Comparison of leaf morphology and anatomy among *Malva sylvestris* ("gerânio-aromático"), *Pelargonium graveolens* ("falsa-malva") and *Pelargonium odoratissimum* ("gerânio-de-cheiro")

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RESUMO: Comparação da Morfologia e da anatomia foliar entre as espécies *Malva sylvestris* (gerânio-aromático), *Pelargonium graveolens* (falsa-malva) e *Pelargonium odoratissimum* (gerânio-de-cheiro). *Malva sylvestris* é comumente confundida com *Pelargonium graveolens* e *Pelargonium odoratissimum* devido às semelhanças na morfologia foliar. As folhas de *M. sylvestris* possuem antocianinas com propriedades citotóxicas, antiinflamatória, antitumoral e antioxidante já comprovadas cientificamente. As folhas de *P. odoratissimum* apresentam óleo essencial com propriedades antibacteriana e espasmolítica, e o óleo essencial da folha de *P. graveolens* possui atividade antimicrobiana e antifúngica. O objetivo deste estudo foi analisar morfo-anatomicamente as folhas destas espécies, apontando diferenças que possam ser utilizadas para esclarecer controvérsias na sua utilização como planta medicinal. Com a finalidade de se comparar anatomicamente a estrutura de cada planta, as amostras foram observadas por Microscopia de Luz e Microscopia Eletrônica de Varredura (MEV). A anatomia foliar entre as espécies foi bem distinta. *Malva sylvestris* apresentou tricomas do tipo capitado, estrelado e tector, além de drusas e células mucilaginosas. A distinção entre *P. graveolens* e *P. odoratissimum* foi observada em relação aos tricomas. Ambas as espécies apresentaram tricomas glandulares e tectores, sendo que *P. graveolens* se diferencia pela maior altura dos tricomas tectores e menor quantidade destes em relação ao *P. odoratissimum.* Este trabalho permitiu constatar diferenças anatômicas, auxiliando na taxonomia e classificação entre estas espécies.
Palavras-chaves: anatomia foliar, *Malva sylvestris*, *Pelargonium graveolens*, *Pelargonium odoratissimum*, plantas medicinais.

Abstract: *Malva sylvestris* is generally confused with *Pelargonium graveolens* and *Pelargonium odoratissimum* due to similarities in their leaf morphology. The leaves of *M. sylvestris* have anthocyanins with scientifically proven cytotoxic, anti-inflammatory, antitumor and antioxidant properties. The leaves of *P. odoratissimum* have essential oil with antibacterial and spasmolytic properties, while the essential oil from *P. graveolens* has antimicrobial and antifungal activity. The aim of this study was to morpho-anatomically analyze the leaves of these species, indicating differences that can be used to clarify controversies about their use as medicinal plants. To anatomically compare the structure of each plant, samples were observed by Light Microscopy and Scanning Electron Microscopy (SEM). Leaf anatomy among species was quite different. *Malva sylvestris* showed capitate starry tector trichomes, as well as druses and mucilaginous cells. *P. graveolens* and *P. odoratissimum* differed as to trichomes. Both species had tector and glandular trichomes, and *P. graveolens* is distinguished for the greater height of tector trichomes and less quantity of the latter relative to *P. odoratissimum*. This study allowed the detection of anatomical differences, assisting in the taxonomy and classification of these species.

Key words: leaf anatomy, *Malva sylvestris*, *Pelargonium graveolens*, *Pelargonium odoratissimum*, medicinal plants.

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INTRODUCTION

The use of medicinal plants by a traditional community, both in the simplest forms such as tea and in the sophisticated manufacturing industry, transforming them into tablets, drops or capsules with the isolated active principle, is motivated by properties to generate beneficial reactions to the body (Lorenzi, 2008). However, any use requires caution, especially the species identification since they are difficult to distinguish when dehydrated. Recently, considering the dental industry, research has shown the promising use of medicinal plants: *in vitro* studies with hydroalcoholic extract from *Myracrodruon urundeuva*, *Psidium guajava* and *Malva sylvestris* showed potential antimicrobial activity against the biofilm former microorganisms, but also antifungal activity on *Candida* strains isolated from the oral cavity (Alves, 2009).

Malva sylvestris belongs to the family Malvaceae, is native to Europe and is cultivated in southern Brazil. The pharmacological properties of *Malva sylvestris* are concentrated in its leaves. Among them is antimicrobial activity, proven by numerous studies involving tests with ethanolic extracts against *Staphylococcus aureus* (Wang et al., 2006; Quave, 2008), *Bacillus subtilis*, *Pseudomonas albuginea*, *Escherichia coli*, *Saccharomyces cerevisiae* (Souza et al., 2004), *Streptococcus mutans* and *Streptococcus sobrinus* (Alves, 2009).

The action of its leaves also include stimulation of phagocytes (Delaveau et al., 1980), muscle relaxation, uterine stimulation (Calegari, 1942), nociception (Esteves, 2009), cytotoxic activity (Alesiani, 2007) and topical anti-inflammatory action on the skin (Chiclana, 2009).

The large number of pharmacological properties of *M. sylvestris* is justified by the complexity of its composition, which consists of tetrahydroxilatated sesquiterpenes and diterpenes, two monoterpenes, six normal-C13 terpenes and eleven aromatic compounds (Cutillo, 2006). Similarly to the anthocyanin in the leaves, which has a natural potential for degrading free radicals, it serves as antioxidant, reducing total cholesterol, triglycerides in the blood and preventing thrombosis and cardio-cerebral angiopathy (Wang, 2005).

The genus *Pelargonium* (Geraniaceae), according to a review by Knuth (1912), comprises about 250 species, of which 80% occur in southern Africa and the rest in eastern Africa, Madagascar and Australia.

In their chemical constitution, *Pelargonium graveolens* and *Pelargonium odoratissimum* have flavonoids such as quercetin, kaempferol and myricetin, and *P. graveolens* is highlighted for its greater concentration of the first two compounds and proanthocyanidins (Williams et al., 2001). Essential oils are also in their chemical composition, and in *P. graveolens* the most important oils are citronellol and geraniol (Shin & Lim, 2004).

After the experiment of Choi et al. (2007), citronellol acted against the resistant strains of *Streptococcus pneumonia*, reducing the concentration of administered antibiotics and the side effects.

P. graveolens essential oil, in synergy with the antibiotic ketoconazole, may also be recommended as a new herbal antimicrobial because of its strong action against *Trichophyton soudanense* and *Tricchophyton schoenleinii*, reinforcing another case of reduction in synthetic antibiotic use (Shin & Lim, 2004). The antifungal potential of the volatile oil was proven in synergism with amphotericin B against *Candida* sp., resulting in inhibition of all species (Rosato et al., 2008), besides absolute fungitoxic activity against toxigenic strains of *Aspergillus flavus*.

Specifically for *P. odoratissimum*, essential oil composition and bioactivity was evaluated by Balchin and Roth (2000), who identified methyl eugenol as main responsible for its antimicrobial activity and effectiveness against *Staphylococcus aureus, S. epidermidis, Proteus vulgaris* and *Bacillus cereus*.

The pharmacological properties of *Malva sylvestris* are especially due to the presence of anthocyanins in its leaves. The pharmacological activity of *Pelargonium graveolens* and *Pelargonium odoratissimum* is due to their essential oils.

Due to the widespread medicinal use of these species - *Malva sylvestris, Pelargonium graveolens* and *Pelargonium odoratissimum* - in the antimicrobial, anti-inflammatory and anti-fungal field and the absence of their anatomical characterization, we carried out a comparative study of the leaf morphology and anatomy among them in order to clarify controversies on their use as medicinal plants, especially when they are dehydrated.

MATERIAL AND METHODS

Adult leaves of the three studied species were collected from the Garden of Medicinal Plants of ESALQ-USP, Piracicaba-São Paulo State, and transversely sectioned at the midrib region.

For the processing of permanent slides, fresh samples were fixed in 70% FAA (formaldehyde, acetic acid and 70% alcohol) and placed under vacuum for 24 hours. The leaves used for diaphanization were previously kept in 70% alcohol until they were processed. For scanning electron microscopy, samples were fixed in Karnovsky buffer with sodium cacodylate, pH 7.2, and prepared with

10% paraformaldehyde and 2.5% glutaraldehyde for 24 hours.

Light Microscopy preparation consisted in cross and paradermal sections by diaphanization. For transversal sections, samples fixed in 70% FAA were dehydrated in an ethanol series (70, 80, 90, 100%). They were then subjected to GMA resin infiltration (glycol methacrylate) and embedded in resin blocks which were sectioned in a rotation microtome to 8-μm cross sections. The dye 1% toluidine blue with 1% sodium borate in 100 ml of distilled water (Gerrits, 1964) was used, followed by Entellan mounting. A parallel test was performed by using immunoperoxidase on the microtome sections; the slides were stained with 2% methylene blue, washed and also mounted in Entellan.

Diaphanization was based on technique II of Felipe & Alencastro (1966), i.e., the leaves were washed in distilled water, placed in 5% sodium hydroxide (NaOH) and then cleared in 20% sodium hypochlorite until the samples become transparent. The used dye was 0.5% aqueous Safranin for 2 hours, followed by ethanol series (30, 50, 60, 70, 80 and 96%) and Entellan mounting.

The sample preparation for visualization under a scanning electron microscope consisted of washing with distilled water and dehydration in ethanol series (30, 50, 70, 90, 100%). Then, the material was dried to the critical point with carbon dioxide and metallic gold. The samples were analyzed under a scanning electron microscope LEO 435 VP Variable Pressure. Preparation and analysis of the samples were performed at the Center for Research Support / Electron Microscopy Applied to Agricultural Research (NAP / MEPA), located at ESALQ-USP, Piracicaba-SP.

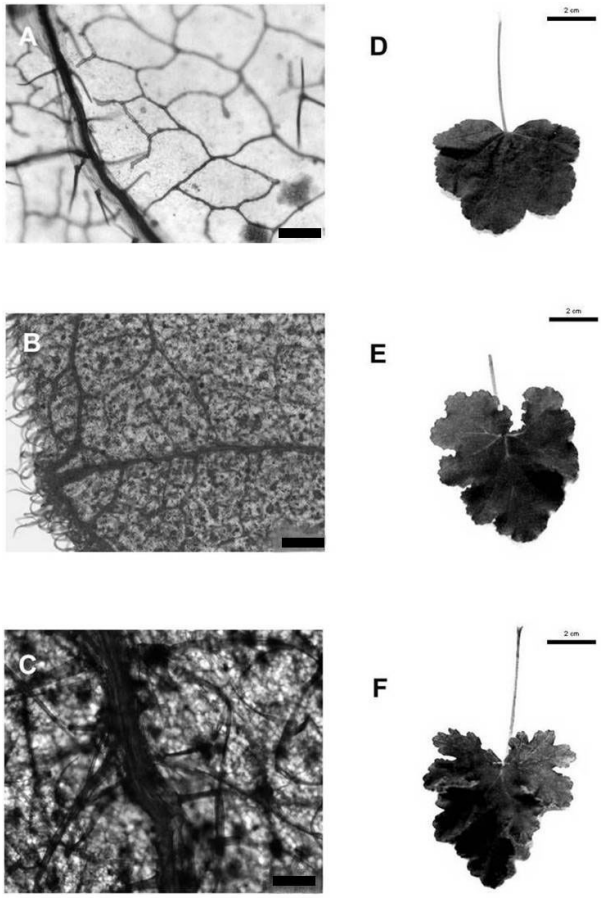

FIGURE 1. Morphology of the three studied species. A and D: *Malva sylvestris*; B and E: *Pelargonium odoratissimum*; C and F: *Pelargonium graveolens*. A-C: Diaphanized leaf cross sections (bar = 100μm) and D-F: General aspect of mature leaves (bar = 2 cm).

RESULTS AND DISCUSSION

As to general morphology (Figure 1, DF), *Malva sylvestris, Pelargonium graveolens* and *Pelargonium odoratissimum* have a large number of similarities such as single leaves, which are symmetrical, with broadly obtuse basal angle and basal petiole. There are differences not only between *M. sylvestris* and *Pelargonium* sp. but also among *Pelargonium* sp. The species *P. graveolens* is distinguished for showing a petiole dilated into a cone shape, while the other two have truncated base. *M. sylvestris* is distinguished for its obtuse angle and apical margin with laminar hair, while others have acute apical base and toothed margins. In diaphanized leaves (Figure 1, AC), the characteristic translucent tissue clearly indicates that *M. sylvestris* leaves are less thick than those of *P. graveolens* and *P. odoratissimum*.

Light microscopic analysis indicated that *Malva sylvestris* have grained biconvex rib, while *Pelargonium graveolens* and *Pelargonium odoratissimum* have concave and convex rib (Figure 2 A-C). *M. sylvestris* has in the rib a large amount of mucilage and capitate glandular trichomes (Figure 2A), while *Pelargonium odoratissimum* has a large number of short trichomes along the midrib and druse (Figure 2B). In *Pelargonium graveolens*, there are also druses and trichomes which are more scattered but longer; capitate trichomes associated with the rib can also be observed (Figure 2C).

The mesophyll of *M. sylvestris* shows the vein, and clusters of calcium oxalate crystals can also be observed (Figure 2D). In the mesophyll of *P. odoratissimum*, besides the structures mentioned in the rib, there are capitate trichomes (Figure 2E); *P. graveolens* has fewer trichomes, with only one druse (Figure 2F). The three species are hypostomatic.

Scanning electron micrographs, due to the broad spectrum of growth on leaf surfaces, allowed

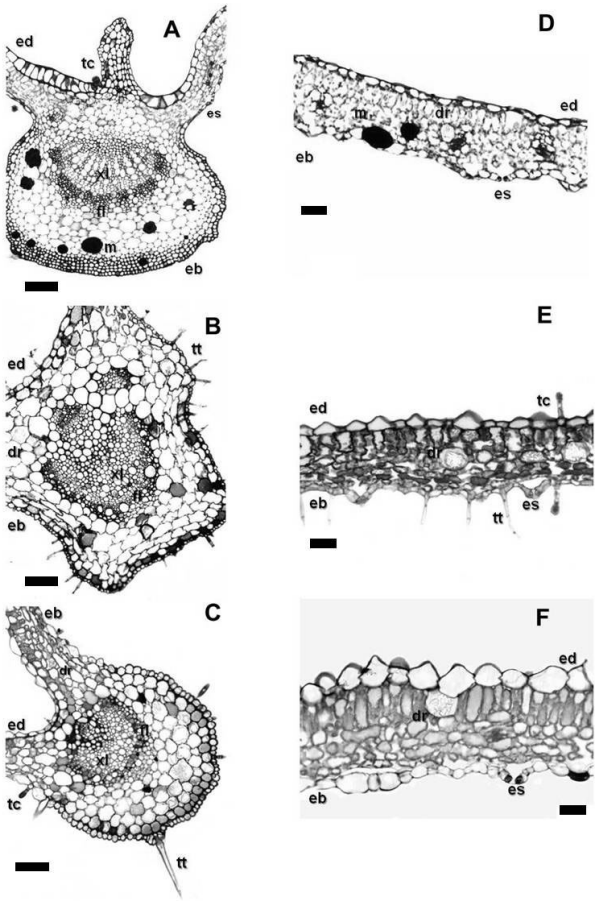

FIGURE 2. Cross sections of the leaf. A and D: *Malva sylvestris*; B and E: *Pelargonium odoratissimum* and; C and F: *Pelargonium graveolens*. A-C: Midrib region (bar= 100 μm) and D- F: Mesophyll region (bar=30μm). ed: adaxial epidermis, b: abaxial epidermis, s: stoma, fl: phloem, xl: xylem, dr: druse, m: mucilage, tc: capitate trichomes and tt: tector trichomes.

the observation of greater complexity and details of ultra-structures, some of which were not visible in the cross-sections, such as starry rib trichome associated with *M. sylvestris* (Figure 3A) and three types of capitate trichomes in *P. graveolens* (Figure 3C). In *P. odoratissimum*, there are numerous tector and a capitate trichome (Figure 3B).

Corroborating this study, which states that mucilage cells, druse with calcium oxalate, trichomes with more than one branch, and glandular peltate and capitate trichomes are characteristic of Malvaceae, studies have point out the same for *Kitaibelia balansae* (Guven & Duman, 2005) and for the genus *Sida* (Shahenn et al., 2009).

As regards the variety of trichomes in Geraniaceae, Stark (1975) noted four different types of trichomes, all on both surfaces of leaves (Waiters, 1989): long tector, short curved, short glandular, and totally glandular, of which the latter is most frequent (Stafford & Gibby, 1992).

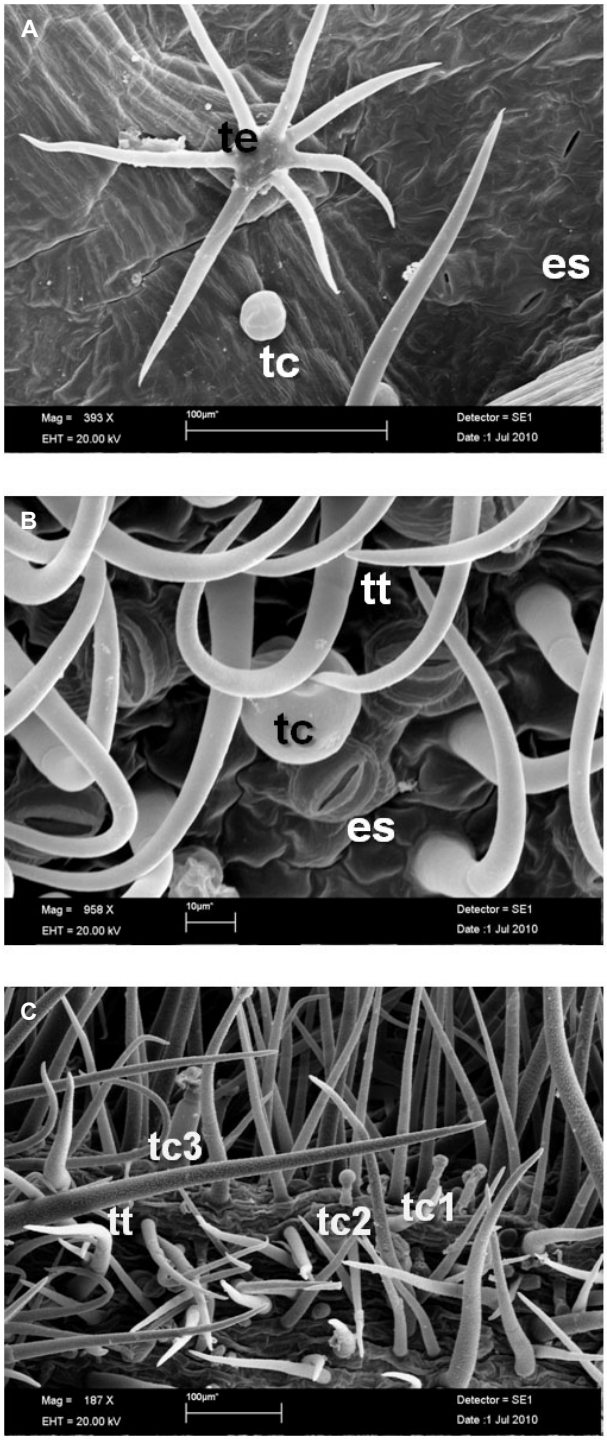

FIGURE 3. Scanning Electron Micrograph of the abaxial surface. A- Malva sylvestris, B- *Pelargonium odoratissimum* and C- *Pelargonium graveolens*. es: stomata, te: starry trichome, tt: tector trichome, tc1: capitate trichome type 1, tc2: capitate trichome type 2, tc3: capitate trichome type 3.

As to trichomes, the anatomical differences between species are very significant. The length of the capitate trichome is one of the most easily visible features, which is longer in *M. sylvestris*, followed

by *P. odoratissimum* and *P.graveolens*, and justified by the scanning electron micrographs (Figure 4 GI). There are also distinction as to the layout of cells in the trichome of *M. sylvestris*; it has a basal cell, two and a stalked apical uniseriate spherical cell (Figures 4A and 4B), while *P. odoratissimum* has a trichome composed of a basal cell, three pedunculated, a paste and a more spherical apical cell (Figures 4C and 4D). *P. graveolens* has trichome composed of a basal cell, two short stalk and an elliptical apical cell (Figures 4E and 4F).

Histochemical examination with methylene blue evidenced the presence of mucilage in the three species (Table 1); in *M. sylvestris* it is in the vein, mesophyll and glandular trichomes, agreeing with the studies of Guven & Duman (2005) and Shahenn et al. (2009) for species of the same family. In *P. odoratissimum* and *P. graveolens*, the mucilage is present only in glandular trichomes; it was also observed by Stark (1975) and Waiters et al. (1989) as a potentially acaricide viscous secretion within the genus *Pelargonium*.

The main anatomical differences between *Malva sylvestris* and *Pelargonium* sp. are horsehair laminar margin; innervation pattern; mucilage in the vein and mesophyll, and druses only in the rib since

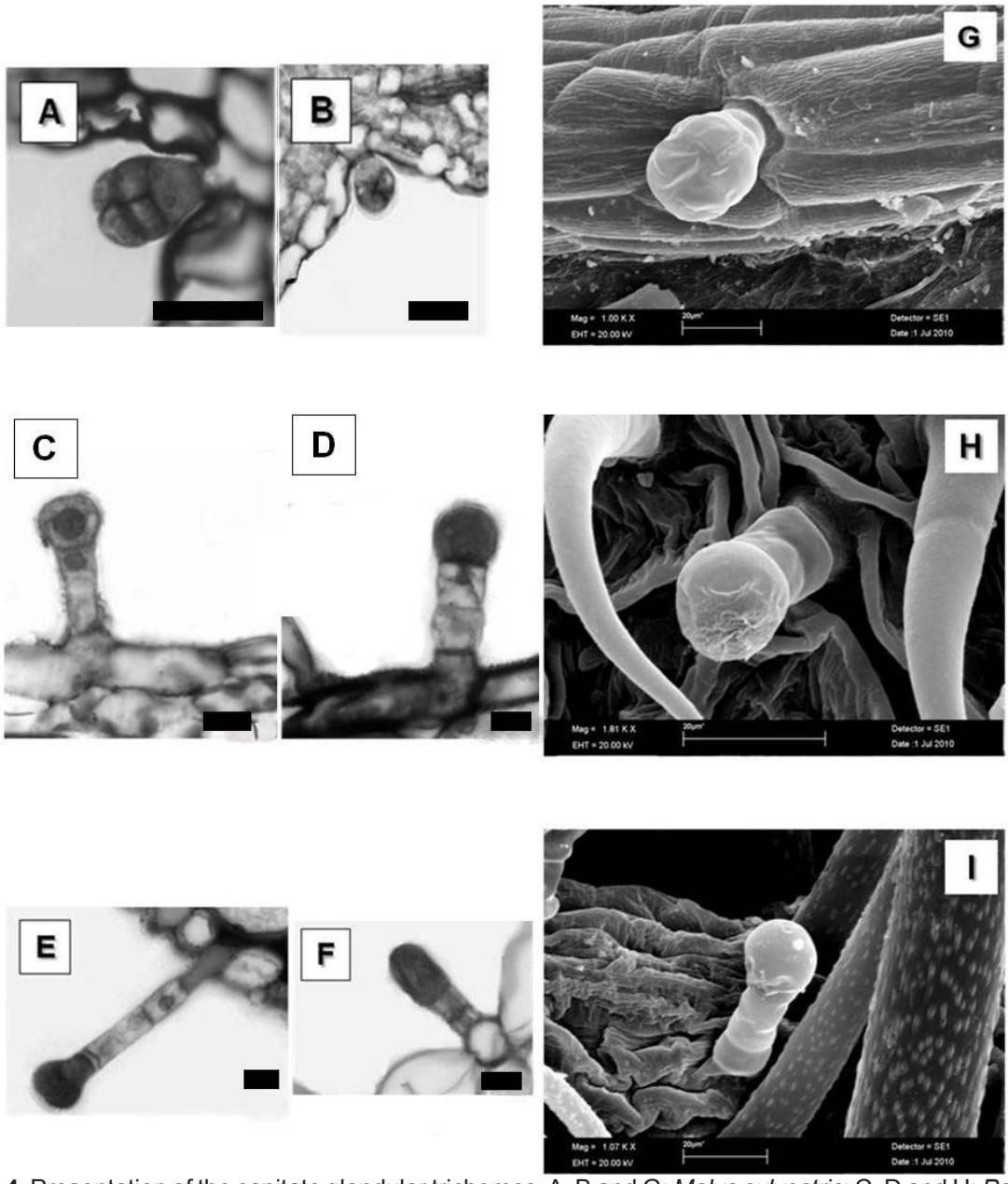

FIGURE 4. Presentation of the capitate glandular trichomes. A, B and G: *Malva sylvestris*; C, D and H: *Pelargonium odoratissimum*; E, F and I: *Pelargonium graveolens* A-F: Transversal sections of the leaf (bar = 10 μm) and G-I: Scanning Electron Micrograph, G and H: adaxial surface, and I: abaxial surface.

TABLE 1. Mucilage location in the leaves of *Malva sylvestris*, *Pelargonium odoratissimum* and *Pelargonium graveolens*.

Structure	*Malva sylvestris*	*Pelargonium odoratissimum*	*Pelargonium gravolens*
Rib	+	-	-
Mesophyll	+	-	-
Glandular trichomes	+	+	+

what distinguishes *Pelargonium odoratissimum* from *Pelargonium graveolens* are: greater length and smaller amount of glandular trichomes.

This study allowed the detection of anatomical differences, assisting in the taxonomy and classification of these species and preventing errors in their use as medicinal plants.

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