Original Research Article

Structural characterization of mint (*Mentha x villosa* Huds) stem and leaf

Aims: The objective of this study was to identify the structural characteristics of the shoots of *Mentha x villosa* Huds. and also, identify the main structures responsible for the production of active principles.
Methodology: The analyzes were performed with stems and leaves from adult and healthy plants, which presented a uniform morphological pattern. The materials selected for the anatomical analyzes were fixed in FAA for 24 hours and after this period conditioned in 70% alcohol. Cross-sections and paradermic sections were manually made with a cutting blade. For sections analysis, 1% sodium hypochlorite was used for discoloration and safranine 10% dye for tissue staining.
Results: The leaf of *Mentha x Villosa* Huds. presents asymmetric mesophyll, formed by palisade and lacunar parenchyma, and uniseriate epidermis. The diacytic stomata are located on the abaxial surface of the leaves with different sizes, all having a substomatal chamber. On both sides of leaves, glandular trichomes were observed in great quantity. The leaf midrib is formed by a large vascular bundle, with xylem facing the adaxial surface and phloem facing the abaxial surface. The stem presents uniseriate epidermis and below it can be found one or two layers of colenchyma. The vascular bundle consists of four main xylem points, and externally to it is found the phloem, which gives the quadrangular shape to the stem. *Mentha x villosa* Huds. has characteristics that are common to aromatic species of the Lamiaceae family, which makes its characterization and differentiation difficult, as for example, its secretory structures which do not have taxonomic importance for differentiation on this species, since they are similar in other species of the same family.
Conclusion: However, *Mentha x villosa* has a larger number of cell layers in the lacunar parenchyma, which is an important characteristic for the differentiation of species.

Keywords: Lamiaceae, plant anatomy, medicinal plant, quality control.

1. INTRODUCTION

The use of medicinal plants is significantly important, according to the World Health Organization (WHO), 80% of the world's population uses traditional medicine to ease or cure diseases [1] Data from the Ministry of Health of 2006, reported that about 37% of the Brazilian population uses products of natural origin, especially plants [2].

Mint is a species (*Mentha x villosa* Huds) with medicinal and aromatic properties, cultivated throughout the Brazilian territory [3]. It is an herbaceous, low-growing plant with erect branches, opposite and short petiolate oval leaves, with strong and characteristic aroma [4]. This plant is a hybrid originated from the cross between *Mentha spicata* L. and *Mentha suaveolens* Ehlh. Due to the numerous hybrids from spontaneous crossbreeding among its species, it always presented conflicts in its taxonomy, where mint can easily be confused with other species, as happens with *Mentha crispa* L. [5; 4].

Mentha x villosa Huds is a plant from subtropical climate susceptible to intense winters. It requires light soils, rich in organic matter and well drained, daily irrigation is recommended, since high temperatures associated with water deficit, reduce the content of essential oils [4].

Mint is usually sold fresh at fairs and supermarkets like other vegetables. Some accessions are rich in piperitenone oxide, an effective active principle against amebiasis and giardiasis [6]. This plant is asexually propagated and there are no seeds of Mentha x villosa Huds on markets. In Brazil there was a drastic reduction in mint production due to problems of soil fertility and management, considering that nutritional conditions are essential for the balance between biomass accumulation and essential oil production, which are indispensable for a profitable agricultural productivity [7; 8].

Although the phytotherapy efficiency has been proven, the absence of quality, adulteration, and incorrectly use interfere on its efficiency and even in the quality of the product [9], a common concern between the health and scientific community.

Among the causes of poor quality of vegetable material, the biological contamination (fungi, bacteria) and physical (soil), associated with the lack of safe agricultural practices, long and discontinuous drying process and, most of the time, inadequate transport and packaging are among the most important. Besides these problems, the contents of the active principle are confusing due falsification by mixing of other plant organs and even other plant species [10].

Therefore, many elements contribute to the product quality: plant material purity (without adulteration), low contamination by bacteria, fungi, pesticides, absence of radioactivity and heavy metals and adequate concentration of active compounds [11]. Therefore, studies that include the morphological and anatomical characterization are important for the determination of the quality control used in the production of herbal products, especially vegetable drugs [12; 13].

The objective of this study was to anatomically characterize *Mentha x villosa* Huds and also identify the plant structures responsible for the production of active principles.

2. MATERIAL AND METHODS

Shoots of *Mentha x villosa* Huds (mint) were collected in an organic production area in the municipality of Alagoa Nova - PB (07º03'07 "S latitude and 35º45'56" W longitude). The samples were then taken to the Laboratório de Biologia of the Departamento de Agroecologia e Agropecuária - Universidade Federal da Paraíba.

The evaluations were performed on stems and leaves from adult and healthy plants, which presented uniform morphological patterns. For this, a brief description of the general anatomical organization of the leaf and stem of the studied species was made, with emphasis on the identification of secretory tissues.

The materials selected for the anatomical evaluations were fixed in FAA (5% formaldehyde, 5% acetic acid and 90% of alcohol at 70%) for 24 hours and then conditioned in 70% alcohol. Cross sections were made manually with a cutting blade in the median region of the leaf and in the region between the third and the fourth node from the apex of the stem, using embaúba (*Cecropia pachystachya*) petiole and styrofoam (expanded polystyrene) as support. For cross sections analysis, 1% sodium hypochlorite was used as decolorant and safranine 10% dye for tissue staining. The material was mounted on semi-permanent glass slides with glycerin and analyzed in a photomicroscope.

3. RESULTS AND DISCUSSION

The leaf cross-section of *Mentha x villosa* Huds, presents asymmetric mesophyll, formed by palisade and lacunar parenchyma and uniseriate epidermis (Figure 1A).

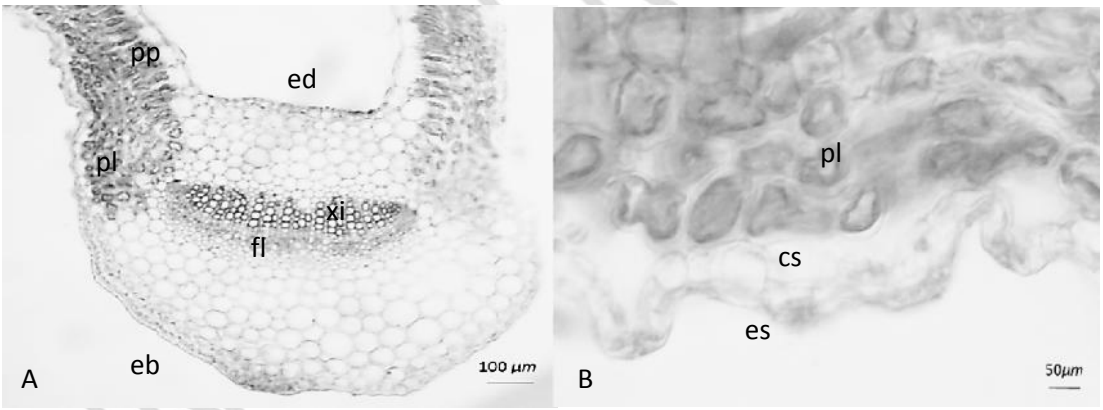

Fig. 1. General structure of the leaf cross-section. A. Leaf of *Mentha x villosa Huds*. B. Abaxial epidermis of the leaf showing stomata with sub-stomatal chambers. (pp - palisade parenchyma, lp - lacunar parenchyma, de - adaxial epidermis, be - abaxial epidermis, ph - phloem, xy - xylem, st - stomata, sc - sub-stomatal chamber).

The palisade parenchyma is formed by one or two elongated cells on the adaxial surface of the leaf, while the lacunar parenchyma is formed by three to five layers of cells of varying sizes. The cells of the epidermis have thick outer periclinal walls and are covered by cuticles. The diacytic stomata are located on the abaxial surface of the leaf and have different sizes, all having a sub-stomatal chamber (Figure 1B). On both leaf surfaces, glandular trichomes were observed in great quantity. The leaf mid rib is formed by a large vascular bundle, which presents two to three layers of collenchyma after the epidermis, with xylem facing the adaxial face and phloem facing the abaxial face.

The stem presents uniseriate epidermis, with the glandular trichomes of various shapes and sizes throughout its surface, and below it there is one to two layers of collenchyma (Figure 2). The vascular bundle consists of four main xylem points, and externally to it is found the phloem, which gives the quadrangular shape to the stem, as already observed in other species of Lamiaceae [14; 15; 16]. The medulla presents rounded parenchyma cells of various sizes.

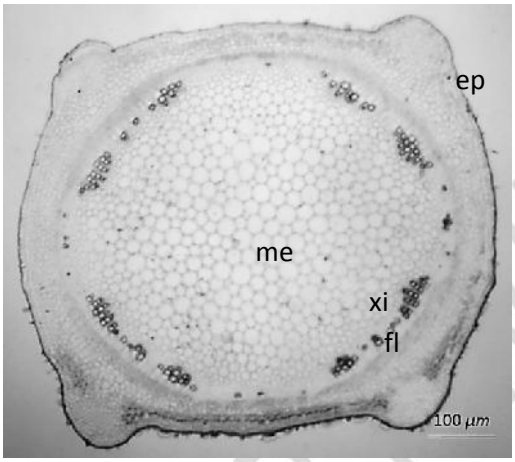

Fig. 2. General structure of Mentha x villosa Huds stem in cross - section (ep – epidermis; me- medulla, xy - xylem, ph - phloem).

Similar characteristics were observed in other species of Lamiaceae. The lamina of *Plectranthus amboinicus* (Lour.) Spreng. presents one to two layers of palisade parenchyma, however, regarding the lacunar parenchyma, *Mentha x villosa* has three to five layers of cells differing from *P. amboinicus* which presents two to three layers of lacunar parenchyma. The stem of *P. amboinicus* also presents characteristics similar to mint, two to three layers of angular collenchyma, below the uniseriate epidermis, formed by tiny cells. The medullary and cortical regions are filled by common parenchyma with meatus [14].

Diacytic stomata are most common in Lamiaceae [17], but anisocytic stomata have already been described on leaves of other species, such as *Hyptis ovalifolia* Benth. and *Hyptis rugosa* Benth. [18].

In the leaves and stem of *Mentha x villosa*, glandular trichomes of two types were found: sessile glandular trichomes with a globular pluricellular secretory apex (Figure 3A) and glandular trichomes with unicellular pedicel and unicellular secretory apex (Figure 3B).

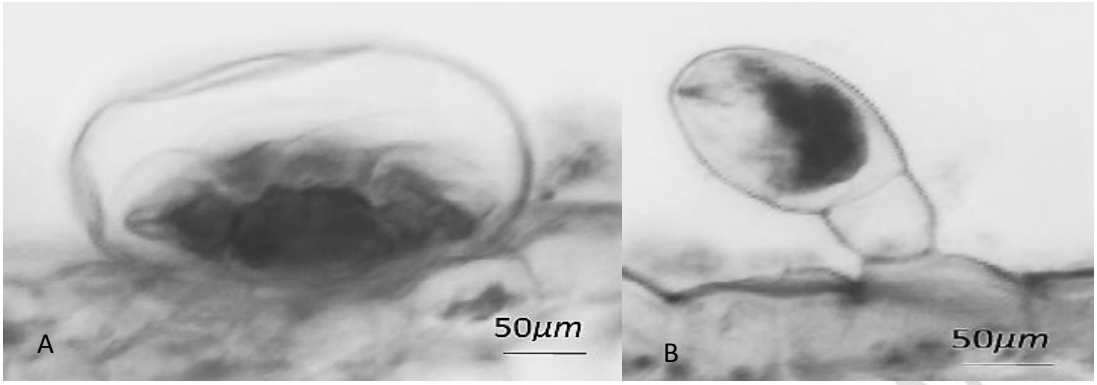

Fig. 3. *Mentha x villosa* Huds secretory structures. A. sessile glandular trichome; B. Glandular unicellular pedicel trichome and unicellular secretory apex.

The trichomes, such as the cuticle and the stomata, are present in different ways according to the conditions offered to the plants, presenting taxonomic importance [19].

The trichomes avoid herbivory and act as an obstacle to those that try to feed on the tissues of the plant, due to its characteristics such as density, shape and size. The glandular trichomes have a vesicular structure that can accumulate substances such as terpenes, gums and tannins, which in contact with predators can trigger several reactions, repelling, provoking limb immobility or even toxicity and death [20].

These results are in agreement with those found by [21], where were found the presence of glandular trichomes pelted and capitated on both surfaces of the epidermis of *Mentha cf. aquatica*, *Mentha x piperita* and *Mentha arvensis*. In addition to glandular trichomes, the species *M. arvensis* presents a high density of tectonic trichomes, a characteristic that distinguishes it from *Mentha x villosa*.

In other species of the Lamiaceae family, such as lavender, several types of tectors and glandular trichomes were found, present in leaves and inflorescences. The tectors trichomes are higher in number than the other types, pluricellular, have sharp, erect or curved tip, and most of them are branched and arborescent [22].

[15], describing *Ocimum basilicum* L. (Lamiaceae), found two types of glandular trichomes: the first located in small depressions in the upper epidermis with bicellular gland and the second located in greater depressions in the inferior epidermis with four gland cells.

4. CONCLUSION

Mentha x villosa Huds. has structural characteristics that are common in aromatic species of the family Lamiaceae, which makes it difficult to characterize and differentiate them, such as their secretory structures which do not have taxonomic importance for the differentiation of this species, since similar structures are found in other species of the Lamiaceae family. However, *Mentha x villosa* has a higher number of cell layers in the lacunar parenchyma, which makes it an important characteristic for the differentiation among species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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