Scientific Papers. Series B, Horticulture. Vol. LXIV, No. 1, 2020
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME ASPECTS OF THE ANATOMICAL FEATURES OF THE MEDICINAL PLANT *AGASTACHE FOENICULUM* (PURSH) KUNTZE (*LOPHANTHUS ANISATUS* (NUTT.) BENTH.)

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Abstract

Agastache foeniculum (Pursh) Kuntze (Lophanthus anisatus (Nutt.) Benth.) is an aromatic plant used for its medicinal and nutritive properties. It is also is a melliferous and ornamental plant. For the first time, the acclimatization of new species, such as Agastache foeniculum (Pursh) Kuntze, was made in 2010 at the Buzău Institute for Research and Development in Vegetable Growing, Romania. In 2019, Agastache foeniculum (Pursh) Kuntze was studied at the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The current study is dealing with the leaf and stem anatomy of Agastache foeniculum specimens obtained from seeds provided by the Buzău Institute. The anatomical analyses were made at different parts of the specimens. The leaf analyses showed the presence of glandular and non-glandular trichomes in the upper and lower epidermis. The trichomes were observed in the epidermis of the stem, as well.

Key words: *leaf and stem anatomy; trichomes.*

INTRODUCTION

Agastache foeniculum (*Lophanthus anisatus* (Nutt.) Benth.) - common name Blue Giant Hyssop, Giant Hyssop, Fragrant Giant Hyssop, Anise Hyssop, Wild Anise, Lofant popular¸ is a member of the *Lamiaceae* family. It belongs to this very large family whose species are to be found all over the world. The *Agastache* genus comprises 22 species of perennial aromatic medicinal herbs. *Agastache foeniculum* (Pursh) Kuntze is known as a medicinal and flavouring spice plant. Extract from giant hyssop shows significant bioactivity: antimicrobial, antiviral, antimutagenic, antiproliferative, antiatherogenic, cytotoxic for cancer cell, anti-inflammatory, antioxidant, relaxant activity on the contractions a.o. (Yashika et al., 2013; Sánchez-Recillas et al., 2014; Mazza and Kiehn, 1992; Mostafa et al., 2018; Omidbaigi and Sefidkon, 2003; 2004; Zielińska and Matkowski, 2014; Mihaylova et al., 2013; Duda et al., 2015; Hashemi et al., 2017; Ivanov et al., 2019). The results obtained from antimicrobial screening revealed that essential oil of *Agastache foeniculum* possesses inhibitory activity against *Staphylococcus aureus*, *Curtobacterium flaccumfaciens*, *Listeria monocytogenes*, *Bacillus subtilis*, *Salmonella* sp., *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*, while *Enterococcus faecalis* remained unaffected (Ivanov et al., 2019). The phytochemical compounds in different extracts could be possibly used in the pharmaceutical industry and also in the production of natural cosmetics. The leaves and flowers of *A. foeniculum* have been reported to be used in cakes, ice creams, and sweets, and can be added as fresh ingredients to salads and desserts (Van Hevelingen, 1994; Vogelmann, 1983; Hopkinson et al., 1994; Lima, 1986; Tucker, 1994).

At the same time, the insecticide effect of *Agastache foeniculum* has been reported (Kim Soon-Il, 2003; Ebadollahi et al., 2010;

Ebadollahi, 2011; Ebadollahi et al., 2013; Zielińska and Matkowski, 2014).

An overview of *Agastache* research has been made by Ayers and Widrlechner, 1994, as well as by Fuentes-Granados et al., 1998, but does not include anatomy-related data.

The anatomical features of the vegetative organs are important in the characterization of *Lamiaceae* taxa (Metcalfe & Chalk, 1950; Abu-Asab & Cantino, 1987; Kahraman et al., 2009; 2010). The glandular trichomes and their distribution, the stomatal distribution together with other anatomical features provide significant taxonomic information (Werker, 1985; 2006; 2010; Tirillini et al., 1997; Dinç & Öztürk., 2008; Celep et al., 2014; Ascensao & Pais, 1998; Baran et al., 2010; Mota et al., 2013; Duarte & Lopez, 2007; Corsi & Botega, 1999; Van Horne & Zopf, 1948; Svidenko et al., 2018; Gul et al., 2019).

The *Lamiaceae* family comprises many species known for their medicinal and economic importance, due to the production of essential oils in the glandular trichomes (Serrato-Valentini et al., 1997; Rodrigues et al., 2013). There is sparse data regarding the anatomy of the species under study world wide (Schulz, 1899; Gurtovenko et al., 2018). *A. foeniculum* has not been studied anatomically either, although taxonomists need such studies for a correct classification of the *Lamiaceae* species as well as for finding any possible anatomic modifications that are adaptations of the studied species to the local conditions in Romania.

MATERIALS AND METHODS

The seed material (Aromat de Buzău) originated from the Buzău Institute for Research and Development in Vegetable Growing, Romania. The studies showed that this species has adapted very well and can be successfully grown in Romania (Vânătoru et al., 2015). Plants of *A. foeniculum* (*Lophanthus anisatus*) were planted in the experimental field of the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV-Bucharest). This material was sectioned by hand using razor blades to obtain semipermanent and permanent slides for microscopic studies. The cross-sectioned plantlets presented 4 internodes. Fresh leaves, stems and petioles were collected for anatomical study. Thereafter the sections were clarified with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Săvulescu and Hoza, 2011; Georgescu et al., 2015). Analyses and observations of these cross-sections were performed at the Center for the Study of Food and Agricultural Products Quality at USAMV-Bucharest. Photos were taken and measurements were made using the Leica DM1000 LED, the Leica DFC295 Video Camera and the Leica S8 APO Stereo Microscope, Optika Microscope, as well as a Sony photocamera. Photos were taken using light microscope with different magnifications.

RESULTS AND DISCUSSIONS

Metcalfe and Chalk (1972) stated that stems of many genera and species of the family Lamiaceae are quadrangular with well-defined collenchyma in the four angles. The stem of this plant ehxhibits the general characteristics of the *Lamiaceae*.

The great diversity of plant trichomes has interested botanists by their adaptive and taxonomic values. The morphology and distribution of glandular trichomes are often applied as taxonomic characters at subfamiliar level in *Lamiaceae* family (Abu-Asab & Cantino 1987; Cantino, 1990).

In this paper, the authors analyze the structure of the vegetative organs (leaf, petiol and stem) of *A. foeniculum* (Figure 1), trying to describe the constant and particular histo-anatomical features of the species. The anatomical studies on stem, petiole and leaf cross-sections are presented, along with leaf surface sections. Glandular trichomes are important taxonomic features in the *Lamiaceae* family (Xiang et al., 2010). Trichomes are considered relevant in comparative systematic investigations and in morpho-diagnosis (Metcalfe & Chalk, 1950; 1988). Peltate glandular trichomes with essential oil are localized on the adaxial and abaxial leaf surfaces.

Figure 1. *Agastache foeniculum*

Werker (1993) mentioned, considering a functional viewpoint, that glandular trichomes produce essential oils which apparently protect the plant against herbivores and pathogens and attract pollinators to the floral parts.

Stem anatomy

The stem has a square shape, with four corners and four faces between them (Figure 2).

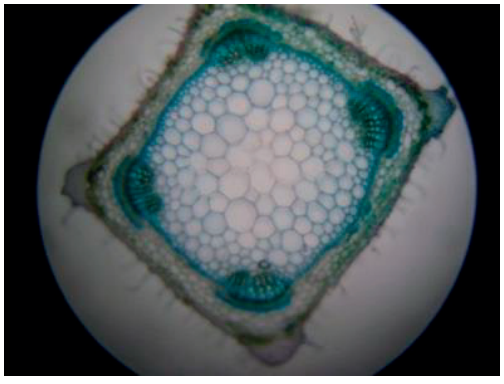

Figure 2. Stem *anatomy*

The epidermis is composed of one oval-shaped or circular cell layer 9.55-9.95 µm thick, a 2.82-4.51 µm cuticle with non-glandular trichomes about 137.8-327.4 µm long, as well as glandular trichomes 172.6-174.6 µm long. Just inwards of the epidermis within each corner there is a region of angular collenchyma cells, 36.25-52.78 µm thick. Cambium is running around the stem and connects the vascular bundles. Inwards of the cortex there is vascular tissue. In the corners, the vascular tissue is made up of prominent vascular bundles (phloem 19.46-22.67 µm, xylem (vessels) 7.13-16.92 µm). The parenchyma tissue has small cells 42.37-46.23 µm and larger cells 74.83-85.69 µm (Figure 3). Pith appears in the centre of the stem.

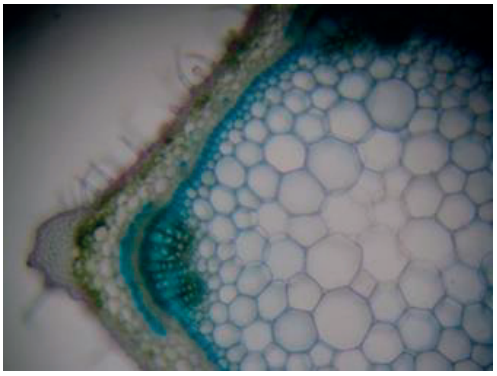

Figure 3. Stem *anatomy*- details

Petiole anatomy

In cross section (Figure 4), the petiole's adaxial and abaxial epidermis consists of a single layer of rectangular and oval cells. The epidermis presents glandular and non-glandular trichomes (Figure 5). There is also cuticle, sclerenchyma tissue, parenchyma tissue with cells about 16-45 mm and a vascular bundle. The non-glandular trichomes are multicellular and long, or unicellular. The glandular trichomes are of the capitate type. Capitate trichomes consist of 1-2 stalk cells and 2 head cells.

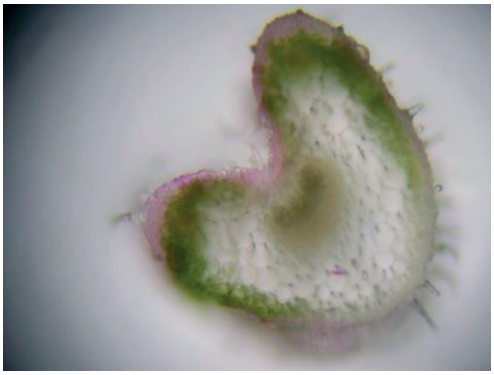

Figure 4. *Petiole* anatomy

Chlorenchyma cells are especially seen at the abaxial side, and vascular bundles are located in the parenchyma tissue. The petiole features 3 vascular bundles, with a large arc-shaped bundle in the middle and a single small vascular bundle in each corner. The median vascular bundle is surrounded by parenchyma tissue.

Figure 5. Petiole epidermis - glandular and non-glandular trichomes

Leaf anatomy (Figures 6 and 7)
The lower epidermis of the leaf is made up of one layer of cells, ranging from 12.38 to14.42 µm, on top of which there is a 3.26-5.08 µm thick cuticle.
The lower epidermis has many glandular and non-glandular trichomes and stomata. Trichomes are considered relevant in comparative systematic investigations and morpho-diagnosis (Metcalfe Chalk, 1988).

Figure 6. Leaf anatomy

The upper epidermis presents one layer of 9.86-13.78 µm thick cells, on top of which there is a 3.08-3.70 µm thick cuticle as well as glandular and non-glandular trichomes (Figures 7, 8).

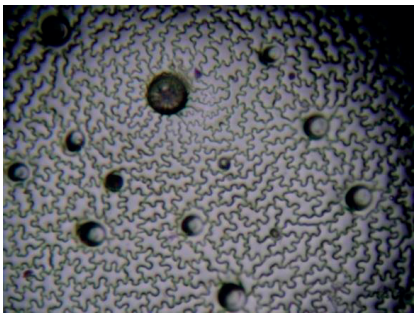

Figure 7. Upper epidermis with glandular trichomes

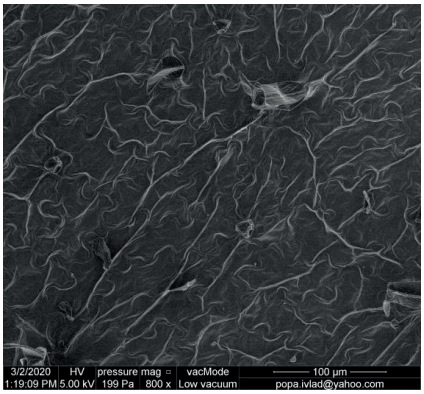

Figure 8. Upper epidermis (SEM)

The glandular (353.2-352.6 µm) and non-glandular trichomes (530-247.5 µm) are present on both the adaxial and the abaxial surfaces.
The glandular trichomes (peltate and capitate) are formed of one basal cell one or two stalk cells, and one head.
The head of mature capitate glandular hairs has two cells (Figure 9).

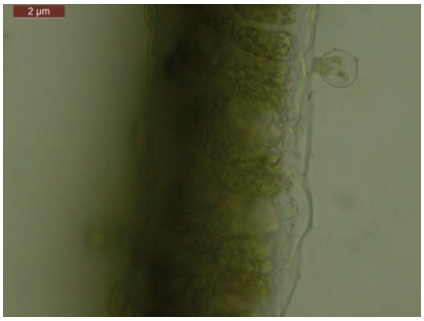

Figure 9. Upper epidermis with glandular trichomes

The leaf width is 657-688 µm. The dorsoventral mesophyll comprises one layer of 350.3 µm long palisade cells and spongy tissue. The cells of the spongy tissue are roundish, with intercellular spaces. The midrib is prominent on the lower side of the leaf and its epidermis has glandular and non-glandular trichomes; the midrib has also parenchyma tissue and a vascular bundle (Figure 10).

Figure 10. Midrib

On both epidermal surfaces there are glandular and non-glandular trichomes similar to those existing on the stem. Stomata are present in the lower epidermis (Figures 11, 12). The blade, in surface view, has epidermal cells with sinuous contours, with glandular and non-glandular hairs.
(Figures 13, 14, 15).

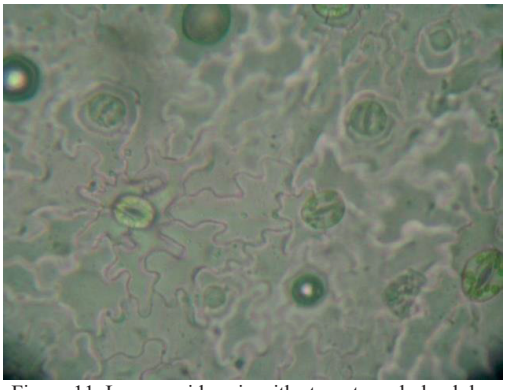

Figure 11. Lower epidermis with stomata and glandular trichomes

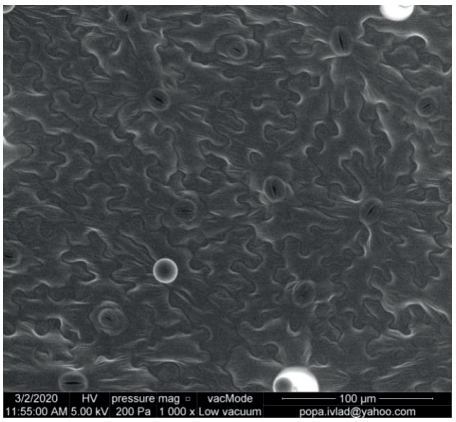

Figure 12. Lower epidermis with stomata and glandular trichomes (SEM)

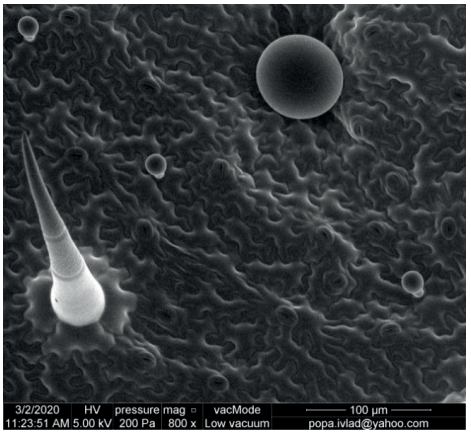

Figure 13. Lower epidermis with trichomes (SEM)

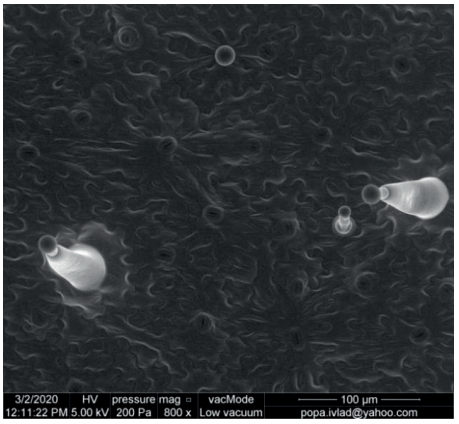

Figure 14. Lower epidermis with stomata and glandular trichomes (SEM)

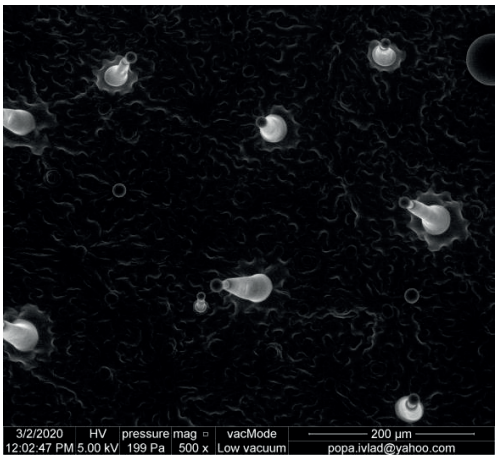

Figure 15. Lower epidermis with and glandular trichomes (SEM)

CONCLUSIONS

This investigation shows that the assembled anatomical characters of *Agastache foeniculum* helps in the identification of this medicinal species.

The micromorphological and anatomical characteristics are of great interest and significance to the discussion of the taxonomy of the species.

The glandular trichomes are important taxonomic features for the *Lamiaceae* family.

The anatomical studies we performed on stem and leaf cross-sections as well as on leaf surface sections demonstrate for the first time the anatomy of the vegetative organs of specimens growing in Romania.

The microscopic observations made on leaves, petioles and stems of *A. foeniculum* show the presence of many types of non- glandular and glandular trichomes.

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