Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 2, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

MICROSCOPIC CHARACTERISTICS OF SOME *LAMIACEAE* SPECIES - A SELECTION OF LIGHT MICROSCOPY IMAGES

Monica ENACHE, Adriana NEAGOIE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: monica.enache@biotehnologii.usamv.ro

Abstract

The Lamiaceae family is well known for its aromatic plants that are important in the food, cosmetics and pharmaceutical industry. Also, there are Lamiaceae species that are important melliferous plants, or are used as ornamental plants. In Romania several Lamiaceae species of pharmaceutical relevance can be found (most are cultivated, but some are also spontaneous plants): Hyssopus officinalis, Lamium album, Lavandula angustifolia, Majorana hortensis, Marrubium vulgare, Melissa officinalis, Mentha piperita, Ocimum basilicum, Origanum vulgare, Rosmarinus officinalis, Salvia officinalis, Satureja hortensis, Thymus serpyllum, Thymus vulgaris.
Lamiaceae species have specific characteristics both at macroscopic level as well as at microscopic level, for example their stem and leaves have numerous secretory hairs that can be analysed using wet mounts of hand-sections, surface preparations or samples of powdered plant material. Thus, the present study illustrates the results of such a microscopic approach, for 10 Lamiaceae species obtained commercially in Bucharest (Romania).

Key words*: Lamiaceae species, light microscopy, microscopic pictures.*

INTRODUCTION

The Lamiaceae (Labiatae) family is one of the major plant families (~4000 species - Sârbu, 1999) in the Lamiales order. Known for the numerous aromatic species that can be easily propagated and are widely cultivated, the "mint family" plants are important in the food, cosmetics or pharmaceutical industry. Also, there are Lamiaceae species that are important melliferous plants, ornamental plants, or have religious significance (for example basil).
The Lamiaceae are commonly herbaceous or shrub plants, rarely trees or vines, with stems that are frequently square in cross section and opposite leaves with no stipules and verticillate inflorescences. Aerial reproductive and vegetative organs are covered with glandular (secretory) trichomes (hairs) that produce volatile oil.
The current study presents a microscopic evaluation of plant material from several Lamiaceae species to record some of the parameters of taxonomic relevance using a simple method.

MATERIALS AND METHODS

Plant material

Fresh plant material and/or dried plant material were analysed, as follows: *Lamium album* (white nettle or white dead-nettle) - herbal tea (*herba*); *Lavandula angustifolia* (lavender or true lavender) - plant in pot and herbal tea (*flores*); *Melissa officinalis* (lemon balm) - herbal tea (*folium*); *Mentha piperita* (peppermint) - cultivated and herbal tea (*folium*); *Ocimum basilicum* (basil) - fresh plant material and herbal tea (*herba*); *Majorana hortensis* sin. *Origanum majorana* (marjoram) - fresh plant material; *Origanum vulgare* (oregano) - fresh plant material; *Rosmarinus officinalis* (rosemary) - fresh plant material; *Salvia officinalis* (sage) - fresh plant material and herbal tea (*folium*); *Thymus serpyllum* - (creeping thyme) - herbal tea (*herba*) (Figures 1-2). Plants in pots were obtained from local shops (Aikaterina flower shop in Bucharest or the Garden Services S.C. situated within the Agronomie Herăstrău University Campus).

Figure 1. Herbal teas used in the present study

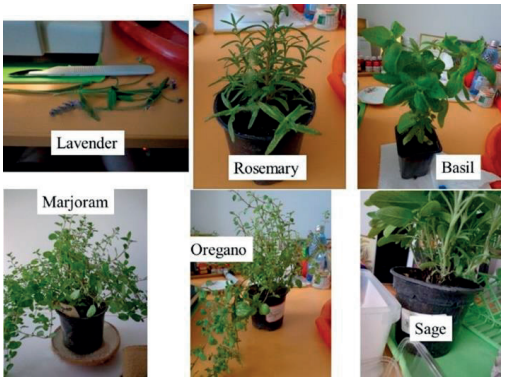

Figure 2. Fresh plant material used in the present study

Microscopic analysis

The analysis was carried out at the Laboratory of Biology, Faculty of Biotechnologies of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Wet mounts of hand-sections, surface preparations or samples of powdered plant material were examined using a Novex Holland optical microscope with ocular micrometer (calibration ratio was 1 µm for ob. 100×, 2.5 µm for ob. 40×, 10 µm for ob. 10×). To colour, toluidine blue was added. Microscopic images were photograped with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens) and were later compared to descriptions found in published data. There is a vast literature in this field, for example AHPA (2014), Azzazy (2019), Bosabalidis & Sawidis (2014), Celep et al. (2011), Choi & Kim (2013), Gîrd et al. (2015), Groşan et al. (2019), Jia et al. (2013), Kahraman et al. (2010), Marin et al. (2006), Popescu et al. (2009), Tozin et al. (2016), Turner et al. (2000), however, most of the descriptions used in the present study are based on the work of Gîrd et al. (2010a, b).

RESULTS AND DISCUSSIONS

Microscopically several specific or common anatomical elements were identified, as follows:

Lamii albi herba: conical unicellular non-glandular hairs (Figure 3); multicellular uniseriate, unbranched non-glandular hairs with a thin apex (Figure 4); peltate glandular trichomes and 3-colpate pollen grains. Oil globules were present also.

Figure 3. Conical non-glandular hairs (white nettle)

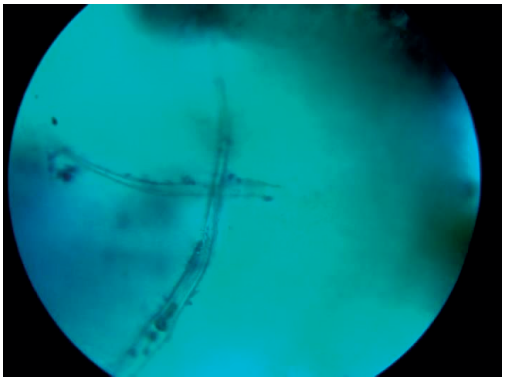

Figure 4. Long, multicellular non-glandular hairs with a thin apex (white nettle)

Lavandula angustifolia **(sin.** ***Lavandula officinalis, Lavandula vera, Lavandula spica*****):** non-glandular multicellular trichomes, uniseriate with warty cuticle and branched in dichotomic manner (star type *hairs*) (Figures 5-6), peltate glandular trichomes and diacytic stomata.

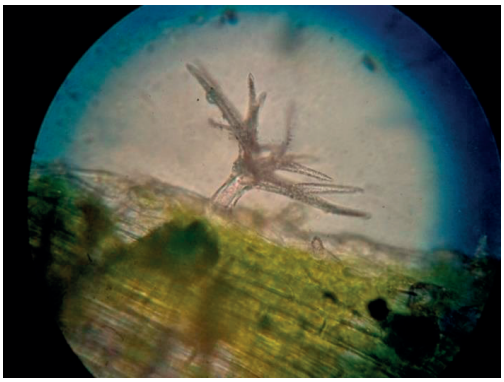

Figure 5. Branched non-glandular multicellular trichome (lavender)

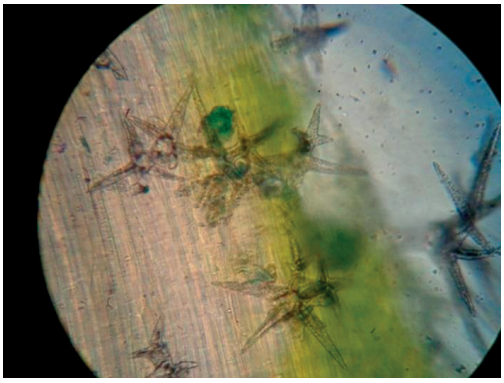

Figure 6. Several branched non-glandular multicellular trichomes (lavender)

In the lavender flower: there were papillae (conical cells) present in the epidermal layer of the corolla (Figure 7); 6-colpate, medium size pollen grains (~ 30 µm) (Figure 8); dense non-glandular trichomes that cover the surface having a protective role (Figure 9). On the ovary there are glandular hairs with elongated stalk and unicellular gland (capitate trichomes) (Figure 10).

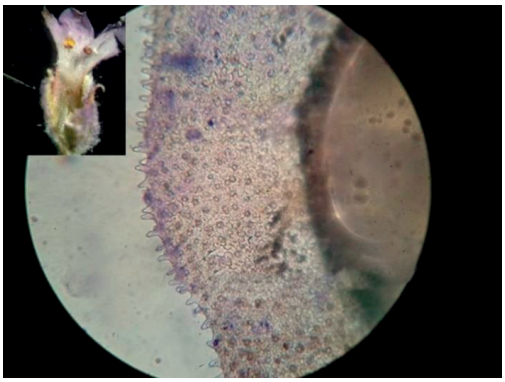

Figure 7. Corolla papillae in the lavender flower

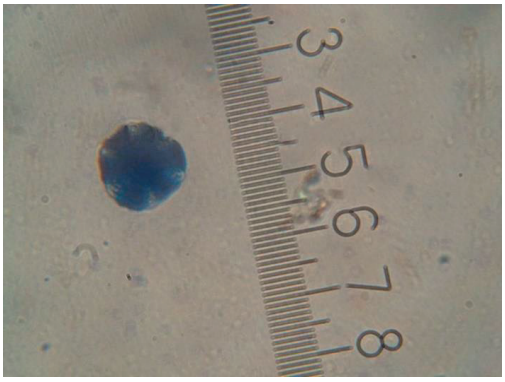

Figure 8. Medium size (~ 30 µm), 6-colpate, pollen grains (lavender)

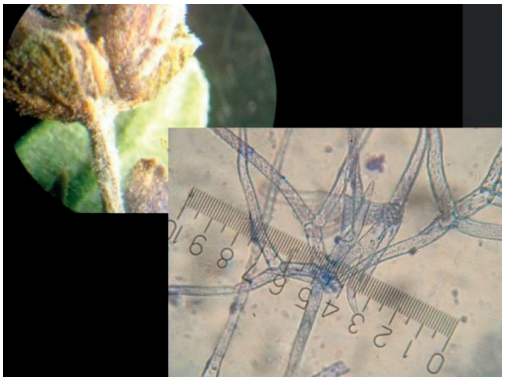

Figure 9. Dense non-glandular trichomes on the lavender flower

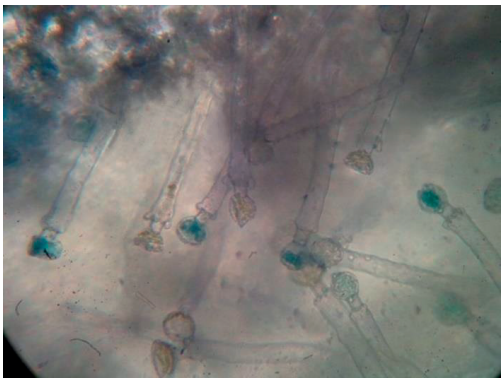

Figure 10. Glandular capitate trichomes in the lavender flower

Lavandulae flores: *numerous non-glandular* multicellular trichomes, uniseriate with warty cuticle and branched in dichotomic manner (star type *hairs*), *that were whole or fragmented* (Figure 11).

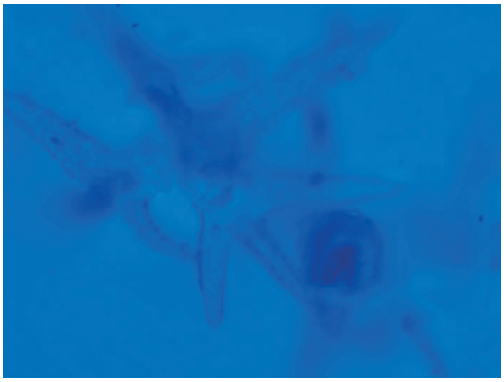

Figure 11. Fragmented star type *hairs* (*Lavandulae flores*)

Melissae folium: epidermal cells and diacytic stomata; non-glandular unicellular trichomes with warty cuticle and a sharp tip, "with the appearance of a canine tooth" (Gîrd et al., 2010), hairs specific to lemon balm (Figure 12); multicellular trichomes with warty cuticle (Figures 13-14); glandular peltate trichomes (Figures 15).

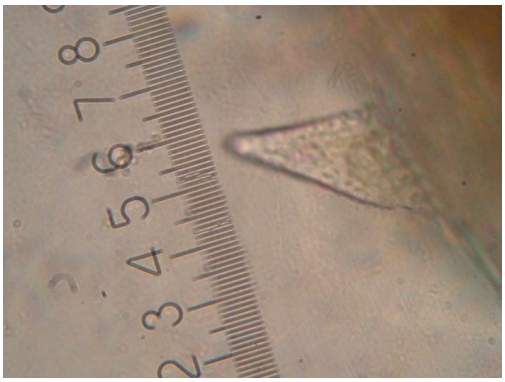

Figure 12. Non-glandular unicellular trichome with warty cuticle and a sharp tip, "with the appearance of a canine tooth" (Gîrd et al., 2010) (*Melissae folium*)

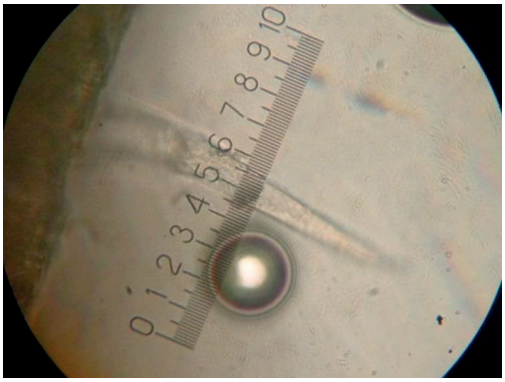

Figure 13. Non-glandular multicellular trichome with warty cuticle (*Melissae folium*)

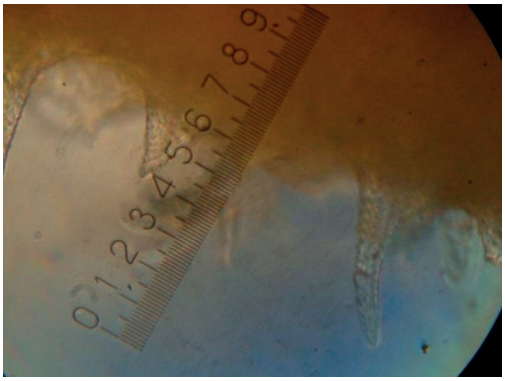

Figure 14. Non-glandular unicellular and multicellular trichomes with warty cuticle (*Melissae folium*)

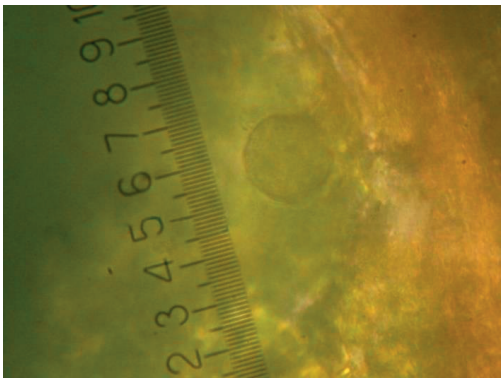

Figure 15. Glandular peltate trichome (*Melissae folium*)

Menthae piperitae folium: non-glandular uniseriate, multicellular, arched hairs with a sharp tip (Figure 16); peltate glandular trichomes.

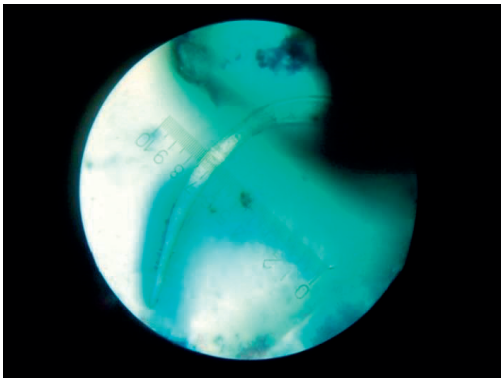

Figure 16. Non-glandular uniseriate multicellular trichome (*Menthae folium*)

Ocimum basilicum: non-glandular trichomes either unicellular or multicellular (Figures 17-18); glandular capitate and peltate trichomes (Figures 19-20); and large size (~ 52.5 µm), 6-colpate pollen grains (Figure 21).

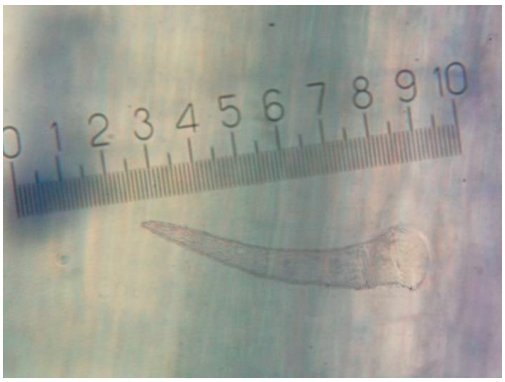

Figure 17. Non-glandular unicellular trichome (basil)

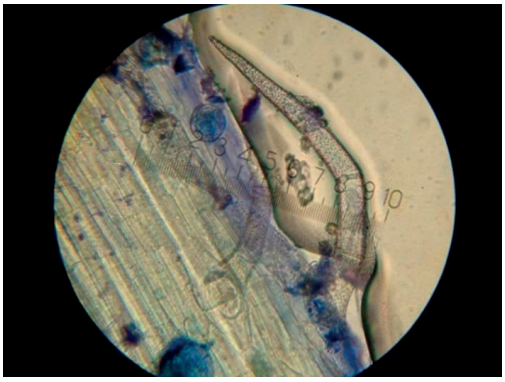

Figure 18. Non-glandular multicellular trichome (basil)

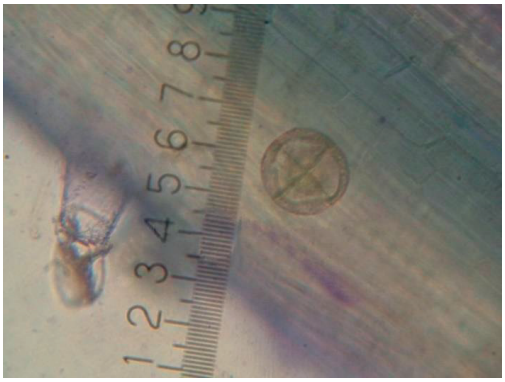

Figure 19. Glandular capitate trichome and glandular peltate trichome with a globular head composed of 4 cells (basil)

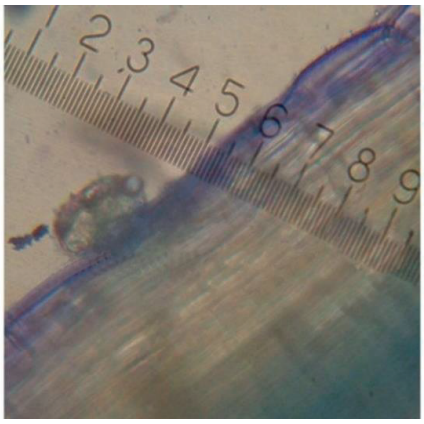

Figure 20. Glandular peltate trichome - side view (basil)

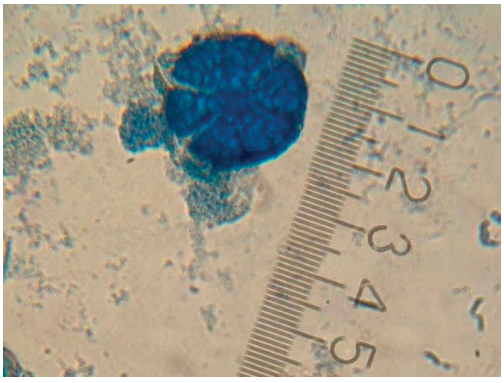

Figure 21. Large size (~ 52.5 µm), 6-colpate pollen grain with reticulate surface pattern (basil)

Basilici herba: non-glandular trichomes (Figure 22); glandular peltate trichomes and epidermal cells with diacytic stomata (Figure 23); oil globules were also present.

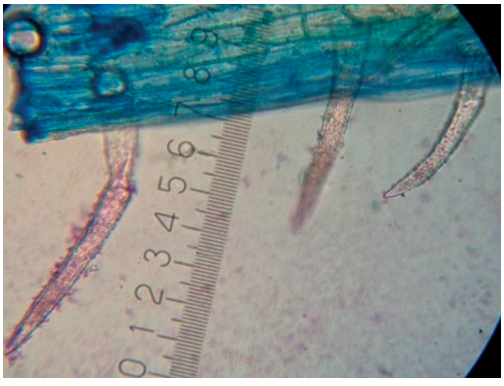

Figure 22. Non-glandular trichome (*Basilici herba*)

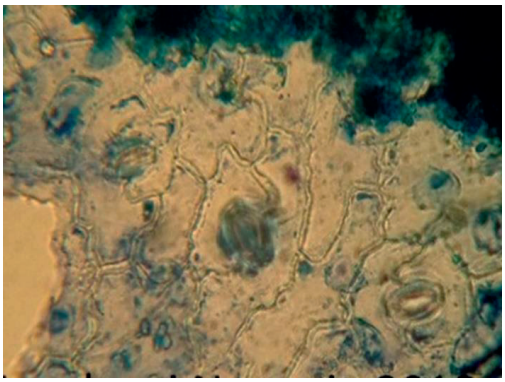

Figure 23. Epidermal cells; diacytic stomata (*Basilici herba*)

Majorana hortensis* sin. *Origanum marjorana*, *Majorana majorana: non-glandular unicellular and multicellular trichomes (Figure 24); numerous peltate glandular trichomes (Figure 25).

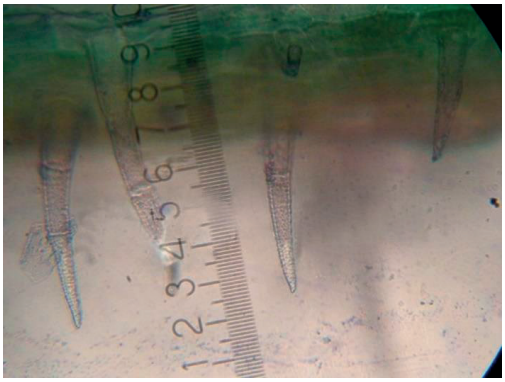

Figure 24. Non-glandular trichomes (marjoram)

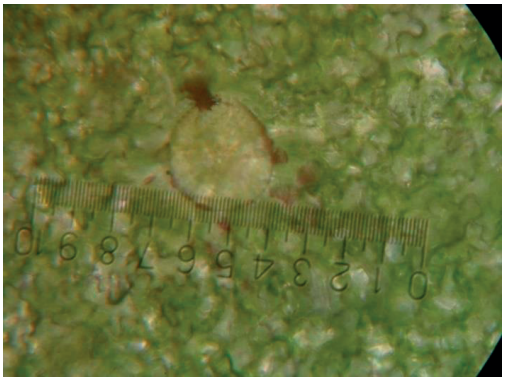

Figure 25. *Peltate glandular trichome* (marjoram)

***Origanum vulgare**:* non-glandular unicellular and multicellular trichomes (Figure 26); peltate glandular trichomes (Figure 27); oil globules were also seen.

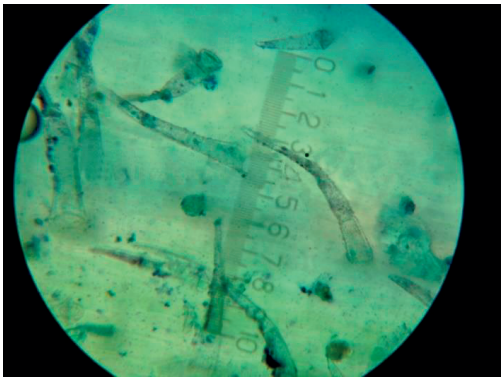

Figure 26. Non-glandular unicellular and multicellular trichomes (oregano)

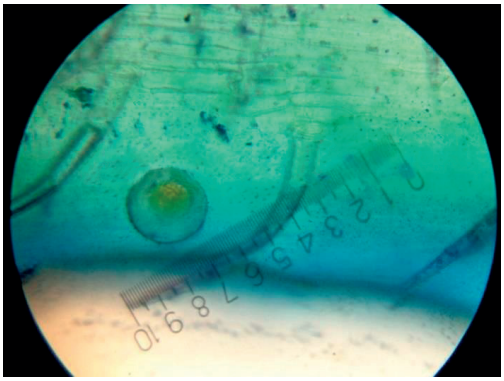

Figure 27. Non-glandular trichomes and peltate trichome (resin sphere visible) (oregano)

Rosmarinus officinalis: numerous non-glandular uniseriate, multicellular, branched trichomes with sharp tips (star type *hairs*) (Figures 28-29); capitate glandular trichomes (Figures 30-31); peltate glandular trichomes (Figure 32); numerous oil globules were also present.

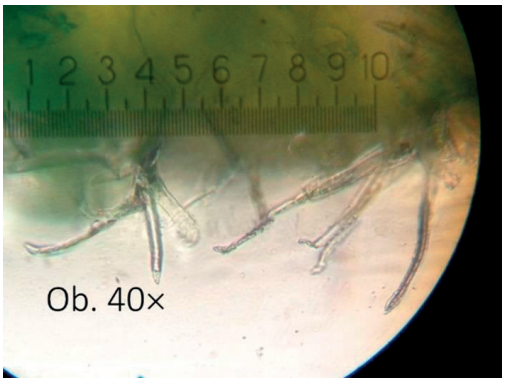

Figure 28. Non-glandular multicellular, branched trichomes with sharp tips (side view) (rosemary)

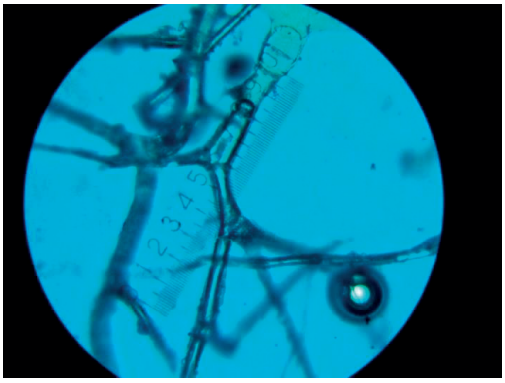

Figure 29. Non-glandular uniseriate, multicellular, branched trichomes with sharp tips (rosemary)

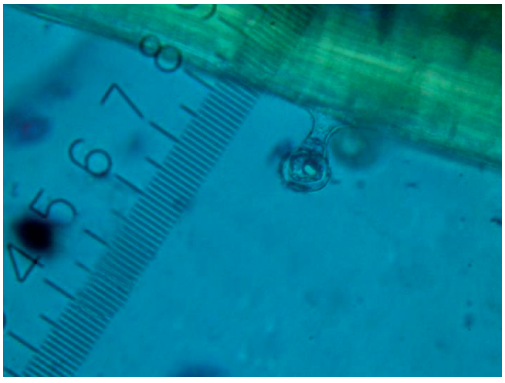

Figure 30. Capitate glandular hair with short stalk (rosemary)

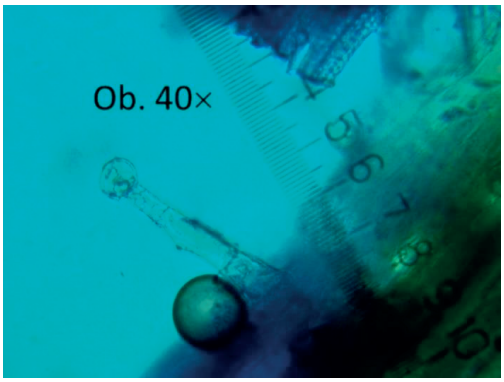

Figure 31. Capitate glandular hair with long stalk (rosemary)

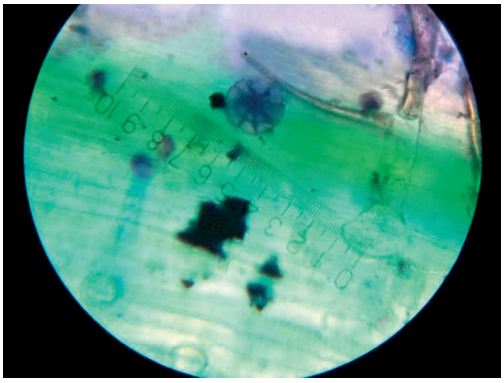

Figure 32. Peltate glandular trichome (rosemary)

Salvia officinalis: numerous non-glandular uniseriate, long and thin, multicellular trichomes with sharp tips (Figures 33-34); capitate glandular trichomes (Figure 35); diacytic stomata.

Figure 33. Numerous non-glandular uniseriate, long and thin, multicellular trichomes with sharp tips (sage)

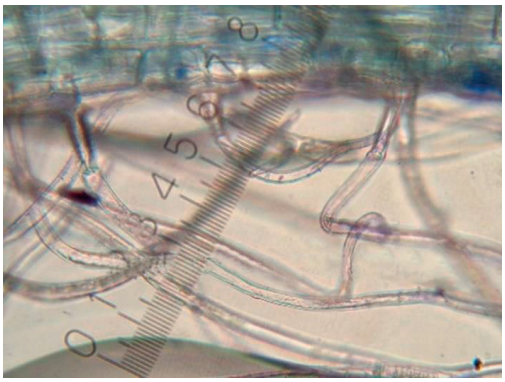

Figure 34. Non-glandular uniseriate, long and thin, multicellular trichomes (sage)

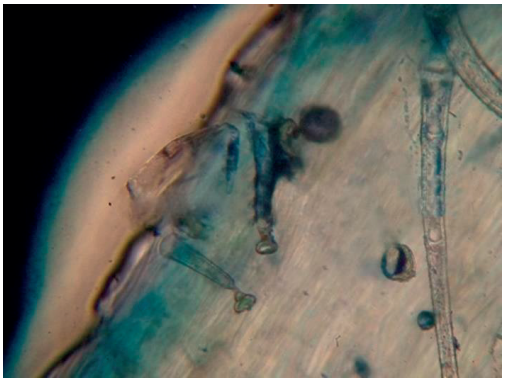

Figure 35. Capitate glandular trichomes (sage)

Salviae folium: non-glandular uniseriate, multicellular trichomes (Figure 36); oil globules (Figure 37).

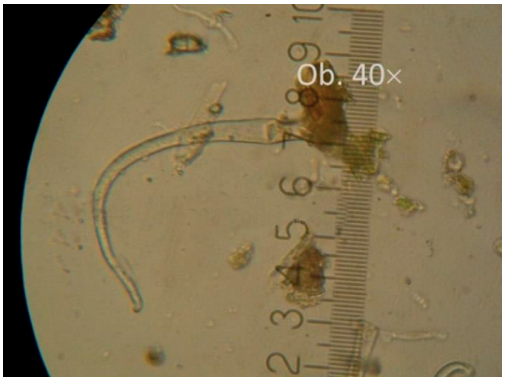

Figure 36. Non-glandular uniseriate trichome (*Salviae folium*)

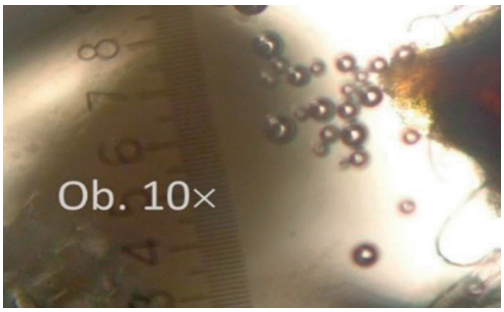

Figure 37. Oil globules (*Salviae folium* - wet mount)

Serpylli herba: 6-colpate pollen grains, oblate in lateral view; non-glandular uniseriate, multicellular trichomes (Figure 38); non-glandular tricellular trichomes with large base (Figure 39); numerous glandular peltate and capitate hairs (Figures 40) and epidermal fragments with diacytic stomata.

Figure 38. Non-glandular uniseriate, multicellular trichome (*Serpylli herba*)

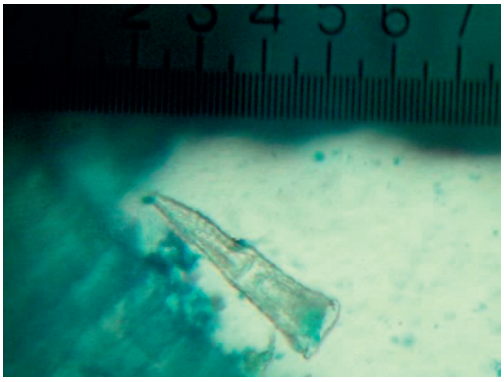

Figure 39. Non-glandular tricellular trichomes with large base (*Serpylli herba*)

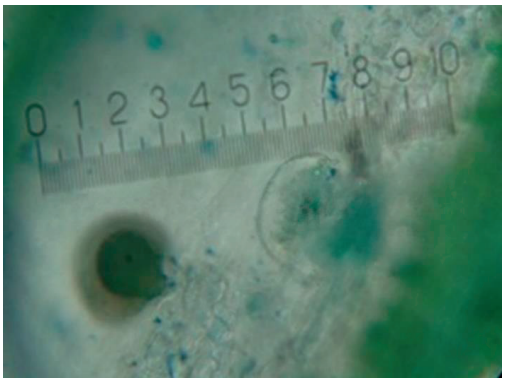

Figure 40. Glandular capitate hair (resin sphere visible) (*Serpylli herba*)

CONCLUSIONS

Three main types of trichomes were observed: glandular peltate trichomes, capitate glandular trichomes and non-glandular trichomes. The two types of glandular trichomes can be determined in light microscopy based on the length of the stalk (which is longer in capitate hairs and short in peltate hairs), while the peltate hairs can also be described further if the number of secretory cells is visible. The non-glandular hairs also showed morphological variety.

The results of the present analysis show some of the structures indicated in the scientific literature for the species that were analysed. Knowledge of the specific tissues and anatomical characteristics of a plant can be applied together with chemical profiling for standardization and quality assurance purposes. Thus, the macroscopic and microscopic analysis of medicinal plants are still used as a preliminary step in the botanical identification of herbal products that is carried out using standard techniques as part of the quality control procedure in pharmaceutical labs and industries.

REFERENCES

AHPA (2014). AHPA Botanical Identity References Compendium. http://www.botanicalauthentication.org/, site accesat în 8 mai 2018.

Azzazy, M. (2019). Micromorphology of Pollen Grains, Trichomes of Sweet Basil, Egypt, 5, 427-433. 10.31031/ACAM.2019.05.000604.

Bosabalidis, A. & Sawidis, T. (2014). Glandular and non-glandular hairs in the seasonally dimorphic *Origanum dictamnus* L. (Lamiaceae) as a means of adaptation to cold stress. *Acta Agrobotanica*, 67: 15-20. 10.5586/aa.2014.010.

Celep, F., Kahraman, A., Atalay, Z. & Dogan, M. (2011). Morphology, anatomy and trichome properties of *Lamium truncatum* Boiss. (Lamiaceae) and their systematic implications. *Australian Journal of Crop Science* 5(2): 147-153.

Choi, J.S. & Kim, E.S. (2013). Structural Features of Glandular and Non-glandular Trichomes in Three Species of Mentha. *Applied Microscopy*, 43(2), 47-53. https://doi.org/10.9729/am.2013.43.2.47.

Gîrd, C.E., Costea, T., Nencu, I., Duțu, L.E., Popescu, M.L. & Balaci, T.D. (2015). Comparative pharmacognostic analysis of Romanian *Ocimum basilicum* L. and *O. basilicum* var. *purpurascens* Benth. aerial parts. *Farmacia,* 63(6): 840-844.

Gîrd, C.E., Duțu, L.E., Popescu, M.L., Iordache, A.T., Tudor, I. & Costea, T. (2010) *Bazele teoretice şi practice ale analizei farmacognostice*, vol. I şi II, Editura Curtea Veche, Bucureşti.

Groșan, A., Ștefănescu, R., Laczkó-Zöld, E., Eșianu, S. & Muntean, D.L. (2019). Identification of the herbal drug Prunellae spica based on macroscopic and microscopic characteristics. *Acta Biologica Marisiensis*, 2(1): 12-20.

Jia, P., Liu, H., Gao, T. & Xin, H. (2013). Glandular trichomes and essential oil of *Thymus quinquecostatus*. *Scientific World Journal*. 2013:387952. doi:10.1155/2013/387952

Kahraman, A., Celep, F. & Dogan, M. (2010). Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *South African Journal of Botany*, 76(2): 187-195.

Marin, M., Koko, V., Duletić-Laušević, S., Marin, P.D., Rančić, D. & Dajic-Stevanovic, Z. (2006). Glandular trichomes on the leaves of *Rosmarinus officinalis*: Morphology, stereology and histochemistry. *South African Journal of Botany*, 72(3): 378-382.

Popescu, M.L., Dinu, M. & Toth, O. (2009). Contributions to the pharmacognostical and phytobiological study on *Leonurus cardiaca* (L.) (*Lamiaceae*). *Farmacia*, 57(4): 424-431.

Sârbu, A. (1999). *Biologie vegetală. Note de curs*. Editura Universității din București.

Tozin, L.R., de Melo Silva, S.C. & Rodrigues, T.M. (2016). Non-glandular trichomes in Lamiaceae and Verbenaceae species: morphological and histochemical features indicate more than physical protection. *New Zealand Journal of Botany*, 54(4): 446-457. 10.1080/0028825X.2016.1205107.

Turner, G.W., Gershenzon, J. & Croteau, R.B. (2000). Development of peltate glandular trichomes of peppermint. *Plant Physiol*., 124(2): 665-680.