

# Leaf anatomy and histochemistry of *Oriza* (*Pogostemon cablin* Beth., LAMIACEAE): Medicinal plant used in community Arari region, Itacoatiara, Amazonas

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**Abstract**— *Lamiaceae* (*Labiatae*) is one of the largest Angiosperm families (with over 7500 species). Many authors confirm that 25 species from this family are medicinal, both native from Brazil and exotic. This work aimed to characterize the anatomy and histochemistry of the leaves of *Oriza* (*Pogostemon cablin*) and identify the key substances of pharmacological importance present in the leaves. The leaves were collected in the Monte Cristo community, Itacoatiara, and were studied via light microscopy and scanning electron microscopy (SEM), following standard anatomy and histochemistry protocols. For electron microscopy, sections were dehydrated in ethanol at different concentrations, undergoing critical point, metallization and then photomicrographed with the SEM. The front view epidermis appeared anfi/hypostomatic (but functionally hypostomatic) with diacytic stomata. The mesophyll is dorsiventral, with palisade parenchyma in a single layer of cells and the spongy parenchyma with 4 or 5 layers, witch two are maiden of parallel cells and the others are irregular. Petiole is non-cylindrical, with a semicircular arrangement of woody fascicles. Histochemically slices reacted to pectin, starch, proteins and phenolic compounds, tannins, acidic lipids, so that oriza showed all the substances assessed in the tests. The presence of the recorded compounds and the anatomical characterization may assist taxonomic pharmacological and allelopathic studies.

**Keywords**— plant cell cuts, taxonomic studies, pharmacognosy, taxonomic studies, electron microscopy medicinal plant.

## I. INTRODUCTION

The use of medicinal plants is a very common practice in many parts of the country, especially in inner cities (Silva 2012). The use of drugs, plants and their derived, directly or indirectly, for the treatment of diseases affecting human beings who seek to achieve physical, mental and social well-being and has been a very common way, especially in developing countries, where the World Health Organization estimates that 80% of the population relies on traditional medicine (Oliveira *et al.*, 2014).

Among the families of plants that have shown greater representation and a large number of species in ethnobotanical surveys in relation to medicinal plants, the Lamiaceae family, Asteraceae and Fabaceae has always been in the top positions (SILVA; FARIA, 2014).

The Lamiaceae family has a cosmopolitan distribution, including about 300 genera and 7,500 species, and of these 23 genera and about 232 species occur in Brazil. Among the genera of this family, *Pogostemon* was introduced in Brazil and consists of 80 to 90 species, including aquatic subshrubs and herbs (BLANK *et al.*, 2013).

The importance of the species of the genus *Pogostemon* is related to allelopathy being found by Souza Filho et al., (2009) the constituents of the essential oils of this species synonymous *P. heyneanus* Benth that identified 15 constituents, with the major compounds alcohol patchouli (21.9%),  $\alpha$ -bulneseno (11.8%),  $\alpha$ -guaiano (8.6%), seicheleno (6.6%) and  $\alpha$ -patchuleno (6.3%) with a repellent action, in addition they are used in folk medicine, have food use and compose incenses in Chinese culture (Ramya et al., 2013).

*Pogostemon cablin* commonly known as oriza is a plant widely used for medicinal purposes, mainly for the treatment of child sickness, headache and heart problems (CASINO, 2010). The species has its center of origin in India and is grown in Indonesia and Malaysia, intensively, as in South America the plant is grown in Paraguay and Brazil, drawing attention by having an essential oil with characteristic odor, persistent and canforáceo (Maia et al., 2001).

In anatomical studies conducted with *Pogostemon cablin*, commonly also known by the common name of patchouli in order to determine the types and micromorphological characteristics of trichomes, the results showed that there were eight distinct trichomes types: two non-glandular six glandular trichomes. The non-glandular trichomes are simple, unicellular and multicellular and glandular trichomes are short, with long peduncle, peltate, fingerlike, filiform and fusiform (RUSYDI et al., 2013). In this study, it was not held histolocalização substances in the leaves, and the nature of substances.

For the diagnosis of vegetable drugs are considered important factors such as the nature of the cellular walls and cellular inclusions of organic and inorganic nature can be revealed by histochemical analysis (Oliveira et al., 2005).

In the presence of chemicals in the plants *Pogostemon* gender and specifically in *Pogostemon cablin*, it is necessary to analyze and identify compounds through immunohistochemical studies to try to justify the therapeutic capacity (SILVA and FARIA, 2014).

In the Monte Cristo community, Itacoatiara, Amazonas, the *Pogostemon cablin* is used mainly to treat heart problems, in the form of tea, which encouraged the interest for this study. Thus, this study aims to characterize anatomically Oriza leaves (*Pogostemon cablin*), identifying the main substances in tissues by immunohistochemistry as a way of providing information for further nafarmacologia research, in addition to the anatomical characterization sheet in general, because

previous studies and available literature concentrate efforts only on the trichomes characterization.

## II. MATERIAL AND METHODS

### Study area and botanical material collection

The botanical material used in this study was collected after an ethnobotanical survey in Arari region, Itacoatiara, Amazonas, in a rural community called Monte Cristo. The criteria used for choosing *Pogostemon cablin* was the crossing of information between plants mentioned for the treatment of heart and other diseases related to the circulatory system, with the historic municipality of diseases that proved that such diseases have caused the greatest number of visits or death incidences in 2014.

Leaves were chosen because they are used in the preparation of tea to cure possible heart problems. The samples were collected in the morning, in a total of three individuals, withdrawing only mature leaves and in good phytosanitary conditions and replicas, fixed in FAA (formaldehyde, acetic acid, ethyl alcohol 70%) for 24 hours. After this period, they were preserved in 70% ethanol for further analysis.

The plant was herborizada and incorporated into the herbarium collection of the Center for Advanced Itacoatiara in Amazonas State University Studies - CESIT / UEA with number of voucher specimen Hitam 4300-4302. The material was identified by specialist INPA herbarium.

### Light microscopy: anatomy and histochemistry

The study of light microscopy was performed in Agroforestry Botany Laboratory of the Federal University of Amazonas - Labaf / UFAM in Manaus, in the period from May to September 2015.

The anatomical analysis was made of the median limb portion of the petiole and leaf set from cross sections made freehand with razor aid in manual microtome. The sections were clarified with sodium hypochlorite solution to 20% safrablau stained and mounted in glycerine.

In the dissociation of the epidermis, sections were taken from the apex, edge of the middle region of the rib base and the leaf blade subjected to the hydrogen peroxide solution and acetic acid in the ratio 1: 1 (Franklin, 1946). After insertion into the solution, the material was kept for 24 hours in an oven at a temperature of 45 ° C. Subsequently, the sections were cleaned with the aid of a soft brush to remove the mesophyll and stained with Safranin O, passing in an ethanol series and retrofitting of the blade. Trichomes and stomata were classified according to the literature (METCALFE, CHALK, 1950;

Appezato-THE-GLORY and CARMELLO-WARRIOR, 2003; CUTTER, 2002).

The epidermis was also obtained through paradermal cuts freehand in order to confirm stomata classification and evidence in greater detail trichomes, epidermal cells and epidermal appendages.

Slides were analyzed with the aid of optical microscope and recorded with imaging obtained with the optical microscope Axioskop MC 80 camera.

For histochemical tests of the leaf sections of fresh material was not subjected to reagents and were photographed to document the original color of the analyzed tissue (white).

With other sections tests for the detection of chemical components of tissue were performed as detailed in Table

Table 1. Histochemical tests applied to identify chemical compounds in the leaves of oriza (*Pogostemon cablin*).

Reagent Test (Author)	Group of detected (reaction)
Red SUDAN III (Pearse, 1972)	Lipids (Orange Blushing)
Iron Chloride III (Johansen, 1940)	Total phenolic compounds (Blushing brown to black)
Red Ruthenium (Johansen, 1940)	Pectins (red blush or color pink)
Lugol (Jensen, 1962)	Starch (purple blush to blackish blue)
Xylidine Ponceau - XP (Berlyn&Miksche, 1976)	Proteins (red heart)
Vanillin Hydrochloric (Mace & Howell, 1974)	Phenolic compound - tannin (red heart)
Blue Nile (Cain, 1947)	Neutral lipids (pink) and acid lipids (blue)
Phloroglucinol (Johansen, 1940)	Phenolic compound - lignin (red or pink blush)
Dichromate Potassium (Gabe, 1968)	Total phenolics (Blushing reddish brown)

For all reactions were made photographic records noting the presence or absence of the analyte.

#### Scanning Electron Microscopy (SEM)

For Scanning Electron Microscopy, cuts the median portion, edge in the middle region and petiole previously dehydrated in ethyl alcohol 90% (10 minutes) and alcohol 95% (15 minutes) and absolute alcohol (twice for 10 minutes) and dried in critical model Bal-Tec CPD 030 - critical point dryer). The samples were then pasted on metal support with silver-based glue and subjected to plating with gold, Bal-TEC SCD 050 apparatus - Sputter Coater, then being examined and photomicrographed in Electron Microscope JEOL Scanning - JSM - 6460 LV - Scanning Electron Microscope. The sample processing stage took place at the Electron Microscopy Laboratory of the National Institute of Amazonian Research and reading in microscopic laboratory of the University of the State of Amazonas - UEA.

### III. RESULTS AND DISCUSSION

The epidermis cells presents in front view with different forms on the adaxial and abaxial being more straight walls

with light windings in the adaxial side (Figure 1A) and more sinuous on the abaxial surface (Figure 1B).

The abaxial epidermis is covered by cuticular ornamentation with random orientation observed only in Scanning Electron Microscopy - SEM (Figure 1C) and adaxial epidermis by a layer of thin and striated cuticle also observed only in SEM (Figure 1A). The cuticular ornamentation was described as CUTLER et al. (2011).

The stomata are diacytic (1A figures, 1B, 1C) and are present on both sides of the sheet, more numerous in the abaxial (Figure 1B), classifying it as amphistomatic, results also confirmed in Scanning Electron Microscopy (Figures 1A and 1C).

The species studied were collected in full sun exposure area, which can influence the varied manifestation of anatomical and also external morphology, such as the wall or thickness of the epidermal cells that correspond to the first barrier to filter solar energy. According Taiz and Zeiger (2004), anatomical features are contrasting in leaves of the same plant, depending only on the different light regimes to which it is exposed.

The species *Leonurus sibiricus* L. Lamiaceae also in Electron Microscopy adaxial epidermis scan showed similar characteristics with papillary cells, trichomes and glandular, differing only in the type of stomata which was identified as anomocytic (DUARTE; LOPES, 2005). The nature of the cell wall may also be related to the metabolic activity of the plant. In analyzes performed with the species *Melissa officinalis* (Lamiaceae) there was an increase in the thickness of the epidermal cells in adaxial and the abaxial were no significant changes (BRANT *et al.*, 2011).

Still on the outline of the cell walls and on the distribution of stomata can be compared with the other species also the Lamiaceae family leaves *Hyptidendron canum* (Pohl x Benth) Harley, whose skin, in front view, had to be different, having hypostomatic sheet and adaxial epidermis in front view, presenting cells with straight anticlinal wall thickened and abaxial epidermis cells with straight wall to wavy, anisocytic and diacytic (FIUZA *et al.*, 2010). diacytic stomata are more common in the family Lamiaceae (METCALFE, CHALK, 1950), but the anisocytic and anomocytic types can also be found, as recorded in the works of CASTRO *et al.*, (2015) and Duarte and Lopes (2005), respectively, for ruddy Hyptis species POHL ex Benth and *Leonurus sibiricus* L.

LIMA (2010), in a study with other Lamiaceae, *Ocimum gratissimum* L. pointed out that the leaf of this species proved amphistomatic presenting stomata diacytic, more frequent on the abaxial surface, differing only in the location of stomata on the same level of epidermal cells.

The leaves of *Scutellaria agrestis* A. St.-Hil. ex Benth also used by riverside communities in the Amazon are amphistomatic with stomata of the diacytic type, being located slightly elevated relative to the other epidermal cells (OLIVEIRA *et al.*, 2011), such characteristics described for this species resemble results found for *Pogostemon cablin*.

*Pogostemon cablin* in epidermal trichomes still exist on both sides, but higher number on the abaxial and also on the center rib (Figure 1D). trichomes and glandular were identified. The trichomes are multistage (two to three cells) and uniseriate and are of tapered shape, with the base made by increased epidermal cells adjacent (1G Figures, 1H, 1I) and glandular with multiseriate base and Split head (3 4 cells) (figure 1K) are pedunculated with one or more cells of different size, with the largest have reduced peduncle and are in epidermal depressions (figure 1J) and smaller feature prominent stem with respect to its size and are at the same level of ordinary epidermal cells (figure 1L).

In a study by other authors with the species in question, the adaxial and abaxial leaves investigated showed numerous glandular trichomes and the bristles. According to the morphology of glandular trichomes, there were three types of trichomes two of the three external trichomes are short stalk, Peltate trichomes and a long stem attached (GUO *et al.*, 2013).

Sandes *et al.*, (2012) study also *Pogostemon cablin*. They turned their attention only to the secretory structures of essential oils, describing and characterizing only the presence and morphology of glandular and glandular trichomes that are distributed in the leaf blade (SANDES *et al.*, 2012).

In observations made with other species of the Lamiaceae family, Faria (2008) identified the presence of glandular trichomes of two types, peltate and capitate and non-glandular trichomes uni simple multicellular. In the study of Milanezi-Gutierrez (2007) with the commonly known as false Boldo plant (*Plectranthus barbatus* Andrews) it was found that, in the limbo of both species occur five types of trichomes in both adaxial as abaxial and *Vitex agnus-castus*, also species belonging to the family Lamiaceae, Braga *et al.*, (2014) considered as glandular or glandular.

The trichomes usually have characteristic shape within species and have taxonomic significance and therefore, is credited also the function is relacionda with the water balance of the plant, one hairy thick surface tends to restrict the flow of air drying (CUTLER, 2011).

For Ramya *et al.*, (2013), the essential oil is an ingredient used as a "base" material in the perfume industry and the kind *Pogostemon cablin* studies report that has application not only in perfumes but also therapeutic, medicinal, like incense and food flavorings. These properties justify the concentration of studies only focused on the morphology of trichomes present in the leaf, because, according to Navarro and El Qualidi (2000), the trichomes may still represent an easy tool for Pharmacognostic characterization of plant to be easy observation and analysis.

In cross-section, of *Pogostemon cablin* leaf mesophyll presents dorsiventral with single-layered epidermis on both sides (Figure 1D). In the region of the center rib, the cells are of regular shape (Figure 1D), and the leaf blades, have rectangular shapes. The upper epidermis is papilhosa (Figure 1E), with stomata slightly above the epidermal cell layer (not shown).

For *Lavandula angustifolia* species Mill and *Lavandula dentata* L. leaves have dorsiventral mesophyll, similar to the species under study, consisting of palisade on the adaxial surface, consisting of a layer of cells,



corresponding to one third of the thickness of the mesophyll, and the cells with rounded shapes with a length corresponding to one and a half length to its width (Riva *et al.*, 2014).

The lower epidermis of *Pogostemon cablin* still displays intussusception in limbo extension on opposite sides, separated by the central rib (Figure 1E). SANDES *et al.*

(2012) observed in both mesophyll faces of *Pogostemon cablin* trichomes and glandular. The non-glandular trichomes are multicellular and uniseriate, formed by two basal cells, one to three intermediate cells and tapered cell at its end.

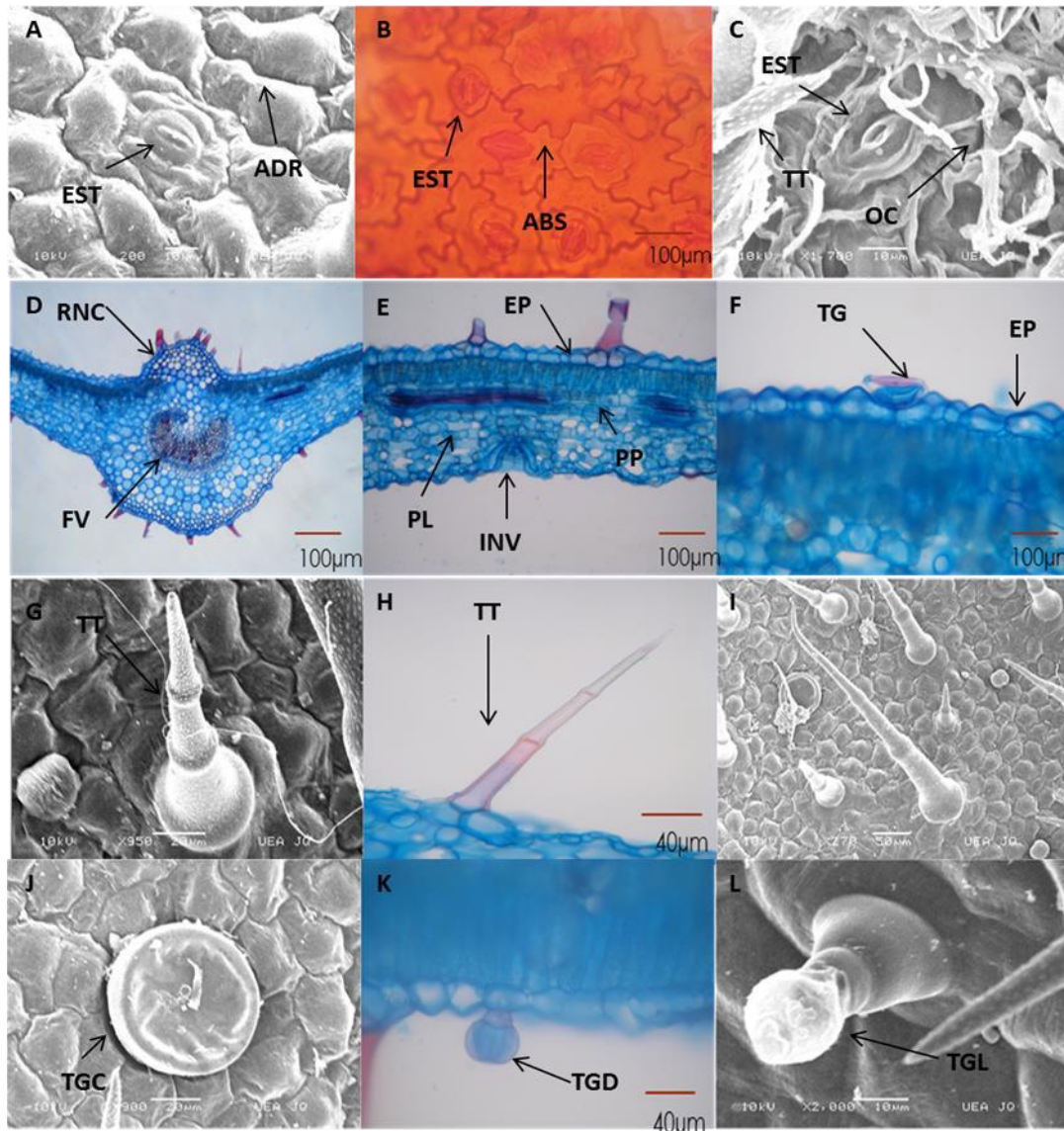


Fig.1: Cross section, paradermico and images for scanning electron microscopy - SEM of the leaf blade of *Pogostemon cablin* Benth. A and B. Characterization of adaxial and abaxial epidermis (ADR - straight adaxial epidermis, ABS - skin winding abaxial, EST - stomata); C. Complexion abaxial SEM (TT - tector trichomes, OC - random cuticular ornamentation); D. Cross limbo Court (RNC - region of midrib, FV - vascular bundles); E. Region outside of the rib in limbo (INV - intussusception, PP - palisade, PL - spongy parenchyma, EP - papillose epidermis). F-G. trichomes observation at different angles and shapes (TG - glandular trichomes, TGC - glandular trichomes with short stalk, TGL - glandular trichomes with long peduncle).

Just below the upper epidermis, the palisade is presented in a single layer of cells arranged in parallel (Fig 1D and 1E) can be interrupted by idioblasts, secondary beams fiber sheath projections or projections (not shown). In the

spongy parenchyma cells are arranged parallel. Are vascular bundles of small arms between the palisade and spongy parenchyma (Figure 1E).

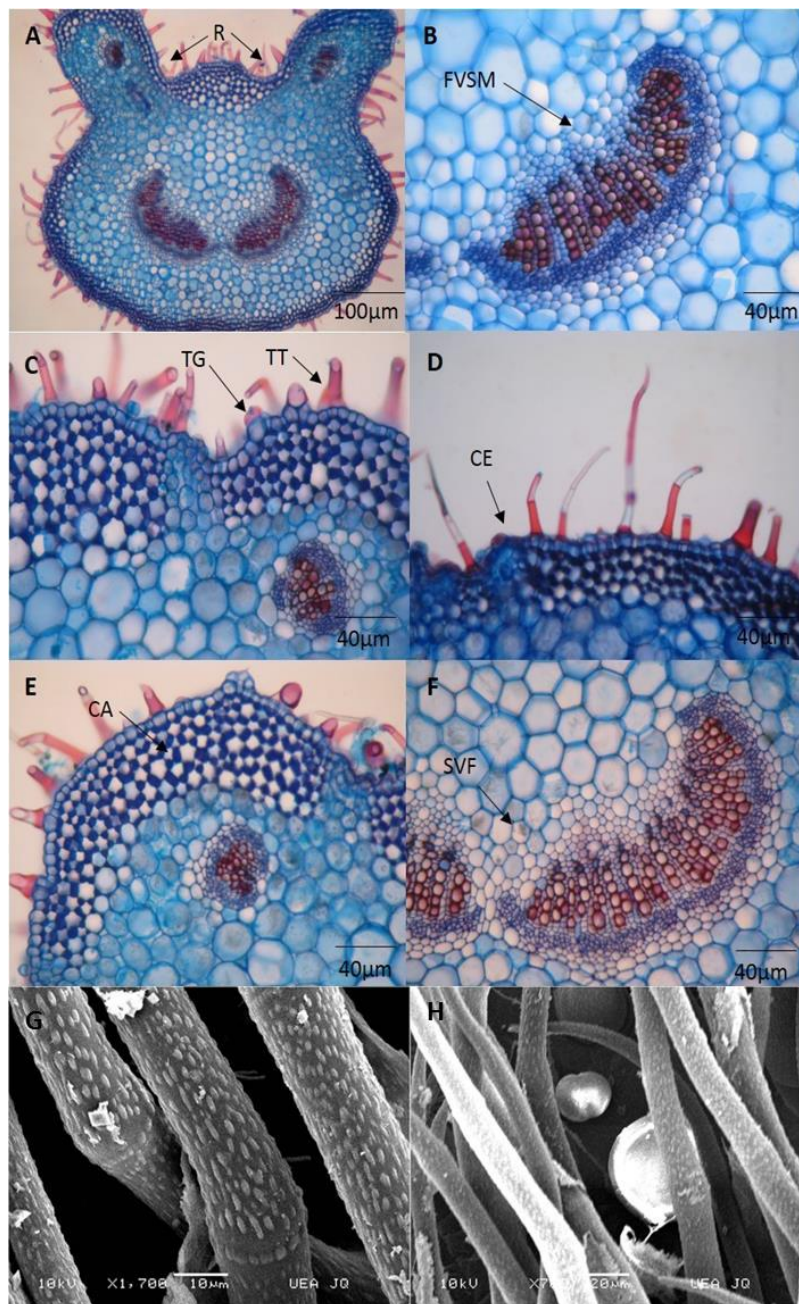


Fig.2: Cross section of the petiole. A. petiole with indentations at both ends; B. Beams in a semicircle; C. Trichomes and glandular on adaxial and abaxial, similar to the limbus; D. Complex stomatal above the epidermal cells; E. Four layers of angular collenchyma; F. vascular system in a horseshoe shape. (R - Reentrâncias the petiole, FVSM - Beams Vascular Semicircle, TG - Glandular trichome, TT - trichome Tector EC - stomatal in petiole, CA - Collenchyma Angle, SVF - Beam Horseshoe Vascular G. Ornamentation of cuticular trichomes SEM. . H. glandular trichomes and glandular SEM.

In the region of the midrib, the appearance is biconvex. The adaxial region of the ridge is more prominent and

more concave than in abaxial and has one to two layers of collenchyma (figura 1D). The epidermis of the two faces



may be interrupted by larger cells located at the base of the trichomes.

The cortex is populated by parenchymal cells constituted of large and intercellular spaces and the vascular bundles are open side (Figure 1D).

Petiole in cross section does not show a cylindrical shape and rather concave, which has the spherical recessed surface presenting with upper recessed at both ends (Figure 2A), therefore the liberolenhosos beams are arranged in an open arc (Figure 2B). Evidence is a uniseriate epidermis, covered with a thin cuticle and trichomes and glandular both adaxial and in abaxial similar to those described for the limbus (Figure 2C). Stomatal complexes occur above the other epidermal cells (Figure 2D).

The non-glandular trichomes present aplenty including preventing you from entering the closest to the skin cell structures (Figure 2G and 2H). In increase, the non-glandular trichomes showed a cuticular ornamentation (Figure 2G) described, according to Cutler *et al.* (2011), and between the trichomes also observed glandular trichomes (Figure 2H). Martins and Martins (2003), in studies of the *Mentha* species *pulegium* in electronic photomicrographs, also found the presence of ornamentation in the cuticle of glandular trichomes.

Table 2. Histochemistry of metabolites present in *Pogostemon cablin*.

Test	Result (leaf and petiole limbo)
Red SUDAN III (Pearse, 1972)	(+)
Iron Chloride III (Johansen, 1940)	(+)
Red Ruthenium (Johansen, 1940)	(+)
Lugol (Jensen, 1962)	(+)
Xylidine Ponceau - XP (Berlyn & Miksche, 1976)	(+)
Vanillin Hydrochloric (Mace & Howell, 1974)	(+)
Blue Nile (Cain, 1947)	(+)
Phloroglucinol (Johansen, 1940)	(+)
Dichromate Potassium (Gabe, 1968)	(+)

Beneath the epidermis was observed up to four layers of angular chollenchyma, interrupted in the regions where the stomata (Figure 2E). In the middle region of the petiole, there is a concave shape on the adaxial and convex face on the abaxial surface. The vascular system in a horseshoe

shape is formed by side beams, showing sclerenchyma tic cap externally to the phloem (Figure 2F).

The results of several authors for *Pogostemon* gender focus in the study of trichomes and glands by the interest in the essential oils and other Lamiaceae family species have varied anatomy. For all histochemical tests applied in the courts that species (Table 2) confirmed the reaction of analytes, showing the results with the positive sign (+).

In chlorophyll vegetables, vegetable double membrane and cytoplasmic is cellulosic and its other constituents, such as peptic substances (pectose, pectic acids, metapéticos and callose). Even when the plant is more mature secondary membranes can be of various natures, lignified, suberous, cutinized, cerificadas with hemicellulose and silicified. Still another important factor in diagnosing the plant cell corresponds to the drug inclusions (starch, aleurone, inulin, oil droplets, silica, and calcium oxalate crystals carbonate and other various contents (Oliveira *et al.*, 2005).

The cuticle is thin at both sides of the sheet. lipid droplets were found in the cytoplasm of cells isolated from tissue parenchyma (Figure 3A). Some cytoplasmic substances in the cells of the adaxial epidermis and palisade reacted to sudan III ethanol, also blushing in glandular trichomes (Figure 3B). In studies by Guo (2013) by histochemical tests for the detection of *Pogostemon* cablin lipid compounds Benth, yielded positive results indicating the presence of total lipids labeled with the dye Sudan black B.

For the Blue Nile both mesophyll cells as the petiole cross-section stained only for acidic lipids, not manifesting peculiar pink color of neutral lipids (Figure 3C and 3D). According to Lima and Martins (2011), the secreted substance is accumulated in periplastidial space trichomes of *Ocimum gratissimum* L. (Lamiaceae) and reacted positively to Sudan Black B tests, Sudan IV and Blue Nile indicating the presence of lipophilic compounds.

Phenolic compounds are expressed by the full extent of the leaf blade, presenting more concentrated in the abaxial epidermis region, the palisade and spongy parenchyma and was also manifested in the area of the midrib (Figure 3E). The glandular trichomes of the petiole reacted showing the presence of the compound (Figure 3F).

Phenolic compounds are substances that are noted for their antioxidant, anti-inflammatory, antitumor and estrogenic, suggesting the action of some phenolic compounds in the prevention of coronary heart disease and cancer (TOMÁS-BARBERAN; ESPÍN, 2001). This information may justify the fact of oriza (*Pogostemon cablin*) be used by Community Arari in the region for the treatment of diseases of the circulatory system, such as the heart.

Chemical analysis of extracts of *Rosmarinus officinalis* L. commonly known as rosemary (Lamiaceae) and can be used for combating circulatory disorders, and as an antiseptic and cicatrizing recommended by HPLC-DAD method revealed the presence of phenolic compounds: Chlorogenic acid, caffeic acid, rutin, rosmarinic acid, quercetin, kaempferol and carnosic acid (FRESHNESS et al., 2013). As *Rosmarinus officinalis*, Lamiaceae other

species present in its chemically flavonoids and phenolic acids, such as Miller *Lavandula angustifolia* (lavender) (caffeic acid and rosmarinic acid), *Stachys officinalis* (L.) Trev. (Brutônica) (caffeic acid, chlorogenic acid and rosmarinic), *Orthosiphon spicatus* Bak. (Ortossifon) (rosmarinic acid, caffeic acid, ursolic acid, glycolic acid and benzoic acid) (Cunha et al., 2012).

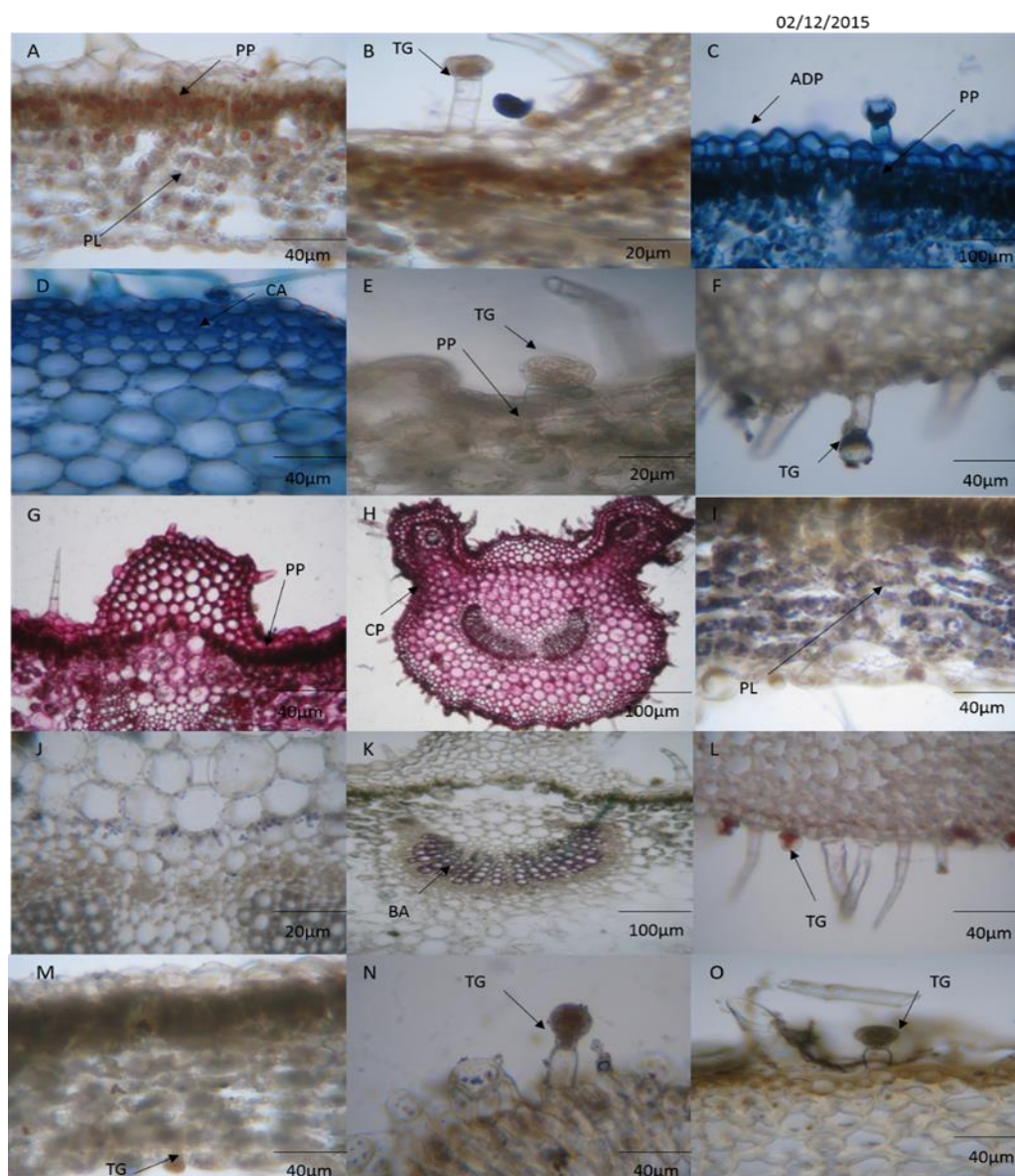


Fig.3: Histochemistry metabolites present in *Pogostemon cablin* leaf cuts Benth featuring reaction. A. Reaction of Sudan III staining the cytoplasm of single cells of parenchyma tissue; B. Reaction in glandular trichomes, presence of lipids; C. Lipids acids in the mesophyll; D. Presence of acid lipids in the petiole; E. Positive reaction to phenolic compounds in palisade and lacunosos parenchyma, and greater concentration in the rib; F. Reaction also in Glandular trichomes and petiole; G. Presence of structural pectins in leaf blade and; H. petiole structural pectins; I. Reaction confirming starch in the leaf blade; J. Starch petiole with formation of starch sheath; K. lignin Presence in conducting vessels of limbo; L. petiole with tannins; M. Reaction confirming proteins in glandular trichomes. (PP - parenchyma palisade, PL - spongy parenchyma, TG - Glandular trichome, ADP - Complexion adaxial papillose, CA - Collenchyma Angle, CP - Wall Cells, BA - Sheath Amilífera).



In the area of leaf blade was detected the presence of structural pectins, including cells of trichomes and the epidermis of the leaf and petiole limbo (Figures 3G and 3H). Unlike what happened with the kind described in issue in *Ocimum* L. species *gratissimum* also the Lamiaceae family, ruthenium red unreacted, featuring the absence of pectins in plant tissue cells (SANTOS, 2013). Starch was found mainly in the parenchyma cells of the leaf blade, the petiole showed the formation of a starch sheath (Figure 3I and 3J).

In another study of *Pogostemon cablin* in applying lugol on various vegetative organs cuts, we observed the presence of starch grains in the roots, leaves and petioles, confirming the results detected in this study for the leaf blade and petiole of this species (Storcks; DESCHAMPS, 2012). Contrary to the results of this, Lamiaceae family species as *Mentha piperita*, not reacted to lugol therefore did not show the presence of starch in the evaluated sheets (CONCEPTION *et al.*, 2009).

For phloroglucin oriza the flushing cuts reacted only in the area of the conducting vessels (Figure 3K and 3L). In histochemical tests phoroglucine for *Hyptis stricta* Benth species, the xylem cells in the midrib region and the petiole showed up lignified (SILVA, 2012), similar to that observed in *Pogostemon cablin*. For the detection of proteins showed reaction only in the glandular trichomes (3M).

In the case of phenolic compounds, applying the test with potassium dichromate, sections were reacted confirming the presence of these compounds in the blade and also in the petiole (Figures 3N and 3O). For the specific identified the presence of tannins in the glandular trichomes of the lamina and petiole of oriza leaves (Figure 3P).

Histochemical tests revealed the presence of phenolic compounds, which provide an indication of the presence of some secondary metabolites, such as, for example, tannins. In the Lamiaceae family, is a kind of tannin that has the ability to precipitate proteins, but it is not suitable for tanning the skin, known as "tannin of Labiatae" (Toledo *et al.*, 2004).

In characterizing ethnobotany and histochemistry of medicinal plants in Goias, Lamiaceae family species were subjected to histochemical tests and obtained as a result of starch, total phenolic compounds, lipophilic compounds and tannins (SILVA and FARIA, 2014) similar to the substances detected in the species that study.

The studies that have been conducted such corroborate the importance of ethnobotanical survey data and ethnopharmacological in the selection of plants with therapeutic potential and bioactivity screening. The results

show a significant contribution to the characterization of essential oils activity, confirming the presence of plant areas where there is production as in other species of the genus, but in need of chemical and pharmacological studies to confirm the real action in traditional medicine, addition of secondary compounds and plant extracts flora which can be used in folk medicine.

#### IV. CONCLUSION

Anatomical nature character of diagnostic values were recorded in this study, such as the presence of glandular trichomes and the presence of primary and secondary compounds confirmed the histochemical tests to complement what exists in the literature for *Pogostemon* genre. The work that has been carried out focus on to describe the morphology of glandular trichomes to be an area of concentration and production of essential oils characteristics of the species, in addition to mentioning the tectores. Complementing what already exists in the literature was recorded here the anatomical characteristics of the sheet as a whole differs from the other work that can contribute to the correct identification of the species. Thus the description showed stomata diacytic characteristic of the Lamiaceae family, with amphistomatic leaves, dorsiventral mesophyll and epidermis in both unistratified faces. Also in the upper epidermis was observed the buds and stomata slightly above the cell layer. A striking feature was the present intussusception in the cross section separated by the central rib, which seems it is a glandular cavity. The petiole is not cylindrical, with liberolenhosos beams in a semicircle and below the epidermis layer there are cells composing the angular chollenchyma. The species reacted positively to all histochemical tests confirming the presence of various substances such as lipids, phenolic compounds, proteins, starch, tannins and pectins. In electron microscopy demonstrated layer of ornate and random cuticle on the abaxial epidermis and also in granular form in trichomes petiole, results not seen in light microscopy. This information can be considered relevant to aid in the identification of the species, contribute pharmacognostic studies, phytochemicals, industrial, food and other involving the economic value of the species, in addition to demonstrating the quality control of it for medicinal use.

#### REFERENCES

- [1] APPEZATO-DA-GLÓRIA, Beatriz; CARMELLO-GUERREIRO, Sandra Maria. Anatomia Vegetal. Viçosa: Editora UFV, 2003.

- [2] BRAGA, Z.V. et al. Morfoanatomia e distribuição de tricomas presentes em folha e caule de alecrim-d'angola (*Vitex agnus-castus*, Lamiaceae) ocorrente na Amazônia Brasileira. Enciclopédia Biosfera, Centro Científico Conhecer. Goiânia, v.10, nº 19; p. 23-68. 2014.
- [3] BRANT, R.S. et al. Adaptações fisiológicas e anatômicas de *Melissa officinalis* L. (Lamiaceae) cultivadas sob malhas termorrefletoras em diferentes intensidades luminosas. Rev. Bras. Pl. Med., Botucatu, v.13, nº 4, p.467-474, 2011.
- [4] CASTRO, A.S. et al. Caracterização morfoanatomia e histoquímica de *Hyptis rubicunda* POHL ex BENTH. (Lamiaceae), ocorrente na Serra Dourada, Goiás, Brasil. Revista Eletrônica de Educação da Faculdade Araguaia, v. 7, p.111-133, 2015.
- [5] CONCEIÇÃO, D.M.; SACRAMENTO, L.V.S.; FURTADO, E.L. Caracterização anatômica e histoquímica de mentas infectadas por *Puccinia menthae* e *Erysiphe biocellata*. 2009. 83 p. Dissertação (Mestrado em Agronomia) Faculdade de Ciências Agrônomicas da UNESP – Campus de Botucatu, Botucatu - SP. 2009.
- [6] CUNHA, A.P.; SILVA, A.P.; ROQUE, O.R. Plantas e produtos vegetais em fitoterapia. Lisboa: Fundação Calouste Gulbenkian, 2012. 731p.
- [7] CUTLER, David F.; BOTHA, Ted; STEVENSON, Dennis Wm. Anatomia Vegetal: uma abordagem aplicada. Porto Alegre: Artmed, 2011.
- [8] CUTTER, E. Anatomia Vegetal Parte 1 - células e tecidos. 2ªed. São Paulo: Roca, 2002.
- [9] DUARTE, M.R.; LOPES, J.F. Morfoanatomia Foliar e Caulinar de *Leonurus sibiricus* L., Lamiaceae. Acta Farm, v. 24, n.1, p. 68 -74, 2005.
- [10] FARIA, T.M. Morfologia, anatomia, histoquímica e fitoquímica de espécies do gênero *Hypenia* (Mart. Ex. Benth.) R. Harley (Lamiaceae) ocorrentes no cerrado de Goiás. Rev. Biol. Neotrop, v. 5, nº 1, p. 71-72, 2008.
- [11] FIUZA, T.S. et al. Estudo das folhas e caule de *Hyptidendron canum* (Pohl ex Benth.) Harley, Lamiaceae. Revista Brasileira de Farmacognosia, v. 20, nº 2, p. 192-200, 2010.
- [12] FRESCURA, V.D.S. et al. Compostos fenólicos em extratos de *Rosmarinus officinalis* L. sob cultivo fora do solo. Enciclopédia Biosfera, Centro Científico Conhecer - Goiânia, v.9, nº 17, p. 755, 2013.
- [13] GUO, J. et al. Development and Structure of Internal Glands and External Glandular Trichomes in *Pogostemon cablin*. PLOS/ONE, 2013.
- [14] OLIVEIRA, A.B.; MENDONÇA, M.S.; MEIRA, R.M.S.A. Estudo estrutural e farmacognóstico de *Scutellaria agrestis* a. St.-Hil. ex Benth. (Lamiaceae): uma planta medicinal utilizada por populações ribeirinhas do Amazonas. 2011. 109p. Tese (Doutorado em Botânica) - Instituto Nacional de Pesquisas da Amazônia, Manaus - Amazonas.
- [15] OLIVEIRA, S.S.; VANZELERA, M.L.A.; CHIG, L.A. Plantas Medicinales: el Uso de *Barbatimao* - *Stryphnodendron adstringens* (Mart.) Coville. UNICIÊNCIAS, v. 18: nº 2, p. 115-122, 2014.
- [16] PINTO, J.E.B.P. et al. Aspectos morfofisiológicos e conteúdo de óleo essencial de plantas de alfazema-do-Brasil em função de níveis de sombreamento. Horti.bras, v.25, nº 2, 2007.
- [17] LIMA, F.J.; MARTINS, F.M. Anatomia foliar, ontogenia e histoquímica de tricomas glandulares de *Ocimum gratissimum* L. (Lamiaceae). In: Congresso Nacional de Botânica. 2011.
- [18] LIMA, Fernandes Jamile. Anatomia foliar de *Ocimum gratissimum* L., com ênfase na caracterização dos tricomas secretores e conteúdo do óleo essencial. 2010. 53 f. Trabalho de conclusão de curso (Graduação em Ciências Biológicas), Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia. Cruz das Palmas, 2010.
- [19] MAIA, J.G.S.; Zoghbi, M.G.B.; ANDRADE, E.H.A. 2001. Plantas aromáticas da Amazônia e seus óleos essenciais. Belém: Museu Paraense Emílio Goeldi. 173p.
- [20] MARTINS, M.B.G.; MARTINS, A.R. Caracterização de folhas de *Mentha pulegium* x *spicata* (Lamiaceae). Rev. Bras. Pl. Med. Botucatu, v.5, nº 2, p.33-39. 2003.
- [21] METCALFE C.R., CHALK, L. Anatomy of the Dicotyledons. Systematic Anatomy of the Leaf and Stem. USA: Oxford University Press, 1950.
- [22] MILANEZE-GUTIERRE, M.A. et al. Caracterização morfológica dos tricomas foliares e caulinares de duas espécies de Lamiaceae conhecidas popularmente como “falso-boldo”. Acta Sci. Biol. Sci. Maringá, v. 29, nº. 2, p. 125-130, 2007.
- [23] NAVARRO, T. “Trichome Morphology in *Teucrium* L. (Labiatae). A Taxonomic Review.” Anales Jardín Botánico De Madrid, v. 57, nº 2, 2000.
- [24] RAMYA, H. G.; PALANIMUTHU, V.; SINGLA, R. An introduction to patchouli (*Pogostemon cablin* Benth.) – A medicinal and aromatic plant: it's importance to mankind. Agric Eng Int: CIGR Journal. v. 15, n.2, 2013.
- [25] RIVA, D.A.; PETRY, C.; SEVERO, A.M.B. Caracterização anatômica de folhas e inflorescências de espécies de *Lavanda* (Lamiaceae) utilizadas como medicinais no Brasil. Ciência e Natura. v. 36, n. 2, p.120-127, 2014.
- [26] RUSYDI, A. et al. Morphology of trichomes in *Pogostemon cablin* Benth (Lamiaceae). Australian Journal of Crop Science. 2013.
- [27] SANDES, S. S. et al. Estruturas secretoras foliares em patchouli [*Pogostemon cablin* Benth]. Scientia. Plena. v.8, nº 5, p.1-4, 2012.
- [28] SANTOS, P. S. et al. Molecular characterization of germplasm of patchouli (*Pogostemon* sp.) by RAPD markers. Scientia plena, v. 9, nº 5, 2013.
- [29] SANTOS, S. N. Estudo anatômico e histoquímico de folhas de *Ocimum gratissimum* L. Trabalho de Conclusão de Curso (Graduação em Farmácia-Bioquímica), Faculdade de Ciências Farmacêuticas de Araraquara, Universidade Estadual Paulista, Araraquara, SP. 2013.
- [30] SILVA, A.F. Identificação morfoanatômica e código de barras genético de *Hyptis stricta* Benth. (Lamiaceae). Dissertação (Mestrado em Ciências Farmacêuticas) Setor de

- Ciências da Saúde, Universidade Federal do Paraná, Curitiba, 2012.
- [31] SOUZA, V.; LORENZI, H. Botânica Sistemática. 3ª.ed. São Paulo: Instituto Plantarum. 2008.
- [32] SOUZA FILHO, A.P.S. et al. Efeitos potencialmente alelopáticos dos óleos essenciais de *Piper hispidinervium* C. DC. e *Pogostemon heyneanus* Benth sobre plantas daninhas. *Acta amazônica*, v. 39, n. 2, p.389- 396, 2009.
- [33] STORCK, R.C, DESCHAMPS, C. Crescimento e alterações anatômicas e bioquímicas de patchouli em função do regime hídrico e bioestimulantes. 2012. 112p. Tese (Doutorado em Ciências), Universidade Federal do Paraná. Curitiba. 2012
- [34] SILVA, R. M.; FARIA, M. T. Caracterização etnobotânica e histoquímica de plantas medicinais utilizadas pelos moradores do bairro Carrilho, Goianésia (GO). *Enciclopédia Biosfera*. Goiânia, v.10, n.19, 2014.
- [35] TAIZ, L.; ZEIGER, E. Fisiologia vegetal. 3ª ed. Porto Alegre: Artmed, 2004. 820p.
- [36] TOLEDO, M.G.T.; ALQUINI, Y.; NAKASHIMA, T. Caracterização anatômica das folhas de *Cunila microcephala* Benth.(Lamiaceae). *Rev. Bras. Cienc. Farm. Braz. J. Pharm. Sci*, v. 40, n. 4. 2004.
- [37] TOMÁS-BARBERÁN, F.A.; ESPÍN, J.C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of food and Agriculture*, London, v.80, p. 1073-1080, 2000.
- [38] J. Clerk Maxwell (1892), *A Treatise on Electricity and Magnetism*, 3rd ed., vol. 2. Oxford: Clarendon, pp.68–73.
- [39] I. S. Jacobs and C. P. Bean (1963), “Fine particles, thin films and exchange anisotropy,” in *Magnetism*, vol. III, G. T. Rado and H. Suhl, Eds. New York: Academic, , pp. 271–350.