyma and collateral vascular bundles either ensheath by fibers or with fiber strands capping phloem. Idioblasts containing druses and raphides are found in the outer cell layers and epidermis (FIGURES 4C-H, K). Paradermal and transverse sections and observations on SEM showed paracytic stomata (FIGURES 4C-D, 5A-B) and idioblasts containing druses and raphides in both adaxial and abaxial epidermis (FIGURES 4D-H).

The leaf blade exhibits uniseriate epidermis in both surfaces, with conspicuous cuticle (FIGURES 4G and 4H). Epidermis showed ordinary cells with polygonal shape and paracytic stomata in both abaxial and adaxial leaf surfaces. Idioblasts with druses and raphides were also present. The mesophyll consists of 2-3 layers of palisade parenchyma and 15-20 layers of spongy parenchyma (FIGURES 4I-J). Several idioblasts containing druses and raphides are present in both palisade and spongy parenchyma. Vascular bundles are observed in the center of the mesophyll, with fiber strands capping phloem and xylem (FIGURES 4I-J). In the leaf margins, we observed larger collateral vascular bundles with larger fiber strands (FIGURES 4J). Proteins and lipids are scattered throughout parenchyma cells of the leaf blade.

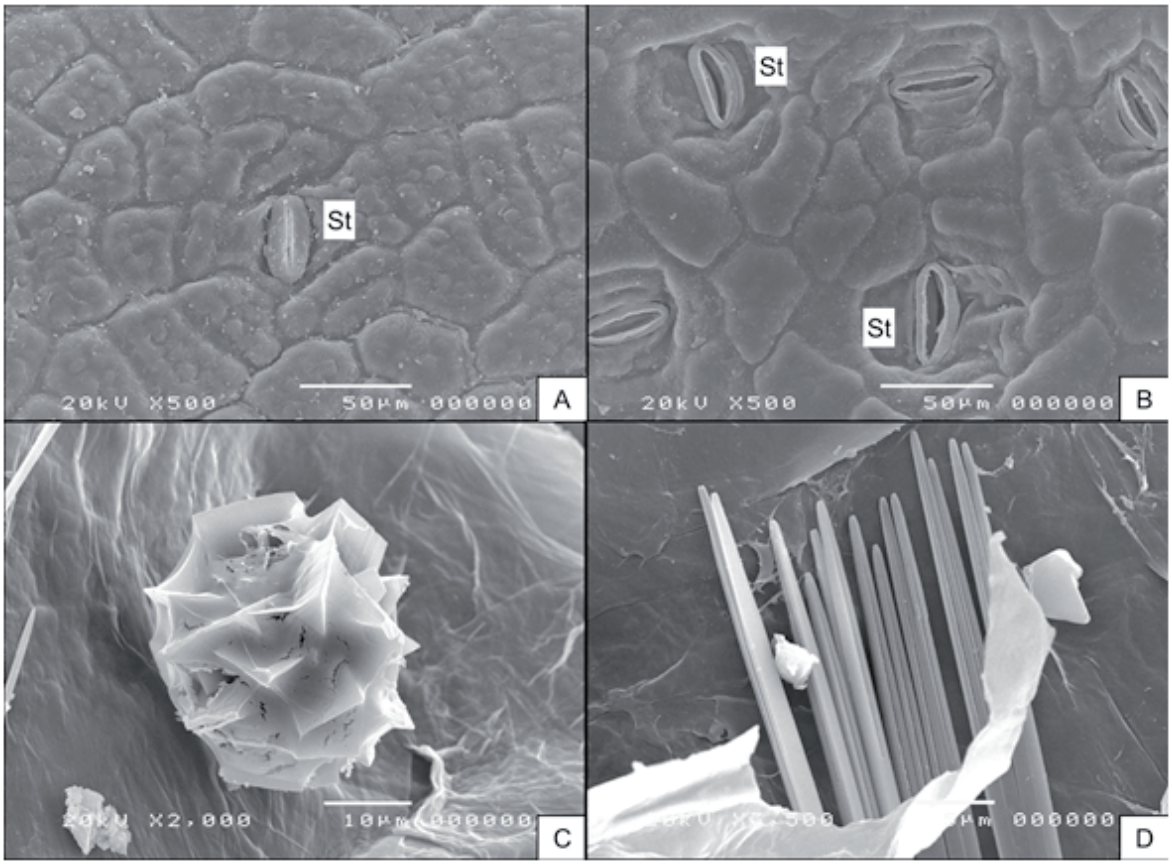
Figure 4. *Anthurium maricense.* Light micrographs of anatomy of leaves. (A) Transverse section (TS) of the middle portion of petiole. (B) TS of the midrib. (C) Details of TS of the midrib region exhibiting several idioblasts with crystals among parenchyma cells. (D-G) Paradermal sections (PS) of the leaf. (D-E) the adaxial and abaxial surfaces, respectively, showing the paracytic stomata and drusa. (F-G) Idioblasts containing drusa and raphides in the adaxial and abaxial faces respectively. (H) TS of the leaf blade showing the presence of sharp drusa in the abaxial epidermis. (I-J) Details of the leaf blade. (J) The leaf margin showing thick cuticle and large vascular bundles. (K) TS under polarized light revealing the presence of raphides next to the abaxial epidermis. Key: Ep= Epidermis; CP= Cortical Parenchyma; PP= Pith Parenchyma; ScR= Schlerenchymatic Ring; AdE= Adaxial Face of Epidermis, AbE= Abaxial Face of Epidermis; Pa= Parenchyma; PaP= Palisade Parenchyma; SP= Spongy Parenchyma; St=Stomata; Braces = Vascular Bundles; Arrow Heads = Drusa; Circles = Raphides. Bars: (A-B): 500μm; (D-H, K): 50μm; (C, I-J): 200μm.



Source: Guimarães (2017)

The histochemical test with Sudan IV did not reveal lipophilic substances inside the idioblasts with crystals. The test with Pizzolato solution showed the crystals in all organs consist of calcium oxalate. Under the SEM, the raphides revealed longitudinal grooves (FIGURE 5D). We also noticed a membrane involving the crystals, especially the drusa (FIGURE 5C).

Figure 5. Scanning electron migrographies of *Anthurium maricense*. (A) Adaxial epidermis. (B) Abaxial epidermis. Note the ordinary cells and stomata. (C) and (D) Two different types of calcium oxalate crystals from the leaf macerate. (C) Druse with a coating membrane. (D) Raphides with visible longitudinal grooves. Key: St= Stomata. Bars: in (A) and (B) = 50μm; in (C) = 10μm; in (D) = 5μm.

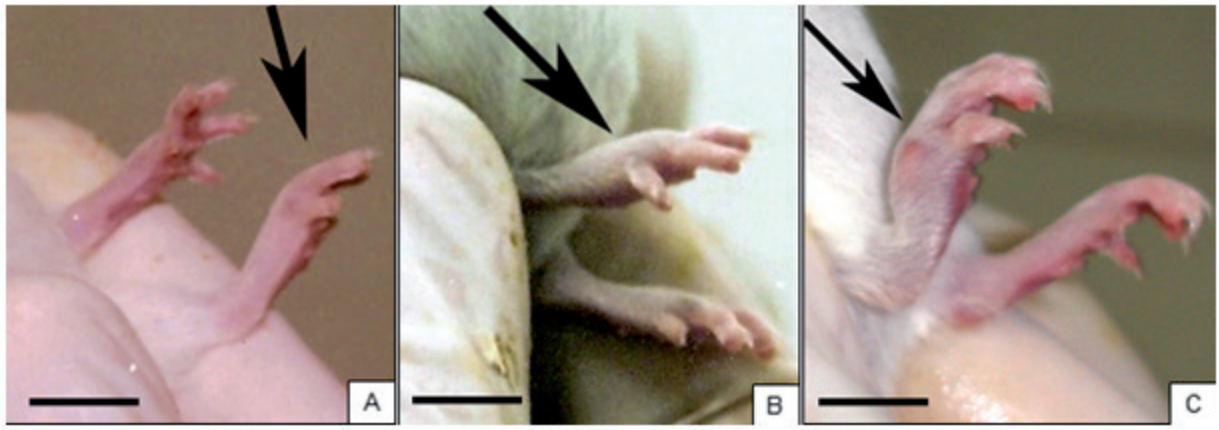


Source: Tanque (2017)

Toxicological analyses

The mouth washings showed negative results in all groups, without any sign of edema in the tongues or oral mucosa of the animals. In the days that followed the procedures, all animals remained alive and consumed water and food normally. The plantar inoculations, however, showed expressive results. In the 24 hours after the experiment, all animals showed level II edema (FIGURE 6B). After 96 hours, the results varied significantly. For the groups that received the centrifuged juices, all edema except one were reduced to level I (FIGURE 6A) and remained that way until the end of the experiment. One animal from the rhizome group had its edema grown to a level III after three weeks (FIGURE 6C). On the groups that received the whole juices, the results were different for each organ: the root group had all edema shrunk to level I after 96 h and showed no alteration at the end of observations. In the rhizome group, three out of the five animals had their edema grown to level III after three weeks and remained that way. On the leaf group, three out of the five animals had their edema grown to level III after one week, but at the end of the experiment, only one still had level III edema. None of the animals showed complete regression of edema during the experiment. Table 1 shows a summary of the data concerning the intensity and duration of paw edema.

Figure 6. The plantar edema in the paws of mice. (A): Level I edema. (B): Level II edema. (C): Level III edema. Note the paws with the edema (arrow), and the other visible paws are the ones in which the control saline solution was injected. Bars: 1cm in all.



Source: Tanque (2017)

Table 1: Results of the plantar inoculations. The number in each cell represents the number of animals that presented each level of edema.

	Whole juice												Centrifuged juice											
	Leaf				Root				Rhizome				Leaf				Root				Rhizome			
Level	0	I	II	III	0	I	II	III	0	I	II	III	0	I	II	III	0	I	II	III	0	I	II	III
A.I.	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-
1h	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-
2h	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-
3h	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-
6h	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-
24h	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-	-	-	5	-
96h	-	-	5	-	-	5	-	-	-	-	5	-	-	5	-	-	-	5	-	-	-	5	-	-
1week	-	-	2	3	-	5	-	-	-	-	5	-	-	5	-	-	-	5	-	-	-	5	-	-
2weeks	-	-	2	3	-	5	-	-	-	-	4	1	-	5	-	-	-	5	-	-	-	5	-	-
3weeks	-	-	2	3	-	5	-	-	-	-	2	3	-	5	-	-	-	5	-	-	-	4	-	1
4weeks	-	-	4	1	-	5	-	-	-	-	2	3	-	5	-	-	-	5	-	-	-	4	-	1

Source: Guimarães et al. (2019)

Discussion

Morphology and anatomy

The root anatomy of *A. maricense* is similar to the anatomy of other species of the genus (KEATING, 2002). It also revealed to be very similar to epiphytic orchids and bromeliads (PITA; MENEZES, 2002; SILVA; MILANEZE-GUTIERRE, 2004). These plants from distinct habits share the presence of velamen in their roots, a tissue responsible for absorbing water and minimizing transpiration in roots (BENZING et al., 1982). Bennet (1983) comments this feature is more

developed in epiphytes and desert plants that live in places under water stress. We did not observe sclerotic hypodermis or resin canals in the roots. Similar results were registered by French (1987a; 1987b), who did not find any of these two tissues in roots of other species of *Anthurium*.

The absence of phellogen and phelloderm indicates the secondary dermal tissue in the roots and rhizome consists of suberized cork (or storied cork), which is predominant in woody monocotyledons (ESAU, 1977). Suberized cork is also observed in other genera of Araceae as *Philodendron* (VIANNA et al., 2001), however, to the best of our knowledge, this is the first record in *A. maricense*.

The majority of studies in stem anatomy aim to characterize aerial parts. Ray (1987; 1988) described the patterns of stem structure in several genera, including *Anthurium*, but only aerial stems have been described in all analyzed species. Nevertheless, French and Tomlinson (1981) described several patterns of the vascular system for stems of Araceae, including subfamily Pothoideae. According to that, the internal architecture of the internode region of *A. polyschistum* is similar to *A. maricense*. Additionally, we also observed similar results to those described for stem anatomy of *Anthurium* (KEATING, 2002).

According to Mantovani et al. (2010), several species of *Anthurium* from Brazilian Atlantic Forest exhibit considerable diversity of the structure of the mesophyll. Some of these species show similar anatomy with the one observed in the present study for *A. maricense.* A similar structure was also observed in *Anthurium scandens* from the arboretum of Botanical Garden of Rio de Janeiro (JBRJ) (LORENZO et al., 2010). Mantovani et al. (2010) also observed similar idioblasts with druses and raphides in leaf epidermis only in *A. minarum.* However, the shape of epidermal cells and the stomatal configuration in *A. maricense* are remarkably distinct from the other species studied by those authors. Additionally, different epidermal cells and stomata were also registered in *A. scandens* (LORENZO et al., 2010).

The presence of a membrane involving the crystals, mainly on the drusa, corroborates the findings that such crystals are formed in organelles named crystal chambers or raphidioblasts (PRYCHID; RUDALL, 1999; FRANCESCHI; NAKATA, 2005). None of the studied organs contained raphides inside idioblasts, called biforine cells, spindle-shaped cells or defensive raphide idioblasts as described for other species of Araceae (SUNELL; HEALEY, 1985; CARNEIRO et al., 1989; LAINETTI et al., 1999). This type of idioblast makes the raphides be ejected at the gentlest touch and suggests a higher level of toxicity (COTÉ, 2009).

Toxicological aspects

The mechanism of intoxication of *A. maricense* is the association of drusa and raphides with proteolytic enzymes and lipophilic substances since the injuries caused by the whole juices were worse than the ones caused by the crystal-free juices. Also, the longitudinal grooves present on the raphides presumably serve to facilitate the penetration of toxic compounds in the wounded tissue (FRANCESCHI; NAKATA, 2005). Some studies with species of Araceae indicated proteolytic enzymes work together with raphides (WALTER; KHANNA, 1972) and sharp drusa of calcium oxalate (BOTHA; PENRITH, 2009). The trigger of the inflammatory response is the combination of mechanical damage by the sharp crystals and the release of proteolytic enzymes, which activates kinin-releasing mechanisms in the body (HANNA, 1986; MURPHY, 1994; KNIGHT; DORMAN, 1997; LORETTI et al., 2003; BOTHA; PENRITH, 2009; MATOS et al., 2011). Nevertheless, other studies with *Philodendron corcovadense* and *Dieffenbachia picta* suggested the edematous process also involved lipophilic substances associated with crystals (CARNEIRO et al., 1985; LAINETTI et al., 1995; LAINETTI et

al., 1999). The anatomical and histochemical analyses indicated the presence of proteins and lipids scattered throughout the roots, rhizomes, and leaves. Therefore, the mechanism of intoxication of *A. maricense* involves both types of chemical substances associated with the mechanical injury of raphides and drusa.

The lack of clear edema on the animals that received the juices orally can be justified by the brief contact of the juices with the tissues of the oral cavity, and it does not reproduce with precision a situation in which the animals would chew a piece of the plant. Nevertheless, several studies demonstrated how dangerous these plants might be. In the clinical case described by Cumpston et al. (2003), a man needed medical assistance in the hospital after chewing a piece of *Dieffenbachia picta* (dumb cane) stem, and had edema in his mouth and upper throat. Even after being treated, the swelling worsened in the three days that followed his admittance. In the study of Dip et al. (2004), the oral edema caused by *D. picta* also progressed over the course of the experiment. Toxicological studies with farm animals revealed *Alocasia macrorhiza* and *Dieffenbachia picta* caused poisonings of variable intensity with sialorrhea and sublingual and submandibular edemas. The latter species induced intense degeneration and necrosis of oral mucosa. Both plants caused immediate clinical symptoms, which evolved for a week, lowing from the eighth day. *Monstera deliciosa* and *Philodendron hastatum* were responsible only for light alterations of oral mucosa (ARMIÉN; TOKARNIA, 1994; TOKARNIA et al., 1996; TOKARNIA et al., 2012). Additionally, several species of Araceae may also cause a lot of damages in the eyes, such as irritation, conjunctivitis, edema, and even injuries in the cornea (MATOS et al., 2011).

The plantar inoculations revealed the potential for poisoning of all vegetative organs of *A. maricens*e. All of them induced moderate edema in all analyzed animals soon after inoculation. After 96 hours, the progression of the edema varied significantly according to each organ and the type of juice (whole or centrifuged). All animals but one that received the centrifuged juices had their edema diminished after 96 h. However, the animals in which we inoculated the whole juices exhibited different edema for each organ. Animals with inoculations of root juice had all edema shrunk to level I after 96 h, similarly to the centrifuged juice. This similar pattern may be related to the presence of isolated raphides and scarce drusa in the cortex of roots. Thus, even when the crystals are present, their amount and distribution are not enough to reinforce the association of mechanical damage and inflammatory substances and induce a more severe inflammatory response in the affected tissues.

Three among five animals with inoculations of rhizome juice had their edema grown to level III after three weeks. These results indicated the greatest potential for poisoning of the rhizomes among all vegetative organs of *A. maricense*. It was probably related to the abundance of idioblasts with raphides and drusa scattered throughout the parenchyma cells of the rhizome. Thus, the inflammatory response was triggered by the combination of mechanical damage by the abundant crystals and the release of proteolytic enzymes and lipids or several parenchyma cells. Furthermore, the worsening of the edema in the animal which received the centrifuged juice of rhizome after 96 h was possibly related to a secondary infection due to the plantar inoculation since similar patterns were not observed in any other analyzed animal.

Three among five animals with inoculations of leaf juice had their edema grown to level III after one week, but at the end of the experiment, only one still exhibited such edema. These results indicated the second greatest potential for poisoning among the vegetative organs. It was probably related to the distribution of idioblasts with raphides and drusa scattered throughout the parenchyma cells, similarly to the rhizome. Nevertheless, the presence of such idioblasts in the leaf epidermis

highlighted how easy this organ could trigger the inflammatory response, especially in contact with the oral mucosa. Additionaly, the better comprehension of the poisoning level of each organ is particular interesting due to the use of aerial and even underground organs of species of *Anthurium* as therapeutic resource (JOLY et al., 1987; ZAMORA-MARTÍNEZ; POLA, 1992; AGRA et al., 2008), and accidental ingestion of these organs from ornamental plants in public or private gardens.

None of the animals showed complete regression of edema during the experiment, which demonstrated the intensity of the poisonings. Other toxicological studies indicated that other species of Araceae, such as *Alocasia macrorhiza* and *Dieffenbachia picta,* induced severe clinical symptoms in animals fed with these plants, which disappeared in 6 to 10 days after their use (ARMIÉN; TOKARNIA, 1994; TOKARNIA et al., 1996; TOKARNIA et al., 2012). Thus, the edema caused by the vegetative organs of *A. maricense* presented lower intensity in contrast with other species of the family, such as *Dieffenbachia*, the attractive seeds of *Arisaema* and the leaves of *Pistia stratiotes* (KEATING, 2002). Nevertheless, they revealed to be chronic, since four weeks after the inoculations no animal had the edema entirely healed. In fact, some animals showed worsening in their conditions. Furthermore, species of Araceae may be responsible for systemic problems if used in the feeding of animals or accidental ingestion by humans, especially children. To the best of our knowledge, this is the first record of such persistent inflammatory response in cases of poisoning with Araceae, which highlights the importance of further studies on this genus, especially due to their use as ornamental and medicinal plants. We stress that the results described in this work are not only a step towards the explanation of the potential for poisoning of Araceae but also a contribution to anatomical patterns of *Anthurium*, especially from Brazilian restinga.

Conclusions

In this paper, we performed a toxicological and anatomical study of vegetative organs of *Anthurium maricense*. Our results revealed the mechanism of intoxication and the different levels of poisoning of each vegetative organ of *A. maricense*. All vegetative organs proved to be toxic and capable of inducing chronic inflammatory responses. We highlight the importance of further studies on this genus, especially due to their use as ornamental and medicinal plants. This paper aims to contribute to further studies of the anatomy of the species of Araceae and their possible toxicological effects.

Estudos toxicológico e anatômico dos órgãos vegetativos de *Anthurium maricense* Nadruz and Mayo (Araceae)

Resumo

Entre outros *taxa* de Araceae, *Anthurium* é um dos principais da família. Algumas espécies têm sido usadas como condimento, ornamentais e medicinais por mais de um século. *Anthurium maricense* é uma espécie endêmica das restingas do Rio de Janeiro, RJ, Brasil. Este trabalho teve como objetivos realizar estudos toxicológico e anatômico dos órgãos vegetativos de *A. maricense*. Essa espécie apresenta um sistema subterrâneo, formado por rizoma e raízes adventícias e folhas completas. As características anatômicas dos órgãos vegetativos são semelhantes àquelas observadas em *Anthurium* e outras espécies de Araceae, destacando-se o velame nas raízes e os idioblastos com

cristais de oxalato de cálcio em todos os órgãos analisados. As análises em MEV revelaram fendas longitudinais nas ráfides. Nos exames toxicológicos, foram usados extratos brutos (com cristais) e centrifugados (sem cristais) de cada órgão. Frações desses extratos foram administradas via lavagem bucal e inoculação nas patas em grupos de 5 camundongos. A formação de edemas foi observada em 1, 3, 6, 24 e 96 horas, além de 7, 14, 21 e 28 dias. As lavagens bucais não induziram desenvolvimento de edemas em todos os grupos. As inoculações nas patas levaram a resultados distintos: os extratos centrifugados induziram edemas moderados regredindo para leves, e os brutos provocaram edemas severos. Nossos resultados indicam que a distribuição e morfologia das ráfides e drusas e a presença de substâncias químicas de natureza diversa estão envolvidas no processo edematogênico. *A. maricense* apresenta potencial para induzir edemas crônicos se comparado a outras espécies de Araceae.
Palavras-chave: Anatomia. Cristais. Restinga. Toxicidade.

References

AGRA, M. F.; SILVA, K. N.; BASÍLIO, I. J. L. D.; FREITAS, P. F.; BARBOSA FILHO, J. M. Survey of medicinal plants used in the region Northeast of Brazil. **Revista Brasileira de Farmacognosia,** v. 18, n. 3, p. 472-508, 2008.

AQUINO, R.; MORELLI, S.; LAURO, M. R.; ABDO, S.; SAIJA, A.; TOMAINO, A. Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. **Journal of Natural Products,** v. 64, n. 8 , p. 1019-1023, 2001.

ARMIÉN, A. G.; TOKARNIA, C. H. Experimentos sobre a toxidez de algumas plantas ornamentais em ovinos. **Pesquisa Veterinária Brasileira**, v. 14, n. 2/3, p. 69-73, 1994.

BENNET, B. C. Primitive habit in Orchidaceae. **Systematic Botany**, v. 8, p. 472-474, 1983.

BENZING, D. H.; OTT, D. W.; FRIEDMAN, W. E. Roots of *Sobralia macrantha* (Orchidaceae): structure and function of the velamen-exodermis complex. **American Journal of Botany,** v. 69, p. 608-614, 1982.

BERLYN, G. P.; MIKSCHE, J. P. **Botanical microtechnique and cytochemistry**. Ames, Iowa: The Iowa State Press, 1976.

BOCHNER, R.; FISZON, J. T.; ASSIS, M. A.; AVELAR, K. E. S. Problemas associados ao uso de plantas medicinais comercializadas no mercadão de Madureira, município do Rio de Janeiro, Brasil**. Revista Brasileira de Plantas Medicinais**, v. 14, n. 3, p. 537-547, 2012. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-05722012000300017&lng=en&nrm=iso>. Access on: 23 July 2018. DOI: http://dx.doi.org/10.1590/S1516-05722012000300017.

BOCHNER, R.; FISZON, J. T.; ASSIS, M. A. **Plantas tóxicas ao alcance de crianças:** transformando risco em informação. Rio de Janeiro (RJ): RioBooks, 2013. 64p.

BOTHA, C. J.; PENRITH, M. L. Potential plant poisonings in dogs and cats in Southern Africa. **South African Veterinary Association**, v. 80, n. 2 , p. 63-74, 2009. Available in: <http://www.scielo.org.za/pdf/jsava/v80n2/02.pdf>. Access on: 22 July 2018.

BRASIL. MINISTÉRIO DA SAÚDE. FIOCRUZ. **SINITOX -** SISTEMA NACIONAL DE INFORMAÇÕES TÓXICO-FARMACOLÓGICAS. (HOMEPAGE NA INTERNET). 2016. DISPONÍVEL EM: <HTTP://SINITOX.ICICT.FIOCRUZ.BR/>. Acesso em: 10 maio 2016.

BRITO, A. S. **Manual de Ensaios Toxicológicos in vivo**. Campinas: Editora da UNICAMP, 1994. 122 p.

BUKATSCH, F. Bemerkungen zur Doppelfärbung Astrablau-Safranin. **Mikrokosmos**, v. 61, p. 255, 1972.

CARNEIRO, C. M. T. S.; NEVES, L. J.; PEREIRA, E. F. R.; PEREIRA, N. A. Mecanismo tóxico de 'comigo-ninguem-pode', *Dieffenbachia picta* Schott. (Araceae). **Anais da Academia Brasileira de Ciências,** v. 57, n. 1, p. 392–393, 1985.

CARNEIRO, C. M. T. S.; NEVES, L. J.; PEREIRA, E. F. R.; PEREIRA, N. A. Mecanismo tóxico de Comigo-Ninguém-Pode, *Dieffenbachia picta* Schott, Araceae. **Revista Brasileira de Farmácia**, v. 70, n. 1, p. 11-13, 1989.

CLARK, G. **Staining procedures**. Baltimore: Williams and Wilkins, 1981.

COELHO, M. A. N. New species of *Anthurium* (Araceae) from Brazil. **Aroideana,** v. 29, n. 1, p. 91-103, 2006.

COELHO, M. A. N. Espécies novas de *Anthurium* e *Philodendron* (Araceae) do sudeste brasileiro. **Boletins do Museu de Biologia Professor Mello Leitão**, v. 28, n. 1, p. 21-40, 2010. Available in: <HTTP://BOLETIM.SAMBIO.ORG.BR/PDF/28_00.PDF>. Access on: 19 July 2018.

COELHO, M. A. N.; CATHARINO, E. L. M. Duas espécies novas de *Anthurium* (Araceae) endêmicas do litoral de São Paulo, Brasil. **Rodriguesia**, v. 59, n. 4, p. 829-833, 2008. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2175-78602008000400829&lng=en&nrm=iso>. Access on: 18 July 2018. http://dx.doi.org/10.1590/2175-7860200859411.

COELHO, M. A. N.; MAYO, S. J. *Anthurium maricense* Nadruz and Mayo: a new species of *Anthurium* Schott (Araceae: tribe Anthurieae) for Brazil. **Aroideana**, v. 23, n. 1, p. 82-88, 2000.

CORRÊA, M. P. **Dicionário das plantas úteis do Brasil e das exóticas cultivadas:** Brasilia: Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal, 1984. 747 p.

COTÉ, G. G. Diversity and distribution of idioblasts producing calcium oxalate crystals in *Dieffenbachia seguine* (Araceae). **American Journal of Botany,** v. 96, n. 7, p. 1245-1254, 2009. Available in: <https://bsapubs.onlinelibrary.wiley.com/doi/10.3732/ajb.0800276>. Access on: 17 July 2018.

CUMPSTON, K. L.; VOGEL, S. N.; LEIKIN, J. B.; ERICKSON, T. B. Acute airway compromise after brief exposure to a dieffenbachia plant. **Journal of Emergency Medicine**, v. 25, n. 4, p. 391-397, 2003.

DAHLGREN, R. M. T.; CLIFFORD, H. T.; YEO, P. F. **The families of Monocotyledons. Structure, Evolution, and Taxonomy.** Berlin, Heidelberg: Springer-Verlag, 1985. 520 p.

DIP, E. C.; PEREIRA, N. A.; FERNANDES, P. D. Ability of eugenol to reduce tongue edema induced by *Dieffenbachia picta* Schott in mice. **Toxicon**, v. 43, n. 6, p. 729-735, 2004.

DRACH, G.; MALONEY, W. H. Toxicity of the common houseplant *Dieffenbachia*. **JAMA,** v. 184, n. 1, p. 1047-1048, 1963.

DUKE, J. A.; VASQUEZ, R. **Amazonian ethnobotanical dictionary**. Michigan: CRC Press, Ann Arber, 1994. 224 p.

ESAU, K. **Anatomy of seed plants**. 2. ed. New York (NY): Jonh Wiley & Sons, 1977. 550 p.

FELIPPE, G. **Venenosas**: plantas que matam também curam. São Paulo (SP): Ed. Senac, 2009. 352 p.

FRANCESCHI, V. R.; NAKATA, P. A. Calcium oxalate in plants: formation and function. **Annual Review of Plant Biology**, v. 56, n. 1, p. 41-71, 2005.

FRANKLIN, G. L. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. **Nature**, v. 155, n. 3924, p. 51, 1945.

FRENCH, J. C. Systematic occurrence of a sclerotic hypodermis in roots of Araceae. **American Journal of Botany**, v. 74, n. 6, p. 891-903, 1987a.

FRENCH, J. C. Systematic survey of resin canals in roots of Araceae. **Botanical Gazette**, v. 148, n. 3, p. 360-371, 1987b.

FRENCH, J. C.; TOMLINSON, P. B. Vascular patterns in stems of Araceae: subfamily Pothoideae. **American Journal of Botany**, v. 68, n. 5, p. 713-729, 1981.

GARDNER, D. G. Injury to the oral mucous membranes caused by the common houseplant, *Dieffenbachia*. **Oral Surgery, Oral Medicine, Oral Patholo**. v. 78, n. 5, p. 631-633, 1994.

GONÇALVES, E. G. *Anthurium macropodum*: a remarkable new species of Araceae from Brazil. **Kew bulletins,** v. 67, n. 3, p. 451-454, 2012.

HANNA, G. Plant poisoning in canines and felines. **Veterinary and Human Toxicology**, v. 28, n. 1, p. 38-40, 1986.

JOHANSEN, D. A. **Plant Microtechnique**. New York: McGraw-Hill Book Co. Inc., 1940. 487 p.

JOLY, L. G.; GUERRA, S.; SEPTIMO, R.; SOLIS, P. N.; CORREA, M.; GUPTA, M., LEVY, S.; SANDBERG, F. Ethnobotanical inventory of medicinal plants used by the Guaymi indians in Western Panama. **Journal of Ethnopharmacology**, v. 20, Part I, p. 145–171, 1987.

JUDD, W. S.; CAMPBELL, C. S.; KELLOGG. E. A.; STEVENS, P. F.; DONOGHUE, M. J. **Sistemática vegetal**: um enfoque filogenético. Porto Alegre (RS): Artmed, 2009. p. 353-355.

KARNOVSKY, M. J. A. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. **Journal of Cell Biology**, v. 27, p. 137-138, 1965.

KEATING, R. C. Acoraceae and Araceae. In: Gregory M, Cutler DF (Eds). **Anatomy of the monocotyledons**. New York (NY): Oxford University Press Inc., 2002. 322 p.

KNIGHT, M. W.; DORMAN, D. C. Selected poisonous plant concerns in small animals. In: DORMAN, D. (Ed.). Symposium. Toxicosis in dogs and cats. **Veterinary Medicine**, v. 92, p. 260-272, 1997.

KRAUS, J. E.; ARDUIN, M. **Manual básico de métodos em morfologia vegetal**. Rio de Janeiro: EDUR, 1997. v. 1, 198 p.

LAINETTI, R.; PEREIRA, N. A.; NEVES, L. J. Mecanismo tóxico de Comigo-Ninguém-Pode – *Dieffenbachia picta* Schott, a "planta armada", e de outras aráceas ornamentais. **Infarma**, v. 4, p. 5-7, 1995.

LAINETTI, R.; VIEIRA, A. C. M.; PEREIRA, N. A. Atividade edematogênica em *Philodendron corcovadense* Kunth. **Revista Brasileira de Farmácia**, v. 80, n. 1, p. 64-65, 1999.

LORENZO, N.; MANTUANO, D. G.; MANTOVANI, A. Comparative leaf ecophysiology and anatomy of seedlings, young and adult individuals of the epiphitic aroid *Anthurium scandens* (Aubl.) Engl. **Environmental Experimental Botany**, v. 68, n. 3, p. 314-322, 2010.

LORETTI, A. P.; ILHA, M. R. S.; RIBEIRO, R. E. S. Accidental fatal poisoning of a dog by *Dieffenbachia picta* (dumb cane). **Veterinary and Human Toxicology**, v. 45, n. 5, p. 233-239, 2003.

MANTOVANI, A.; FILARTIGA, A. L. D. P.; COELHO, M. A. N. Anatomia comparada da folha e espata de espécies de *Anthurium* (Araceae) ocorrentes na Mata Atlântica. **Revista Brasileira de Botânica,** v. 33, n. 1, p. 185-200, 2010. Available in: <http://www.scielo.br/pdf/rbb/v33n1/16.pdf>. Access on: 17 July 2018.

MATOS, F. J. A; LORENZI, H.; SANTOS, L. F. L.; MATOS, M. E. O.; SILVA, M. G. V.; SOUSA, M. P. **Plantas tóxicas** – Estudo de Fitotoxicologia Química de Plantas Brasileiras. São Paulo, SP: Instituto Plantarum, 2011. v. 1, 256 p.

MAZIA, D.; BREWER, P. A.; ALFERT, M. The cytochemical staining and measurement of protein with mercuric bromophenol blue. **The Biological Bulletin**, v. 104, n. 1, p. 57-67, 1953.

MURPHY, M. J. Toxin exposures in dogs and cats: Pesticides and biotoxins. **Journal of American Veterinary Medicine Association**, v. 205, n. 3, p. 414-421, 1994.

OCCHIONI, P.; RIZZINI, C. T. Ação Tóxica de Duas SP de *Dieffenbachia*. **Revista Brasileira de Medicina**, v. 1, n. 1, p. 10, 1958.

PASA, M. C.; SOARES, J. J.; GUARIM NETO, G. Estudo etnobotânico da comunidade de Conceição-Açu (Alto da Bacia do Rio Aricá Açu, MT, Brasil). **Acta Botanica Brasilica**, v. 19, n. 2, p. 195-207, 2005. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-33062005000200001&lng=en&nrm=iso>. Access on : 15 July 2018. DOI: http://dx.doi.org/10.1590/S0102-33062005000200001.

PITA, P. B.; MENEZES, N. L. Anatomia da raiz de espécies de *Dyckia* Schult. f. e *Encholirium* Mart. ex Schult. and Schult. f. (Bromeliaceae, Pitcairnioideae) da Serra do Cipó (Minas Gerais, Brasil), com especial referência ao velame. **Revista Brasileira de Botânica**, v. 25, n. 1, p. 25-34, 2002. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-84042002000100005&lng=en&nrm=iso>. Access on: 16 July 2018. DOI: http://dx.doi.org/10.1590/S0100-84042002000100005.

POLI, L. P.; TEMPONI, L. G.; COAN, A. I. Anatomical aspects of the gynoecium of species of *Anthurium* sect. *Urospadix* Engl. (Araceae, Alismatales). **Flora**, v. 207, n. 8, p. 615-621, 2012.

PRYCHID, C. J.; RUDALL, P. J. Calcium oxalate in monocotyledons: a review of their structure and systematic. **Annals of Botany**, v. 84, n. 6, p. 725-739, 1999.

RAY, T. S. Diversity of shoot organization in the Araceae. **American Journal of Botany**, v. 74, n. 9, p. 1373-1387, 1987.

RAY, T. S. Survey of shoot organization in the Araceae. **American Journal of Botany**, v. 75, n. 1, p. 56-84, 1988.

RADFORD, A. E.; DICKISON, W. C.; MASSEY, J. R.; BELL, R. **Vascular plants systematic**. New York, NY: Harper and Row. 1974. p. 83-152.

REEVE, R. M. Histological and Histochemical Changes in Developing and Ripening Peaches. II. The Cell Walls and Pectins. **American Journal of Botany**, v. 46, n. 4, p. 241-248, 1959.

RIZZINI, C. T.; OCCHIONI, P. Ação tóxica das *Dieffenbachia picta* e *D. seguine*. **Revista Brasileira de Medicina**, v. 20, n. 1, p. 5-26, 1957.

SASS, J. S. **Botanical Microtechnique**, 2 ed. Ames: Iowa Press. 1951. 248 p.

SACHVARTSMAN, S. **Plantas Venenosas e animais peçonhentos**. São Paulo, SP: Sarvier. 1992. 288p.

SILVA, C. I.; MILANEZE-GUTIERRE, M. A. Caracterização morfo-anatômica dos órgãos vegetativos de *Cattleya walkeriana* Gardner (Orchidaceae). **Acta Scientiarum Biological Sciences**, v. 26, n. 1, p. 91-100, 2004. Available in: <http://periodicos.uem.br/ojs/index.php/ActaSciBiolSci/article/view/1664> Access on: 17 July 2018.

SILVA, A. J. R., ANDRADE, L. H. C. Etnobotânica nordestina: estudo comparativo da relação entre comunidades e vegetação na Zona do Litoral-Mata do Estado de Pernambuco, Brasil. **Acta Botanica Brasilica**, v. 19, n. 1, p. 45-60, 2005. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-33062005000100006&lng=en&nrm=iso>. Access on: 20 July 2019. DOI: http://dx.doi.org/10.1590/S0102-33062005000100006.

SILVEIRA, M. Preparo de amostras biológicas para microscopia eletrônica de varredura. In: SOUZA W. (ed.). **Manual sobre técnicas básicas em microscopia eletrônica**. Rio de Janeiro: Sociedade Brasileira de Microscopia Eletrônica. 1989. p. 71-79.

SILVER, V. L.; PRICE, J. L. Demonstration of calcium oxalate crystals in plant tissues by the Pizzolato ($AgNO_3$-H_2O_2) method. **Stain Technology**, v. 44, n. 5, p. 257-259, 1969.

SOUZA, V. C.; LORENZI, H. **Botânica sistemática**: guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APG II. Nova Odessa, SP: Instituto Plantarum, 2005. 640 p.

SUNELL, L. A.; HEALEY, P. L. Distribution of calcium oxalate crystal idioblasts in leaves of taro (*Colocasia esculenta*). **American Journal of Botany**, v. 72, n. 12, p. 1854-1860, 1985.

TEMPONI, L. G.; COELHO, M. A. N. Two new species of *Anthurium* sect. Urospadix (Araceae) for Brazil. **Rodriguesia**, v. 62, n. 2, p. 315-320, 2011. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S2175-78602011000200315&lng=en&nrm=iso>. Access on: 14 July 2018. DOI: http://dx.doi.org/10.1590/2175-7860201162208.

TOKARNIA, C. H.; ARMIÉN, A. G.; PEIXOTO, P. V.; BARBOSA, J. D.; BRITO, M. F.; DÖBEREINER, J. Estudo experimental sobre a toxidez de algumas plantas ornamentais em bovinos. **Pesquisa Veterinária Brasileira**, v. 16, n. 1, p. 5-20, 1996.

TOKARNIA, C. H.; BRITO, M. F.; BARBOSA, J. D.; PEIXOTO, P. V.; DÖBEREINER, J. **Plantas tóxicas do Brasil**. Rio de Janeiro: Helianthus, 2012.

TWARDOWSCHY, C. A.; TEIVE, H. A. G.; SIQUINELI, F.; DIETZEL FILHO, L.; ENTRES, M., TWARDOWSCHY, A.; WERNECK, L. C. Trigeminal sensory neuropathy and facial contact dermatitis due to *Anthurium* sp. **Arquivos de Neuro-Psiquiatria**, v. 65, n. 3, p. 822-825, 2007. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004-282X2007000500017&lng=en&nrm=iso>. Access on: 12 July 2018. DOI: http://dx.doi.org/10.1590/S0004-282X2007000500017.

VIANNA, W. O.; SOARES, M. K. M.; APEZZATO-DA-GLÓRIA, B. Anatomia da raiz escora de *Philodendron bipinnatifidum* Schott (Araceae). **Acta Botanica Brasilica**, v. 15, n. 3, p. 313-320, 2001. Available in: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-33062001000300003&lng=en&nrm=iso>. Access on: 23 July 2019. DOI: http://dx.doi.org/10.1590/S0102-33062001000300003.

WALTER, W. G.; KHANNA, P. N. Chemistry of the aroids. I. *Dieffenbachia seguine, amoena and picta*. **Economic Botany**, v. 26, n. 4, p. 364-372, 1972.

WHITTIER, D. P.; PETERSON, R. L. The cuticle on *Psilotum* gametophytes. **Canadian Journal of Botany**, v. 73, n. 8, p. 1283–1288, 1995.

ZAMORA-MARTÍNEZ, M. C.; POLA, C. N. P. Medicinal plants used in some rural populations of Oaxaca, Puebla and Veracruz, Mexico. **Journal of Ethnopharmacology,** v. 35, n. 3, p. 229-257, 1992.

Received: January 7, 2018
Accepted: April 18, 2018