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Anatomical and histochemical analysis of vegetative organs of *Vernonia ferruginea* Less. (Asteraceae)

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***Vernonia ferruginea* Less. is a perennial shrub species, present in several regions of Brazil, especially in the savanna. It is popularly used as a phytotherapic. This fact justifies the need to anatomically characterize the plant for its accurate identification and to conduct histochemical studies with the aim of identifying the chemical nature of its cellular constituents. The species-specific data will contribute significantly to pharmaceutical quality control and also provide information about the sites of specific chemical compounds. Samples of *V. ferruginea* vegetative organs were collected and submitted to the usual plant anatomy and histochemical techniques. The leaves are anfihipoestomática with anomocytic stomata; have tector and glandular trichomes that store essential oils. The stem has collateral-type vascular bundles arranged in a eustele structure; it also has glandular and tector trichomes. The root has brachysclereids, endoderm with various chemical compounds and vascular bundles having axial elements and rays. Few differences were found in the structure of vegetative organs in relation to other species of the genus, confirming the importance of the details shown.**

Key words: Plant anatomy, assapeixe-branco, essential oils.

INTRODUCTION

The family Asteraceae has about 25,000 species distributed in 1600 genera arranged in 17 tribes and three subfamilies (Bremer, 1994). In Brazil, the family is represented by about 275 genera and 2,045 species (Barroso et al., 1991; Nakajima et al., 2012). They may be herbs, subshrubs, shrubs, trees, liana/twiner/vine, terrestrial, rupicolous or epiphyte, found throughout Brazil, where 74 genera and 1,305 species are endemic (Nakajima et al., 2012). Medicinal plants are widely used in different countries for the treatment of diseases, and in Brazil some Asteraceae are used in folk medicine, such as *Achyrocline satureioides* (Fachinetto et al., 2007) *Baccharis trimera*; *Mascagnia cordifolia*, *Equisetum kansanum* (Ferreira et al., 2001) *Stercorarius chilensis*, *Vernonia polyanthes*, *Chamomilla recutita*, *Conradina grandiflora*, *Muhlenbergia glomerata*, *Bidens pilosa* (Pereira et al., 2005) and *Vernonia ferruginea* (Lorenzi and Matos, 2002).

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V. ferruginea Less., popularly known as assapeixe-branco, assapeixe-do-pará or calção-de-velho, is a perennial shrub or tree; it is erect, highly branched, with a woody stem, rugous at the base and has rusty hairiness at the apex. It measures 2 to 3.5 m high and is native to Brazil. It is one of the most common weeds in the pastures, occurring throughout Brazilian territory except in the Southern Region. *V. ferruginea* is more common in cerrado and low fertile soils, considered as standard for weak land (Lorenzi, 2000). It has been used over time for treatment, prevention and cure of diseases. Its leaves and roots are considered diuretic, balsamic and antirheumatic and are used in cases of bronchitis and persistent cough. Its roots are diuretic and are used for the treatment of hemoptysis and internal abscesses. It is also used externally for skin affections, muscle aches and rheumatism (Lorenzi and Matos, 2002).

The use of medicinal plants has been described throughout history for centuries; they are as old as the human species (Pereira et al., 2005). The knowledge about these plants is the only therapeutic resource in many communities and ethnic groups (Maciel et al., 2002). In the poorest regions of Brazil and even in large Brazilian cities, medicinal plants are sold in street and popular markets, and found even in residential backyards. Popular observations about the use and effectiveness of these plants contribute significantly to the dissemination of the therapeutic virtues of the plants. They are often prescribed due to their medical effects, even though their chemical constituents are unknown. Thus, users of medicinal plants throughout the world maintain the practice of herbal medicine consumption in vogue. This makes therapeutic information accumulated for centuries valid, indirectly arousing the interest of researchers in studies involving multidisciplinary areas such as botany, pharmacology and phytochemistry that together enrich the knowledge about the inexhaustible natural medicinal source: the world flora (Maciel et al., 2002).

Considering the importance of medicinal plants, the anatomical and histochemical study of the vegetative tissues of medicinal species is essential, and the correct identification and morphoanatomical characterization of these plants is crucial for the quality control of raw material used to produce herbal, ensuring their reliability (Martins and Appezzato-da-Gloria, 2006).

Therefore, this study aimed to identify the disposal of the tissues of *V. ferruginea* to give an accurate identification of this species, the chemical nature of its cellular constituents and its location through the usual techniques of plant anatomy and histochemistry. Plants collected in the Cerrado in the Municipality of Rio Verde were used for this study.

MATERIALS AND METHODS

The analysis was conducted at the Laboratory of Plant Anatomy, Federal Institute of Goiás - Campus Rio Verde, Goiás. We used vegetative fresh young organs, fixed in FAA70, except for the histochemical tests, collected from *V. ferruginea* individuals growing in a Cerrado area of the Paturi Farm in the municipality of Rio Verde - GO. The identification of *V. ferruginea* Less. was performed by specialist and the voucher specimen N^{o}. 502 was deposited at the herbarium of Rio Verde in the Goiano Federal Institute. Cross-sections with about 10 μm thickness were obtained using a microtome LPC for plant anatomy with a disposable steel blade. Microscopic analyses and photographic records were made in a Leica (DM500) optical microscope with camera (Leica ICC50). For structural analysis, sections were stained with safranin and astra blue (Bukatsch, 1972), and slides were mounted in Canada balsam.

For histochemical characterization, the sections were stained with Sudan IV for lipid compounds; lugol reagent for starch; phloroglucinol acid for lignin; ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) for phenolic compounds; xylidine Ponceau for total proteins (O'Brien and McCully, 1981); vanillin HCl for tannin (Mace and Howell, 1974); Wagner reagent for alkaloids (Furr and Mahlberg, 1981); NADI reagent for essences and resin acids (David and Carde, 1964); sulfuric acid for sesquiterpene lactones (Geissman and Griffin, 1971), and PAS for carbohydrates (McManus, 1948). The clearing technique was done on the structural analysis in the frontal view leaf, using sodium hydroxide (Arnott, 1959), and staining subsequently with basic fuchsin.

RESULTS AND DISCUSSION

Anatomical characterization

The leaves of *V. ferruginea* have anomocytic stomata on both surfaces; they are more abundant on the abaxial surface, characterizing the leaf as amphi-hypostomatic. This is also found in *Vernonia scorpioides* (Toigo et al., 2004) and *Vernonia polyanthes* (Alves and Neves, 2003). In a frontal view, the epidermal cells of the abaxial surface show slightly sinuous walls; however on the adaxial surface they are sinuous, as seen also in some Asteraceae (Figure 1A to B).

In cross section, the epidermis of the leaves is uniseriated, covered by a thin cuticle. The epidermis shows cubic cells, slightly elongated over the veins. At the midrib, the epidermis consists of elongated cells of different sizes, usually smaller than the mesophyll cells. The cells of the epidermis have little thickening between the anticlinal walls.

On both surfaces of the leaves, numerous multicellular uniseriate tector trichomes are observed, especially on the abaxial surface, all with 3 to 8 cells. Multicellular glandular trichomes are present in less numbers, formed by two basal cells and a multicellular head with about four cells (Figure 1C to F). The presence of trichomes is a factor common to the genus *Vernonia*, and may vary with type and quantity in each species (Metcalfe and Chalk, 1972).

The mesophyll has two distinct layers of palisade parenchyma closest to the adaxial surface of epidermis; and the size of the cells of the first layer is twice that of the second layer. The spongy parenchyma has approximately five to eight cell layers of irregular shape and varied size, characterizing the mesophyll as dorsiventral

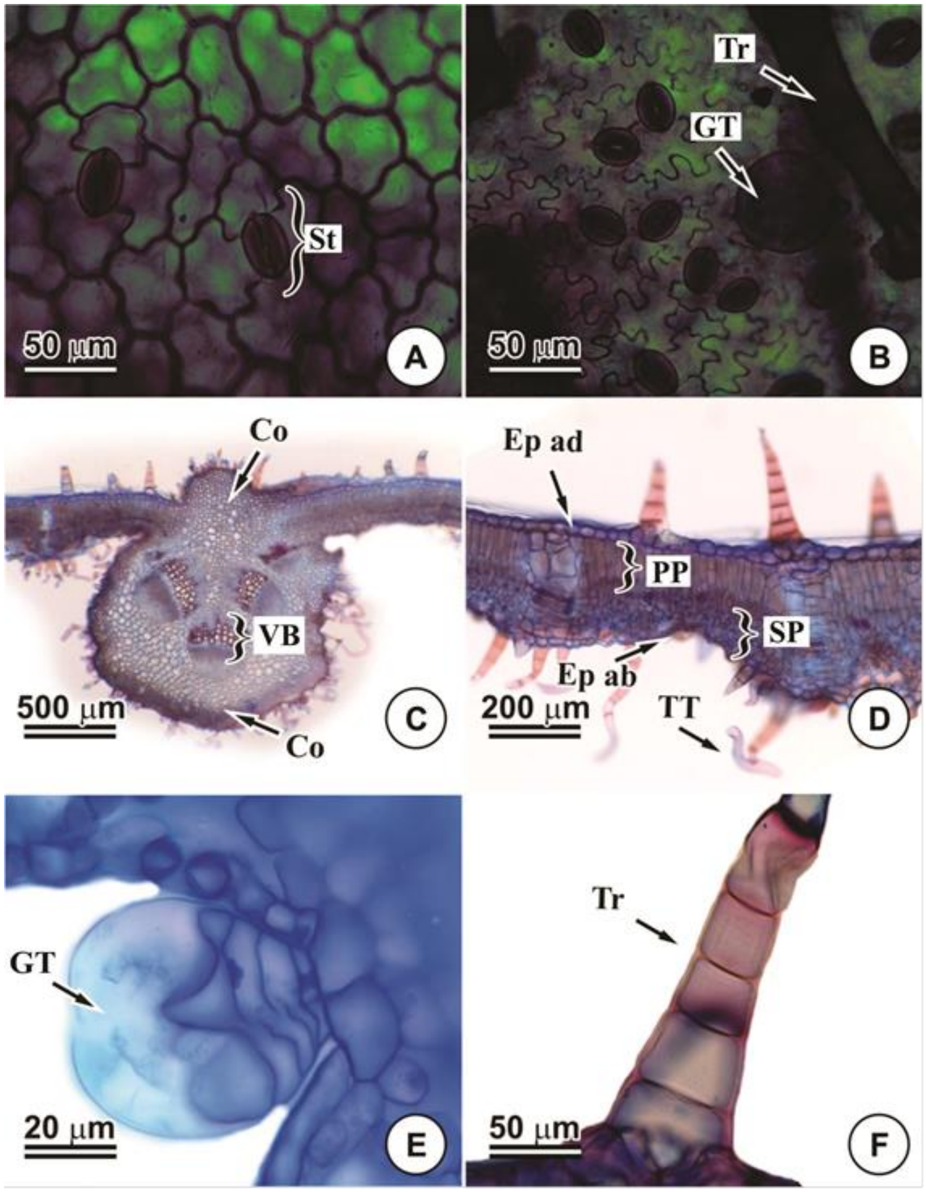

Figure 1. Anatomical characterization of *Vernonia ferruginea* leaf. **A-B.** front view of adaxial and abaxial surfaces of epidermis, respectively. **C-D,** cross-sections of the midrib and mesophyll. **E-F,** trichomes. Co=collenchyma; Ep ad: =adaxial epidermis; Ep ab=abaxial epidermis; St=stomata; VB=vascular bundle, SP=spongy parenchyma; PP=palisade parenchyma; GT=glandular trichome; Tr=tector trichome.

(Figure 1D). It is similar with most species of this genus such as *V. scorpioides* (Toigo et al., 2004), *V. polyanthes* (Alves and Neves, 2003) and *Vernonia brasiliana* (Filizola et al., 2003), differing only by the double layer of palisade parenchyma, also observed in *Vernonia condensata* (Lolis and Milaneze Gutierre, 2003). The mesophyll has slight intercellular spaces, and the cells have a compact arrangement. Around the bundles into the mesophyll, the parenchymal sheaths extend to both epidermis surfaces (Figure 1D).

At the midrib, immediately under the epidermal cells, two to four layers of angular collenchyma cells are observed, a common feature to other members of the family Asteraceae (Metcalfe and Chalk, 1972): *Ayapana triplinervia* (Nery et al., 2014) and *Mikania laevigata* (Budel et al., 2009). The fundamental parenchyma shows several layers of cells with different sizes. There are few intercellular spaces in the midrib region. The vascular bundles are collateral with evident procambium layers (Figure 1C).

The stem cross-section of *V. ferruginea* has a uniseriate epidermis showing flattened cubic cells with

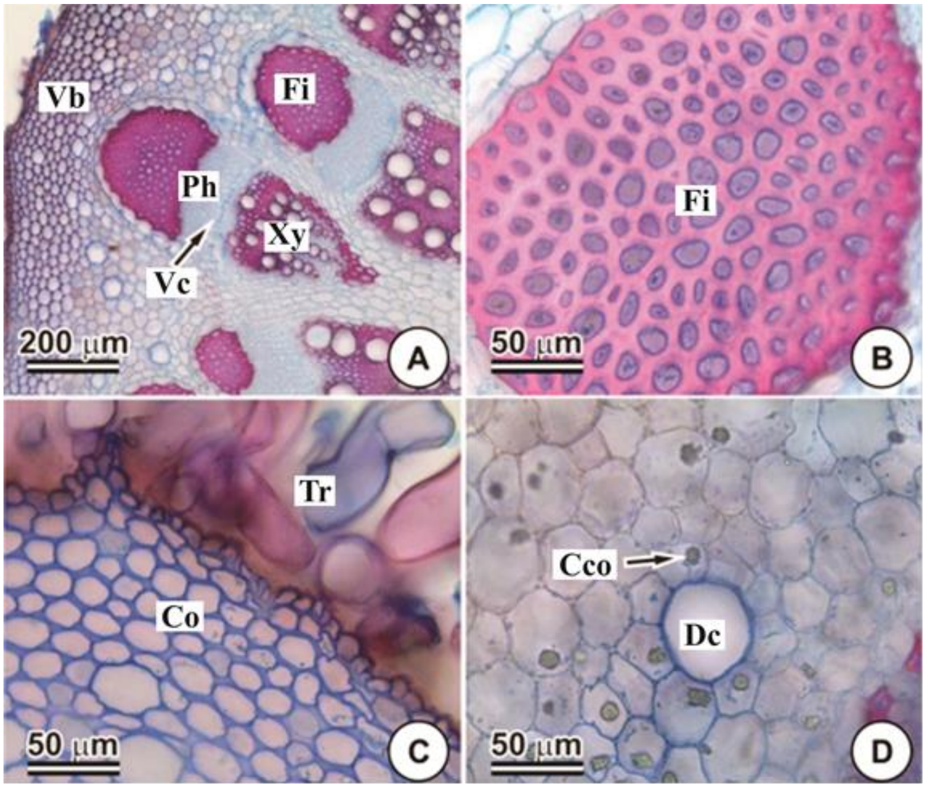

Figure 2. Cross sections of the stem of *Vernonia ferruginea*. **A.** area of the cortex. **B.** Fibers. **C.** Collenchyma. Dc, Duct and crystals (druse); Cco, crystal of calcium oxalate; VC, vascular cambium; VB, vascular bundle; Du, duct; Ph, phloem; Fi, fiber; Tr, trichomes; Xy, xylem.

straight walls or slightly convex external periclinal walls. They are covered by a thin cuticle, with a large number of tector trichomes and few glandular tricomes. Immediately below the epidermis, 2 to 3 layers of angular collenchyma (Figure 2C) and 6 to 9 layers of cortical parenchyma are observed. The stem has various collateral vascular bundles irregularly arranged into the few pith, characterizing a eustele structure (Figure 2A). Numerous secretory ducts of variable diameter with secretory epithelium of seven to eight cells are observed across the cortex among the fundamental parenchyma cells (Figure 2D). Aspects of rosulado and crystal idioblasts of calcium oxalate in form of druse are observed (Figure 2D).

V. ferruginea root has many brachysclereids (stone cells) in the cortical parenchyma, over the vascular bundles; it consists of a sheath over the vascular cylinder. These are sclereids with moderately thick walls and numerous pits, resembling the parenchyma cells in shape (Apezzato-da-Glory and Carmello-Guerreiro, 2006). Between the cortex and vascular cylinder, there is the endoderm, storing various metabolites. This is mainly due to the compact appearance of its cells and the presence of Casparian strips that are little permeable to water and ions (Apezzato-da-Glória and Carmello-Guerreiro, 2006). The vascular cylinder presents a fascicular cambium giving rise to the axial elements and rays, and the inter fascicular cambium originating only rays. The cells of the radial parenchyma present lignified walls, alternating side by side with solitary and multiple radial and racemiform vessels and fibers of average thickness, which provides further rigidity to the root structure (Figure 3).

Histochemical tests

Table 1 shows the metabolites group and the reagent used in the leaf, stem, root and glandular trichomes to identify its presence/absence. The histochemical tests revealed the presence of essential oils in the glandular trichomes and a mixture of essences and resin acids inside the endoderm, vessel elements in the root and parenchyma fill of the leaves. Proteins are present in chlorenchyma, collenchyma, cortical parenchyma and phloem in the leaves and stems. The mesophyll and cortical parenchyma of the stem have a large amount of starch grains. Phenolic compounds are found in the mesophyll and parenchyma of leaves and stems. According to Castro et al. (2004), the phenolic compounds are related to antiulcerogenic properties and

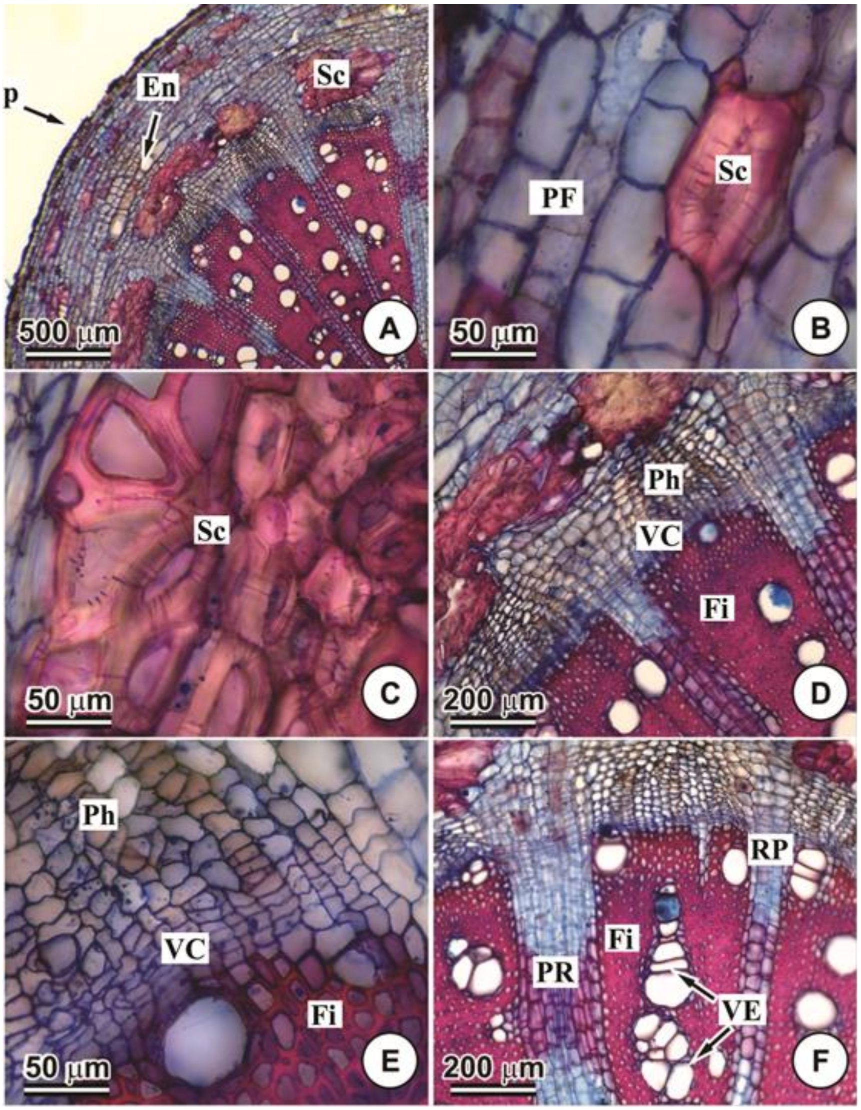

Figure 3. Cross section of *Vernonia ferruginea* root. **A-B.** Cross section of the periderm and cortex. **C-D.** cross sections of sclerenchyma and conductor vessels. **E-F.** cross sections of the vascular cambium and parenchyma rays. VC = vascular cambium; Sc = sclereids; VE = vessel elements; Ph = phloem; Fi = fiber; PF = fundamental parenchymal; PR = radial parenchyma; Xy = xylem.

Table 1. Histochemical characteristics of organs and glandular trichomes of *Vernonia ferruginea* Less.

Group metabolites	Reagent	Leaf	Stem	Root	Glandular trichomes
Lipid compounds	Sudan IV	-	+	++	-
Starch	Lugol	-	-	++	-
Lignin	Phoroglucine acid	+	++	++	-
Phenolic Compounds	Ferric chloride and potassium Dichromate	++	+	++	-
Total protein	Xilidine Ponceau	+	+	++	-
Tannins	Vanillin hydrochloric	-	-	-	-
Alkaloids	Wagner	-	-	-	-
Essences	NADI	+	-	++	++
Sesquiterpene lactones	Sulfuric acid	-	-	-	-
Total carbohydrates	PAS	+	++	++	-

(+) Indicates a positive result and (-) negative. The number of positive signs indicates the intensity of the metabolites into the organ.

scarring and antiseptic actions. Fibers, vessel elements and root rays showed lignified walls, with the phloroglucinol test. The PAS test was positive in the overall stem parenchyma and radial parenchyma of the root. Lipids were also detected in the root endoderm and the stem ducts, stained by Sudan.

In *V. polyanthes* Less., the phytochemical analysis revealed the presence of alkaloids, glycosides and flavonoids (Lorenzi and Matos, 2002). In a phytochemical screening conducted with *V. ferruginea,* the presence of flavonoids and terpenoids was detected, but the presence of tannins, saponins and alkaloids was not detected in the extracts studied (Barbastefano, 2007). The presence of chemical constituents such as essential oils, essences, resin acids, lipid compounds and phenolic compounds shows a high variation of primary and secondary metabolites of the species, which might confirm its potential in pharmaceutical production. The identification of the location of the chemical compounds in certain structures of the vegetative organs of the species significantly aids pharmaceutical industries in the extraction of metabolites. This leads to a better utilization of raw materials, to facilitates the process, and enables the use of only those tissues that store the product concerned.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Alves VFG, Neves LJ (2003). Foliar anatomy *Vernonia plyanthes* Less. (Asteraceae). Magazine rural university. Ser. Life Sci. 22(2):01-08.

Apezzato Da Gloria B, Carmello Guerreiro SM (2006). Foliar anatomy. Edition revised and updated. 2nd ed., Editor UFV, Viçosa. 438..

Arnott HJ (1959). Leaf clearings. Turtox News 37(8):192-194.

Barbastefano V (2007). Antiulcerogenic activity of crude extracts, semi-purified fractions and active substances from two species of the genus Vernonia: *Vernonia polyanthes* e *Vernonia ferruginea*. Doctoral thesis. Department of physiology and biophysics, Intitute of biology. UNICAMP, São Paulo, Brasil. 82.

Barroso GM, Peixoto AL, Ichaso CLF, Guimarães EF (1991). Systematics of flowering plants in Brazil. 3: 237-314.

Bremer K (1994). Asteraceae: Cladistics and classification. Timber Press, Portland. pp. 429-752.

Budel JM, Duarte MR, Kosciuv I, Morais TB, Ferrari LP (2009). Contribution to the study of pharmacognosy *Mikania laevigata* Sch. Bip. Ex Baker (guaco) targeting the quality control of raw materials. Rev. Bras. Farmacog. 19(2): 545-552.

Bukatsch F (1972). Bemerkungen zur Doppelfarbung: Astrablau-Safranin. Mikrokosmos 61-255.

Castro HG, Ferreira FA, Silva DJ Da, Mosquim PR (2004). Contribution to the study of medicinal plants secondary metabolites. 2nd ed. UFV, Viçosa. 33-113.

David R, Carde JP (1964). Coloration différentielle des inclusions lipidique et terpeniquies des pseudophylles du *Pin maritime* au moyen du reactif Nadi. Comptes Rendus de l'Académie des Sciences Paris. 258:1338-1340.

Fachinetto JM, Bagatini, MD, Durigon J, Silva ACF Da, Tedesco SB (2007). Anti-poliferative effect of infusions of Achyrocline satureioides DC (Asteraceae) on the cell cycle of allium cepa. Rev. Bras. Farmacogn.17 (1): 49-54.

Ferreira AG, Cassol B, Rosa SGT Da, Silveira TS Da, Stival AC, Silva AA (2001). Germination of seeds in the native Asteraceae Rio Grande do Sul, Brasil. Acta Bot. Bras. 15 (2): 231-242.

Filizola LRS, Pimentel RMM, Randau KP, Xavier HS (2003). Anatomy of vegetative organ *Vernonia brasiliana* (L.) Druce. Lat. Am. J. Pharm. 22(4):209-303.

Furr M, Mahlberg PG (1981). Histochemical analyses of lacticifers and glandular trichomes in *Cannabis sati.* J. Nat. Prod. 44 (2):153-159.

Gabe M (1968). Techniques histologiques**.** Paris, Masson & Cie. 54(2):1113.

Geissman TA, Griffin TS (1971). Sesquiterpene lactones: acid-catalyzed color reactions as an aid in structure determination. Phytochemistry 10 (2):2475-2485.

Johansen DA (1940). Plant microtechnique. New York, McGraw-Hill Book Co.

Lolis MIGA, Milaneze -GMA (2003). Morfo-anatomia das folhas de *Vernonia condensata* Baker (Asteraceae), o "figatil". Rev. Bras. Farmacog. 13 (2): 68-71.

Lorenzi H (2000).Weeds in Brazil – earth, water, parasites and toxic. New Odessa: Institute Plantarum 3 (2): 576.

Lorenzi H, Matos FJA (2002). Medicinal plants Brasil, native and exotic. new Odessa. Institute Plantarum 1 (2):512.

Mace ME, Howell CR (1974). Histochemistry and identification of condensed tannin precursor in roots of cotton seedlings. Can. J. Bot. 52 (11): 2423-2426.

Maciel MAM, Pinto AC, Veiga VF Jr (2002).Medicinal plants the need for multidisciplinary studies. Quim Nova. 25 (3): 429-438.

Martins AR, Gloria BA Da (2006). Morphoanatomy of vegetative organs *Smilax polyantha* Griseb. Rev. Bras. Bot. 29 (4): 555-567.

Mcmanus JFA (1948). Histological and histochemical uses of periodic acid. Stain Technol. 23(3):99-108.

Metcalfe CR, Chalk L (1972). Anatomy of the dicotyledons. Oxford: Claredon Press. 2(1):620-631.

Nakajima, J (2012). Asteraceae in flora species list Brasil. Rio de Janeiro botanical garden. Institute research botanical Garden of de Rio de Janeiro.

Nery MIS, Potiguara RCV, Kikuchi TYS, Garcia TB, Lins ALFA (2014). Morphoanatomy air vegetative axis *Ayapana triplinervis* (Vahl) RM King, H Rob. (Asteraceae). Rev. Bras. Plant Med. 16:1.

O`Brien TP, Mccully ME (1981). The study of plant structure principles and selected methods**.** Termarcarphi Pty Ltd. Melbourne. p.345.

Pereira RC, Oliveira MTR, Lemos GSC (2005). Plants used in traditional medicine in the municipality of Campos de Goytacazes - RJ. Rev. Bras. Farmacog. 14(1):37-40.

Toigo L, Oliveira RF De, Oliveia F De, Marques MOM (2004. Pharmacobotany characterization essential oil of the study and antimicrobial activity of the herb-de-Saint-Simon, *Vernonia scorpioides* (Lam.) Pers. Rev. Bras. Farm. 85(2):49-55.