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# Contribution to the Microscopic Study of Three Plant Species (Parsley, Spanish Scolyme and White Marrube) Commonly Used in Traditional Algerian Medicine

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**Abstract**

The present work aims to study the anatomical characteristics of three plant species used in Algeria for their diuretic and antilithiasic properties. Also, to summarize the results obtained in detailed drawings of each part studied as well as diagrams with conventional tissue signs. Therefore, *Petroselinum crispum* (Mill.) Fuss (Apiaceae), *Scolymus hispanicus* L. (Asteraceae) and *Marrubium vulgare* L. (Lamiaceae) were obtained from the Tlemcen (northwestern Algeria) region. In the study, stems and leaves of *P. crispum* and *M. vulgare*, and leaves of *S. hispanicus* were used. Once the species was identified and examined, cross-sections were taken and stained using the double staining technique, then observed under an optical microscope. It was generally observed that the parsley leaf did not have any secretory trichomes, and the secretory duct in the midrib was located between the phloem and the lower epidermis. It was also noted that there were no secretory trichomes in the Spanish scolyme. In addition, branched covering trichomes and secretory trichomes with octacellular head were observed in the leaf of white horehound. These findings are certainly going to help enrich the rare bibliographic data available on the anatomy of these three species.

**Keywords:** Algerian flora, *Marrubium vulgare*, *Petroselinum crispum*, Plant anatomy, *Scolymus hispanicus*.



## 1. Introduction

Parsley, Spanish scolyme and white horehound are among the most widely used plants in traditional Algerian and North African medicine (Belakhdar, 1998; Elyebdri et al., 2017) for their diuretic and anti-lithiasic properties (Lahsissene et al., 2010; Tahri et al., 2012; Zeggwagh et al., 2013; Ouis et al., 2014).

According to Bentham (Agyare et al., 2017), Algeria is among the countries where parsley originated. This plant is widely used in the country's cuisine. It should be noted that its fresh leaves are an excellent anti-inflammatory and a very good antioxidant. In addition, its seeds are often used for disinfection and relief from insect bites (Stitou, 2016). Parsley is an excellent anti-allergic plant because it inhibits the secretion of histamine (Pharmacopée française, 2012). Several studies have also shown that parsley has an anti-cellulite action, as it promotes the decrease in fat reserves accumulated in the

body. On the other hand, according to the results from recent research, it could even limit the risk of cancer, by helping the body to get rid of its toxins on a daily basis (Pharmacopée française, 2012). Furthermore, *Scolymus hispanicus* L. is widely distributed in the country (Vázquez, 2000). Its extract is also believed to have antispasmodic and antioxidant effects (Benchiheub, 2015). In Algeria, white horehound is very widespread; it grows mostly in rubble and hedges. Its ethanolic extract was shown to possess mild anti-inflammatory properties. As for marrubin, it would have expectorant and bronchial fluidifying properties (Djahra, 2014). In tachyarrhythmias and arrhythmias, white horehound is used for its cardiosedative effect (Aït Youssef, 2006).

Despite the wide availability of these species and the numerous studies conducted on the chemical composition and biological activity of their extracts (Sahpaz et al., 2002; Zhang et al., 2006; Boudjelal et al., 2012; Snoussi et al., 2016; Aboukhalaf et al., 2020; Kandil et al., 2020), it was not easy to find sufficient data on their anatomy, which made their microscopic identification difficult. Thus, the present study aims to highlight the anatomical characters of the used parts of these species in order to facilitate their identification and avoid confusion with other similar species.

## 2. Material and Methods

The aerial parts of *Petroselinum crispum*, *Marrubium vulgare* and *Scolymus hispanicus* were obtained from herbalists in the wilaya of Tlemcen (northwestern Algeria). They were then identified according the data published in The Flora of Algeria (Quezel et Santa, 1963). Samples were stored in the Pharmacognosy laboratory – Pharmacy department of Tlemcen. The scientific names of the species were verified against the database (http://www.theplantlist.org/).

The studied parts were stored in a mixture of equal volume of ethanol and glycerin (Ruzin, 1999).

Afterwards, several cross-cuts were made by performed on these samples, using a blade, in the thinnest possible way. They were then put in distilled water to prevent them from drying out, then in sodium hypochlorite to empty the cells of their contents. They were subsequently placed in 10% acetic acid to allow fixation of the dyes (Langeron, 1949) .

Once they were washed with distilled water, they were stained using the Mirande double staining technique (Langeron, 1949) and placed successively in two dyes, i.e. iodine green and alum carmine, for 1 min and 20 min, respectively. It was made sure that they were carefully washed after each step with distilled water.

The sections were then placed between slide and coverslip with a drop of glycerin so they can be observed using a Leica DM300 optical microscope. The diagrams have been made with the conventional tissue signs.

## 3. Results and Discussion

### 3.1. Parsley; *Petroselinum crispum* (Mill.) Fuss

#### 3.1.1. Leaf transverse section

The midrib protrudes on both sides, forming a triangle at the top and bottom. It is characterized, from the outside to the inside, by:

- Two upper and lower epidermises formed of more or less rounded cells, with a thin cellulose wall (figures 1- c, 1 - b). It is covered by a thick cuticula. The round sub-epidermal collenchyma is formed by several layers of thick cellulose-walled cells in the protruding parts of both sides (Fig. 1- a, b, c). The cortical parenchyma is found next. It is made up of several layers of large, rounded to polygonal thin-walled cells, leaving inter-cellular spaces between them. It is rich in

chloroplasts. A single secretory duct is located between the phloem and the lower epidermis (1-c) which is characteristic of Apiaceae (Perrot, 1944). This position is an important taxonomic feature(Akpulat et al., 2014). The rounded vascular bundle is in the middle (1-d). In the leaf blade, the two epidermises consist of thin-walled rectangular cells, larger than those of the midrib, like those found in other Apiaceae (Akpulat et al., 2014). The mesophyll is dorsiventral, criteria common to Apiaceae according to Perrot (1944). It is very rich in chloroplasts, with a single layer of palisade parenchyma (Figure 1 - e). The absence of secretory trichoms is also common to Apiaceae (Em. Perrot, 1944) . These results are similar to those found in the same species collected in Brazil in the Alta Floresta region (Larocca, 2013).

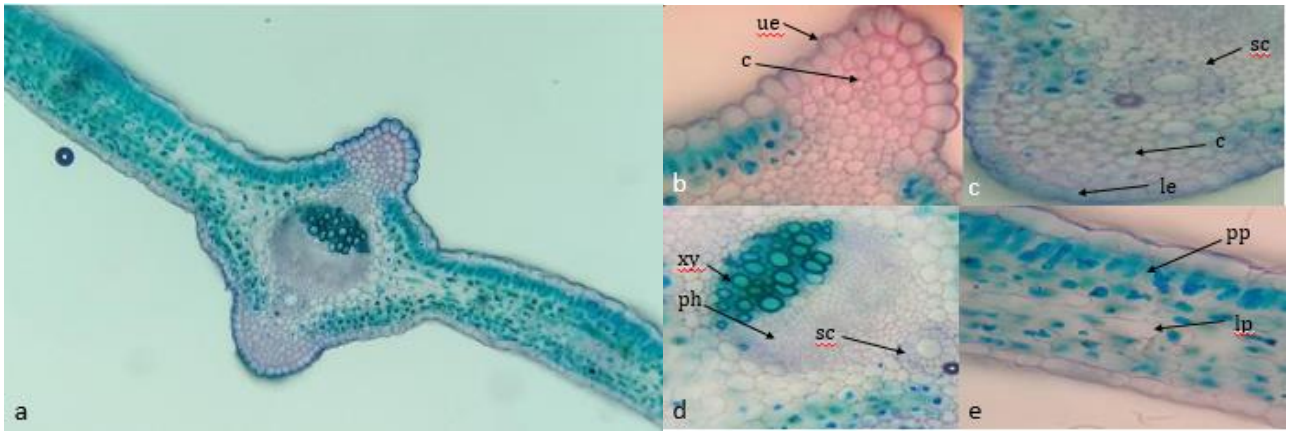


**Figure 1. Anatomical characterization of the transverse section of the leaf of *P. crispum***

*1- a: General view at magnification (4 x10); 1- b: Upper epidermis with collenchyma; 1- c: Lower epidermis with collenchyma and secretory canal; 1-d: Vascular bundle; 1-e: Dorsiventral mesophyll.ue: upper epidermis; c: collenchyma; sc: secretory canal; le: lower epidermis; xy: xylem; ph: phloem; pp: palisade parenchyma; lp: lacunar parenchyma.*

### 3.1.2. Stem transverse section

This part presents a pentagonal shape with bilateral symmetry (figure 2 - a). It includes, from the outside to the inside, a cortical part that is delimited by an epidermis formed of cells, more or less rounded, with a cellulose wall.

After the angular sub-epidermal collenchyma at the angles (Figure 2 - b), there is the cortical parenchyma with intercellular spaces. It is formed by several layers of round to polygonal shaped cells, with a large size thin wall, in which several secretory ducts are found (Figure 2 - c). It is a feature that is common to Apiaceae (Perrot, 1944).

In addition, there are seven vascular bundles in the central cylinder, where the phloem and the xylem are superimposed. Below the xylem, there is an inner phloem (figure 2 - e). The rest is filled with a medullary parenchyma that is composed of polygonal to round shaped cells with a thick wall, leaving spaces between them. Although a hollow pith in the stems of species belonging to the family Apiaceae is often found (Metcalfe et al., 1950), no stem void has been observed in this species. The medulla is devoid of secretory ducts. These features have also been found in another species of the Apiaceae family: *Peucedanum graminifolium* Boiss. (Akpulat et al., 2014).

In this context, Svoboda et al, (2000) reported that Apiaceae have secretory ducts extending from the roots through the stem, leaves and fruits. This was found in cross sections of the stem and leaf of *P. sativum*. Furthermore, there is a single vascular bundle and secretory duct in the center of the leaf of the species studied, a characteristic feature of the Apiaceae (Mavi et al., 2019).

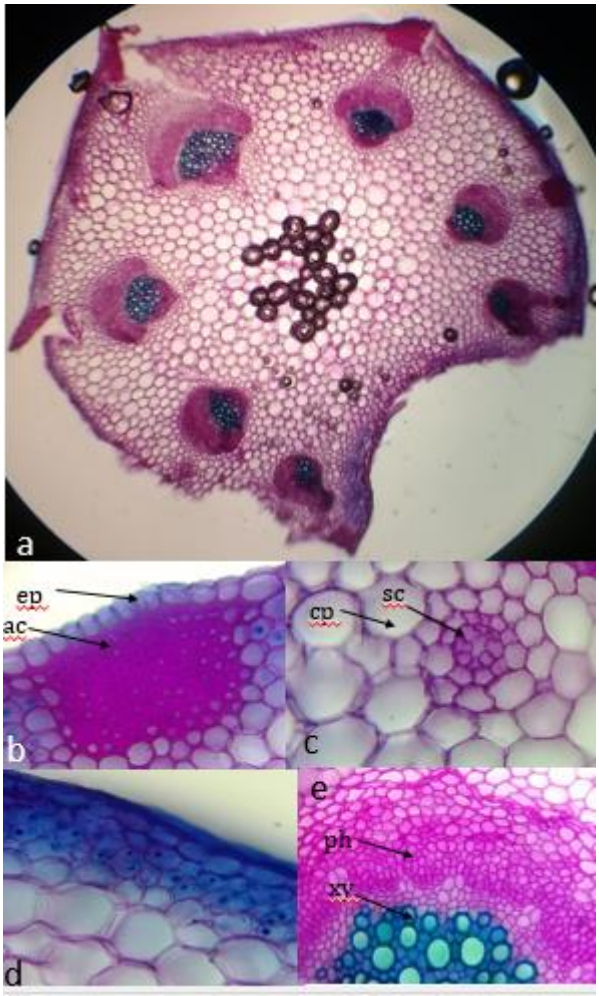


**Figure 2. Anatomical details of the transverse section of the stem of *P. crispum***

*1- a: General view at magnification (4 x10); 2 - b: Epidermis with angular collenchyma; 2 - c: Cortical parenchyma with secretory canal; 2 - d: Parenchyma rich in chloroplasts; 2 - e: Details of the vascular bundle ep : epidermis ; ac : angular collenchyma ; sc : secretory canal ; cp: cortical parenchyma; ph: phloem; xy: xylem.*

### 3.2. Spanish Scolyme; *Scolymus hispanicus*

The transverse section of the leaf of *S. hispanicus* exhibits a triangular midrib that is prominent on the upper part, and very prominent on the lower part (Figure 3 - a). Under the two epidermises comprising polygonal cells with a thick cellulose wall, there are two to three layers of angular collenchyma (Figure 3 - b). Next, we find the cortical parenchyma formed by more or less round cells with a thin wall (Figure 3 - c). In the middle of this parenchyma, each one of the three vascular bundles is surrounded by a sheath of angular collenchyma (Figures 3-d, 3-e). It was clearly noticed that the glandular trichomes are absent, contrary to what is usually found in the Asteraceae (Perrot, 1944). While the covering trichomes are abundant on both sides of the leaf. These trichomes are uniseriate, multicellular, and smooth-walled. Sometimes the basal cells are biseriate (Figure 3 - f). In the leaf blade, between the two epidermises with flattened cells, there is a dorsiventral mesophyll with a palisade parenchyma layer and lacunar chlorophyll parenchyma layers (Figures 3 - g, 3 - h). This mesophyll is common of the Asteraceae (Kadereit et al., 2007; Ozcan, 2015; Metcalfe et al., 1950). Many researchers have reported the existence of accessory vascular bundles in Asteraceae species (Ozcan, 2015). Their presence meets the requirements for translocation under unfavorable conditions (Sidhu et al., 2011).

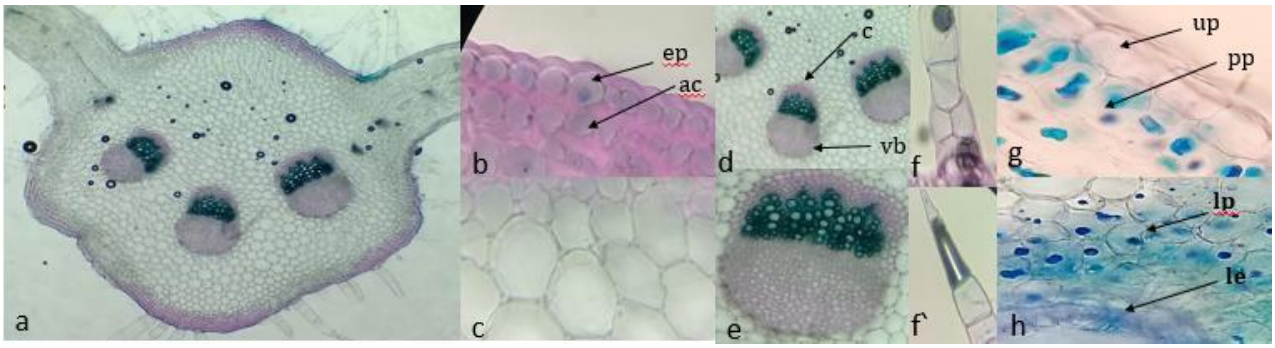


**Figure 3. Anatomical characteristics of the transverse section of the leaf of *S. hispanicus***

3 - a: General view at magnification (4 × 10); 3 - b: Epidermis with angular collenchyma; 3 - c: Cortical parenchyma; 3 - d: Collenchyma surrounding the vascular bundle; 3 - e: Vascular bundles; 3 - f: Tector trichomes; 3 - g: Upper epidermis of the leaf blade and palisade parenchyma; 3 - h: Lower epidermis of the leaf blade and lacunous parenchyma; ep: epidermis; ac: angular collenchyma; c: collenchyma; vb: vascular bundle; up: upper epidermis; pp: palisade parenchyma; lp: lacunar parenchyma; le: lower epidermis.

### 3.3. White horehound; *Marrubium vulgare*

#### 3.3.1. Leaf transverse section

The midrib is very prominent on the lower face and depressed on the upper face. The leaf blade in not very thick (Figure 4 - a). In the midrib, the two upper and lower epidermises, formed by thin-walled contiguous rectangular and rounded cells, and covered by a thin cuticula, surround the round collenchyma, thus occupying a larger area in the upper part (Figures 4 - b, 4 - c). Oval or rectangular epidermal cells were also encountered in species of the same genus as *M. trachyticum* (Akçin et al., 2018). A cortical parenchyma with intercellular spaces, rich in chloroplasts, and made up of several layers of more or less rounded cells with thin walls is found just below (Figure 4 - c).

This parenchyma contains the vascular bundle (Figure 4 - d). The epidermal cells in the leaf blade are larger than those in the midrib. In contrast, the cells of the lower epidermis are smaller than those of the upper one. There is also a bifacial mesophyll, usually found in Lamiaceae (Perrot, 1944), with a single palisade parenchyma layer (Figure 4 - e). The presence of trichomes was observed on both sides of the leaf blade. They were more frequent on the underside and more numerous than in the midrib. These were secretory trichomes with octacellular head and unicellular foot (Figure 4 - g) common to Lamiaceae (Gul et al., 2019). They are embedded in the epidermis, which is in accordance with the findings of Gul et al. (2019).

This type of trichomes was also found by Haratym et al. in a study conducted on the species *M. vulgare* from the Lublin region in Poland. Studies conducted on the chemical composition of their secretory contents have shown the presence of lipids, polysaccharides, polyphenols and terpenes (Haratym et al., 2017).

Also secretory trichomes with bicellular head and unicellular foot (Figure 4 - f), uniseriate and unicellular smooth-walled covering trichomes (Figure 4 - i), and branched covering trichomes (Figure 4 - h) which have previously been reported in other genera of Lamiaceae such as *Stachys* L.(Salmaki et al., 2009), *Nepeta* L. (Jamzad et al., 2003), *Lavandula* L. (T. Upson et al., 2004), and in species of the same genus as *M. trachyticum* (Akçin et al., 2018), *M. friwaldskyanum* and *M. peregrinum* (Gyuzeleva et al., 2022). It is worth noting that the stomata were present on both sides of the leaf.

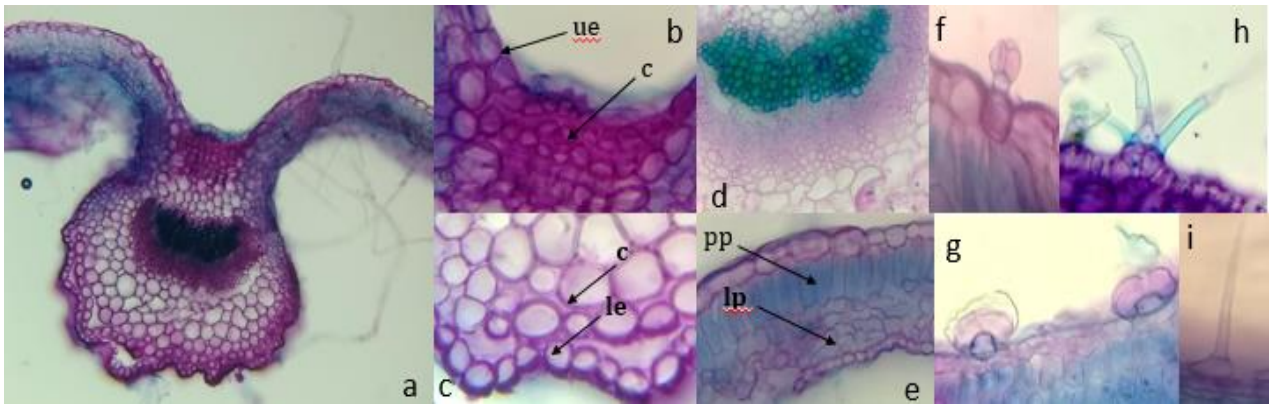


**Figure 4. Anatomical features of the transverse section of the leaf of *M. vulgare***

4 - a: General view at magnification (4 × 10); 4 - b: Upper epidermis and collenchyma of the midrib; 4 - c: Collenchyma and lower epidermis; 4 - d: Vascular bundle; 4 - e: Leaf blade (palisade and lacunous parenchyma); 4 - f: Glandular trichome with unicellular foot and bicellular head; 4 - g: Glandular trichome with octacellular head; 4 - h: Branched tector trichome; 4 - i: Unicellular uniseriate tector trichome.
ue: upper epidermis; c: collenchyma; le: lower epidermis; pp: palisade parenchyma; lp : lacunar parenchyma.

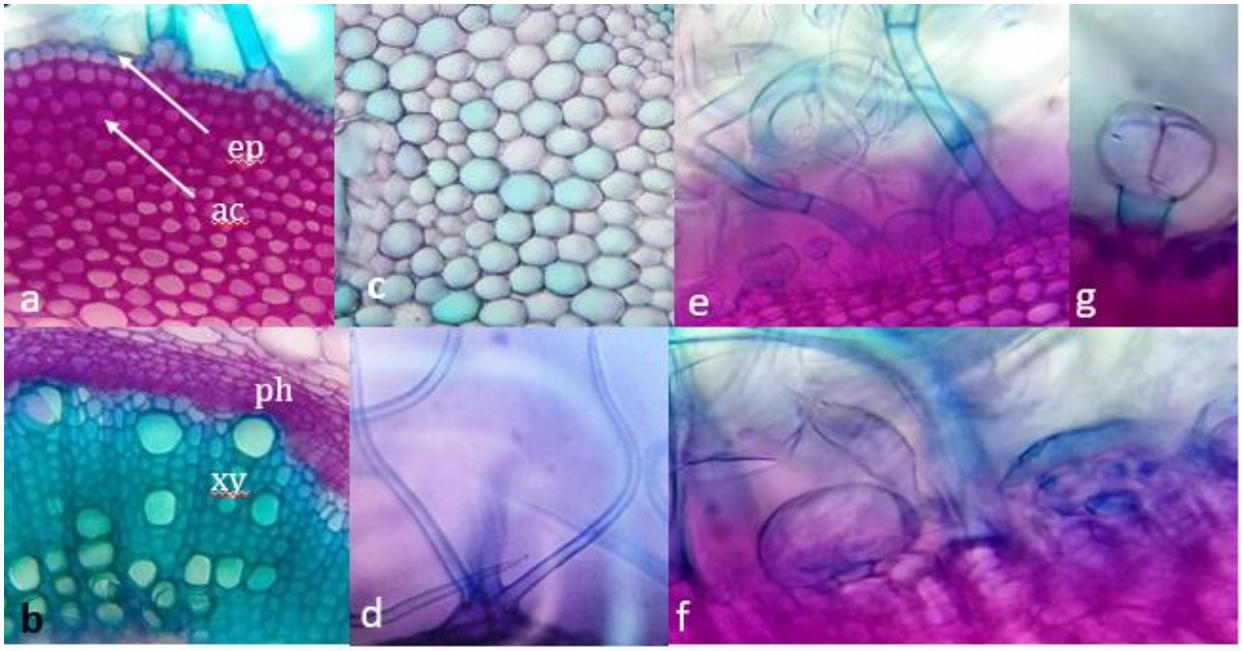


**Figure 5. Anatomical features of the transverse section of the stem of *M. vulgare* at magnification (4 × 10)**

5 - a: Epidermis and angular collenchyma; 5 - b: Vascular tissues; 5 - c: Parenchyma with intercellular spaces; 5 - d: Branched covering trichome; 5 - e: Multicellular uniseriate covering trichome; 5 – f: 5-g: Glandular trichome with unicellular foot and octacellular head;
Ue: upper epidermis; c: collenchyma; le: lower epidermis; pp: palisade parenchyma; lp: lacunar parenchyma.

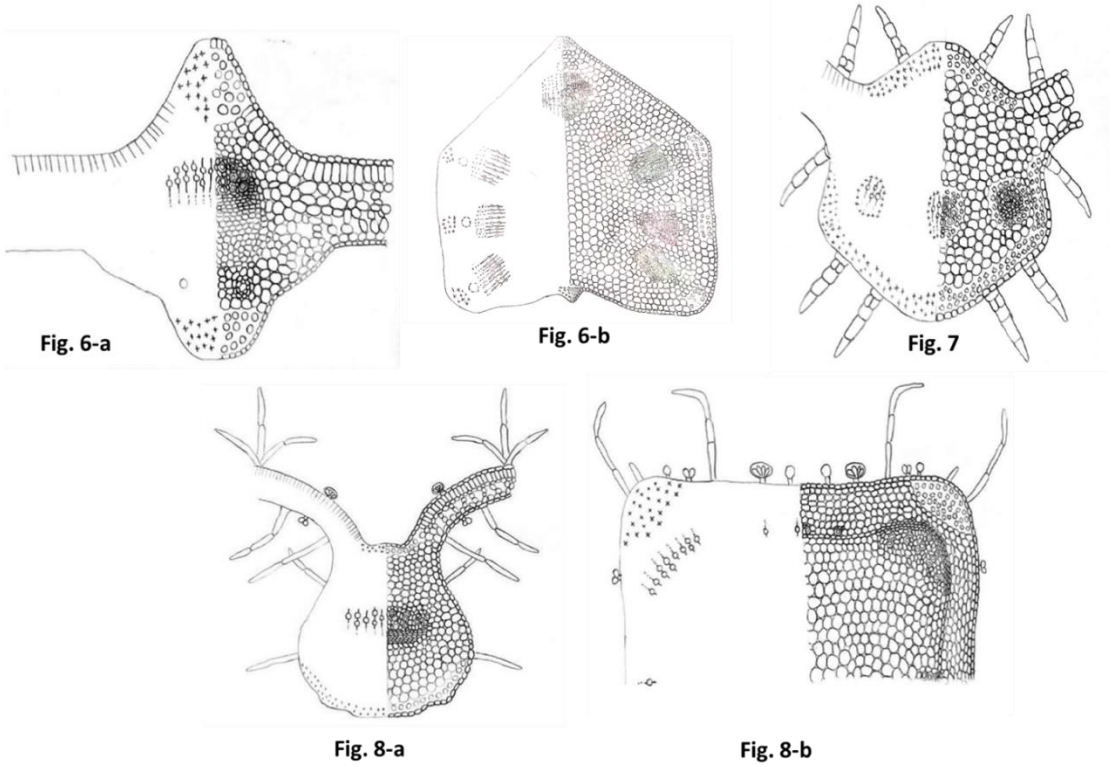


**Figure 6- a:** Drawing and general diagram of the transverse section of the leaf of *P. crispum* (Apiaceae) at magnification (40 × 10); **Figure 6-b:** Drawing and general diagram of the transverse section of the stem of *P. crispum* (Apiaceae) at magnification (40 × 10); **Figure 7:** Drawing and general diagram of the transverse section of the leaf of *S. hispanicus* (Asteraceae) at magnification (40 × 10); **Figure 8-a:** Drawing and general diagram of the transverse section of the leaf of *M. vulgare* (Lamiaceae) at magnification (4 × 10); **Figure 8-b:** Drawing and general diagram of the transverse section of the stem of *M. vulgare* (Lamiaceae) at magnification (4 × 10)

**Table1. Anatomical characters of the three species studied**

| Character | Localisation and description |
|---|---|
| *P. sativum*<br>**Leaf** | |
| Trichomes | Absent |
| Calcium oxalate crystals | Absent |
| Secretory duct | Only one located between the phloem and the lower epidermis |
| **Stem** | |
| Trichomes | Absents |
| Secretory canals | In cortical parenchyma |
| Vascular bundle | In the number of seven |
| Internal phloem | Below the xylem |
| *S. hispanicus*<br>**Leaf** | |
| Trichomes | Abundant on both sides |
| Vascular bundles | In the number of three |
| *M. vulgare*<br>**Leaf** | |
| Trichomes | -Secretory trichomes with octacellular head and unicellular foot<br>-Secretory trichomes with bicellular head and unicellular foot<br>-Uniseriate and unicellular smooth-walled tector trichomes<br>-Branched tector trichomes |
| **Stem** | |
| Trichomes | -Branched tector trichomes<br>-Uniseriate multicellular tector trichomes<br>-Secretory trichomes with unicellular foot and bicellular head<br>-Secretory tricomes with unicellular foot and octacellular head |
| Vascular bundles | Continuous ring |

### 3.3.2. Stem transverse section

The cross section of the white horehound stem is quadrangular, which is characteristic of both Lamiaceae (Perrot, 1944) and the genus *Marrubium* (Buyukkartal et al., 2016). It has a cortical part on the outside with an epidermis formed by a single layer of more or less rounded cells with a thin cellulose wall (Figure 5 - a). The epidermis is rich in branched tector trichomes (Fig. 5 - d) and uniseriate multicellular tector trichomes (Figure 5 - e). It also includes secretory trichomes with unicellular foot and octacellular head (Figures 5-g ; 5 - f).

- Several layers of angular collenchyma are located at the angles of the stem, followed by the cortical parenchyma formed by large size round to polygonal cells with a thin wall (Figures 5 - a, 5 - c). Unlike the species *M. bourgaei* and *M. heterodon*, in which a

lamellar collenchyma is found ( Büyükkartal al., 2016).

- In the middle of the stem the vascular tissus form a continuous ring with several layers of phloem surrounding those of the xylem. In the middle, the medullary parenchyma with meats and a hollow in the center of the stem can be seen.

The main characteristics of each species are summarized in Table 1. Detailed drawings of each part as well as diagrams with conventional signs are grouped in the appendix (Fig. 6-a; Fig. 6-b; Fig. 7; Fig. 8-a; Fig. 8-b).

## 4. Conclusion

To our knowledge, no publication presenting microphotographs of the anatomy of *P. crispum, M. vulgare* or *S. hispanicus* from Algeria is available to date. Our work, therefore, provides additional knowledge on these species, which are particularly interesting from a medicinal point of view.
Some of the anatomical characteristics obtained could be guidelines for future micromorphological and phytochemical investigations, necessary to complete this study in order to better define the characteristics of these three species and provide relevant data to facilitate their identification.

## Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Hasnia Medjahed], [Khawla Yazid],[Sara Hassaïne] and [Nassima Elyebdri]. The first draft of the manuscript was written by [Sara Hassaïne] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Conflict of Interest

The authors declare that they have no conflict of interest

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