GLANDULAR TRICHOMES AND ESSENTIAL OIL CONSTITUENTS OF *PEROVSKIA ATRIPLICIFOLIA* BENTH.

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Abstract: The structure and micromorphology of the glandular trichomes and the chemical composition of the secreted essential oil of *Perovskia atriplicifolia* Benth. were studied. The leaves present numerous glandular trichomes of three morphological distinct types, all of them having a unicellular foot and stalk: 1) capitate hairs with bi-cellular head, 2) peltate hairs with tetra-cellular head and 3) peltate hairs with multicellular (10-celullar) head. Qualitative and quantitative GC-MS analyses of the essential oils revealed limonene (18 %), γ – terpinene (16 %), β - caryophyllene (13 %), α - caryophyllene (12 %) and cymene (11%) as the main constituents.

INTRODUCTION

Perovskia is a small genus from *Lamiaceae* family. *Perovskia atriplicifolia* Benth. is a deciduous perennial with upright, greyish white stems and lobed, silvery-grey leaves 5 cm long and 2,5 cm wide. This plant is used as a cooling medicine in the treatment of fevers and in folk medicine as an analgesic in rheumatic pains. Also, this species is suitable for forming a decorative hedge of moderate height (Pourmortazavi et al., 2003). Many species of *Lamiaceae* produce volatile oils which are secreted by glandular trichomes on aerial vegetative organs and some reproductive organs (Moon et al., 2009). The studies in which these glandular trichomes have been investigated, comprising morphological, structural and micromorphological characteristics of trichomes as well as chemical composition of volatile oils are numerousness (Autunes and Sevinate-Pinto, 1991, Bosabalidis, 1990, Bosabalidis and Tsekos, 1982, Moon and Hong, 2003 Werker et al., 1985 Kaya et al., 2007).

The purpose of this study is to investigate the morpholology and distribution of non-glandular and glandular trichomes on *Perovskia atriplicifolia* Benth. leaves and the composition of the volatile oil produced by the plant in vegetative stage, depending on environmental factors in which plant grows.

MATERIAL AND METHODS

The plant material was collected from fields of SC Miroslava, Iasi in August 2009. For histo-anatomical analysis the vegetal material (leaves) was fixed and conserved in ethylic alcohol 70%. Cross sections of the leaves (from the top and the middle of the stem) were used. Free hand sections were performed using a razor blade. The sections were coloured with Iodine Green and Ruthenium Red. The photos were taken with an Olympus E-330 photo camera, using an Olympus BX51 research microscope. Scanning electron microscopy (SEM) investigations: the investigated material consists in small pieces of leaves in different developmental stages. The vegetal material were fixed in glutharaldehide for 2 hours, washed with distilled water and stored in 70% ethanol. After dehydration in a graded ethanol series (80%, 90% and 100%) and acetone, the material was critical point dried with CO_2 (using a EMS 850 Critical Point Dryer), sputter-coated with a thin layer of gold (30 nm) (using a EMS 550X Sputter Coater) and, finally,

examined in a scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 27.88 kV. The measurements of the glands dimensions and trichomes density were made using biometrical software from Nikon (NIR-Demonstration). For the investigations regarding volatile oils composition, the biological material consisted of whole plant freshly harvested from plants in the vegetative stage, collected from S.C. Miroslava Iasi. The analyses were performed in *„HORTICAL"* - centre for the study of fruits and vegetable quality, belonging to the Faculty of Horticulture from University for Agriculture Sciences and Veterinary Medicine, Bucharest. The work protocol included: volatile oils extraction using a Clevenger hydro distillation process, with a plant material/water ratio being of approximately 1:3, in a 3 hours extraction time; separation of volatile oil components by a gas chromatographic method combined with the mass spectrometry one, by using a GC-MS Agilant 6890; identification of volatile oils possible by using the NIST spectra bank and Kovats indexes.

RESULTS AND DISCUSSIONS

The leaves present non-glandular and glandular trichomes on both sides of the lamina. the non-glandular trichomes were multicellular and branched (figure 1 A). The following types of the glandular trichomes can be recognized: capitate and two types of peltate trichomes. The peltate glandular trichomes were predominantly on the abaxial surface and located in epidermal depressions. They consisted of one basal epidermal cell, a unicellular stalk cell and a tetracellular (figures 1B, 2D) or multicellular secretory head (figures 1C, E, 2A, B).The capitate trichomes were situated on the adaxial and abaxial leaf surface, and they were more numerous than peltate trichomes. Capitate trichomes consisted of a basal cell, a short unicellular stalk and bi-celullar secretory head (figure 1D, 2C).

At maturity, the gland secretory multicellular presents a large subcuticular space. This cavity is filled with volatile oil with start to discharge. Cellular space that makes these birth increases as the oil is secreted and released in these cavities. The secretion products pass through the plasma membrane and cell wall and accumulate in this cavity. When the cuticle breaks, the secretion products are released. At the peltate hairs with multicellular gland, the subcuticular space is higher than the glandular cells themselves (figure 1 F). The diameter of the gland is greater on the upper epidermis, both for the peltate and the capitate trichomes (Table 1). Their density is higher on the lower epidermis. In general, a high variability in leaf coverage by both non-glandular and glandular trichomes has been reported by Bosabalidis and Skoula (1998), who suggested that trichomes may appear on one side only or both sides, equally or unequally.

Analyzing the composition of volatile oils produced by the whole plant in the vegetative stage, 27 characteristic compounds, were identified. Among the identified volatile oil compounds, 11 compounds (for example, limonene (18%), γ terpinene (16%), β caryophyllene (13%), α caryophyllene (12%) and cymene (11%) with the biggest percentage give the specific aromatic properties of the tested material (figures 3, 4).

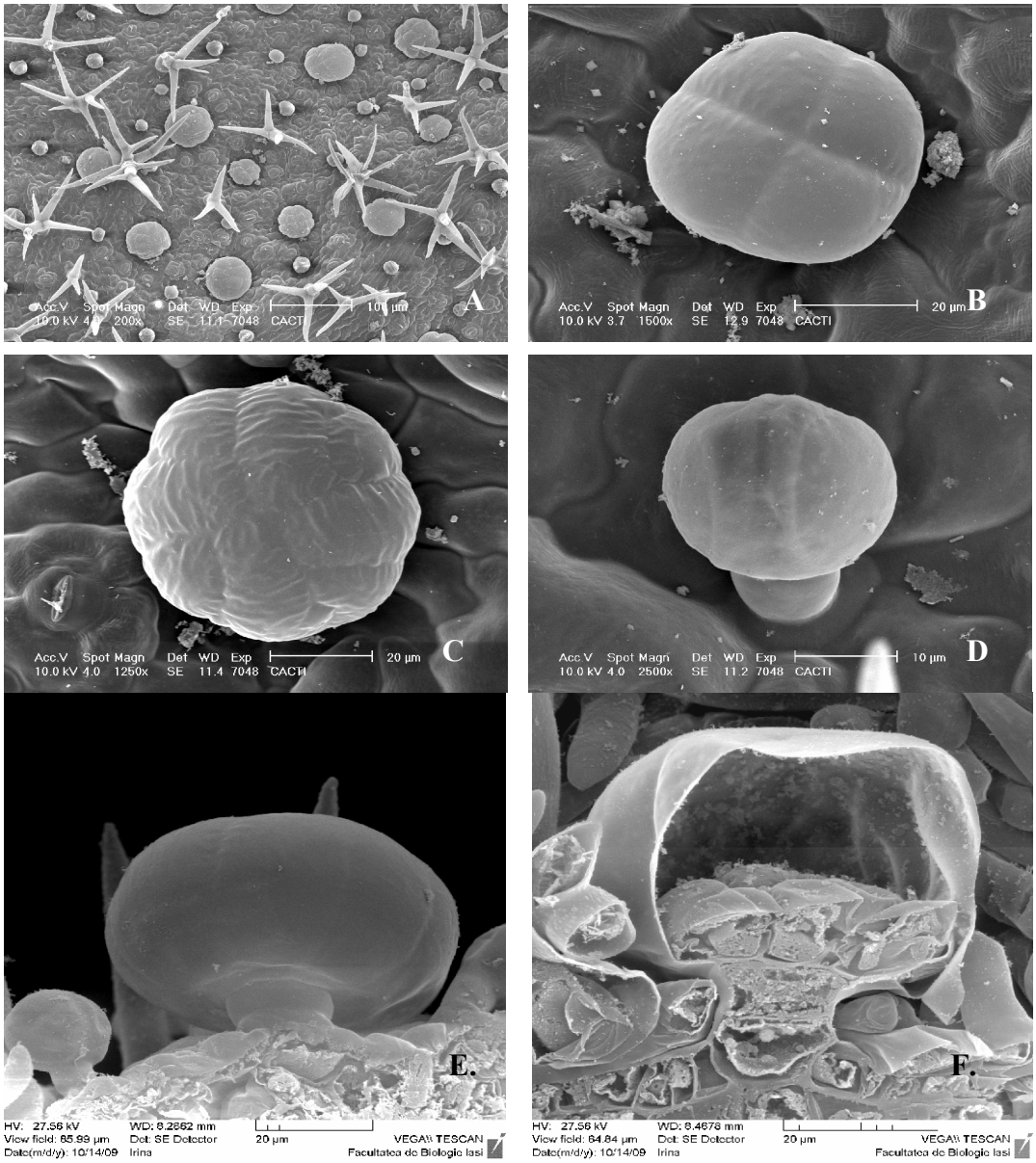

Fig. 1 – SEM images of glandular trichomes on the middle third of the stem leaves: A - lower epidermis, B – peltate glandular trichome with tetra-cellular gland on upper epidermis, C, E - peltate glandular trichome with multi-cellular gland on the lower epidermis, D - capitate glandular trichome on the upper epidermis, F – cross section through a peltate glandular hair with multi-celullar gland (original)

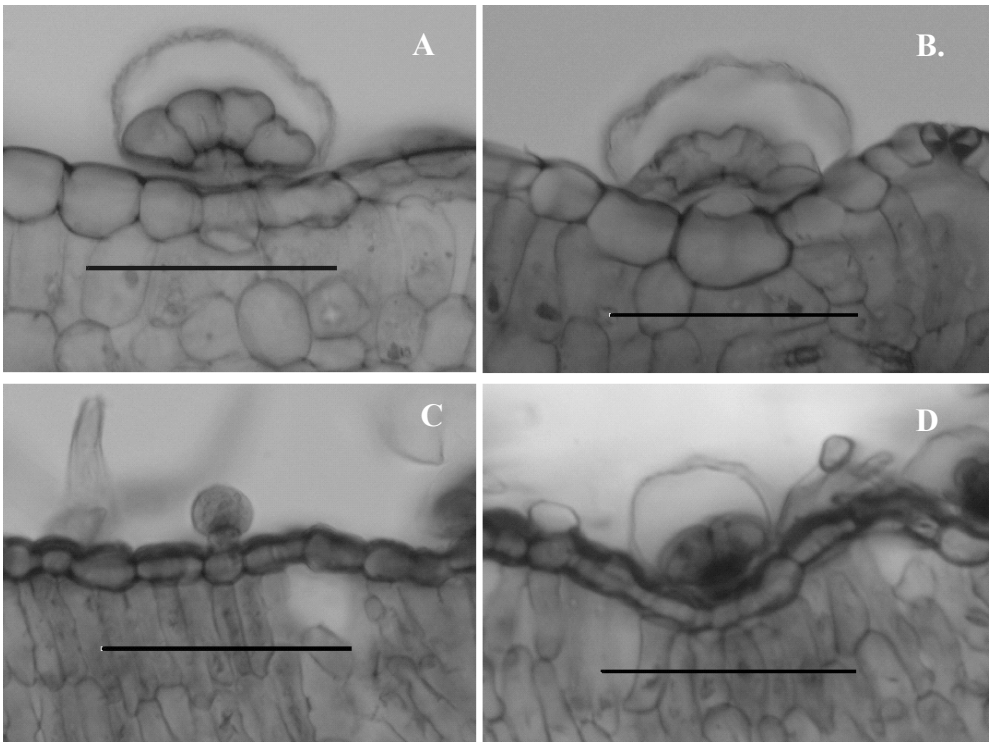

Fig. 2 – Light microscopy images of glandular trichomes on the middle third of the stem leaves: A, B - peltate glandular trichome with multi-cellular gland lower epidermis, C - capitate glandular trichome, D – peltate glandular trichome with tetra-cellular gland (scale bar 50 μm)(original)

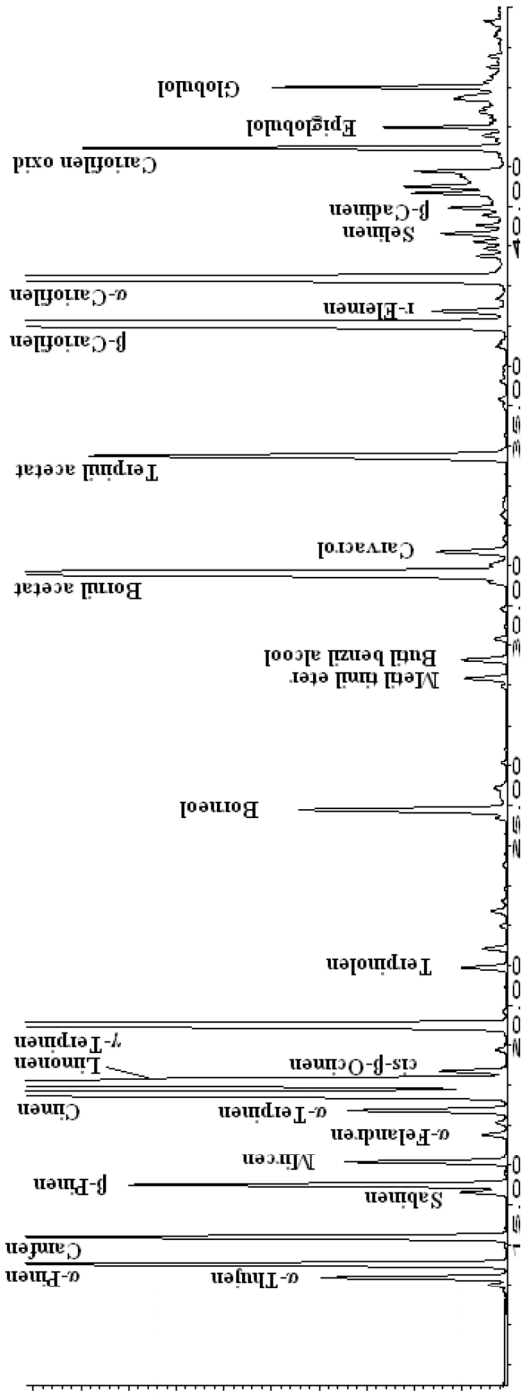

Fig. 3. Composition of *Perovskia atriplicifolia* Benth. volatile oil produced by whole plant in vegetative stage

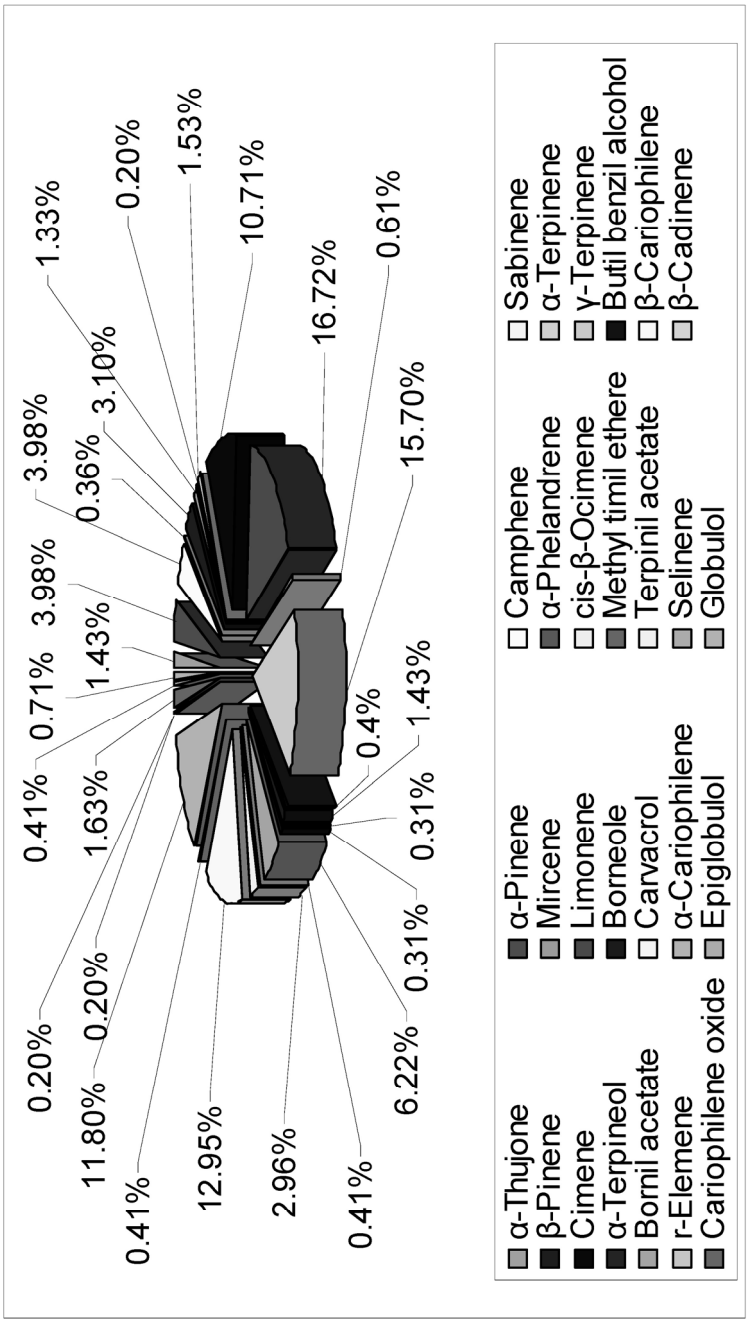

Fig. 4. Percentage composition of *Perovskia atriplicifolia* Benth. volatile oil, produced by whole plant in the vegetative stage

	Lower epidermis	Upper epidermis
Multi-cellular gland diameter (μm)	48.39±7.23	56.09±4.64
Bi-cellular gland diameter (μm)	17.02± 2.2	18.94± 2.7
Peltate trichome with multi-cellular gland density (nr/μm^2)	52.56±5.87	44.54±3.65
Capitate trichome with bi-cellular gland density (nr/μm^2)	92.45±11.34	64.81±9.45

Table 1 - Variation in gland diameter and density of the glandular trichomes on young leaves (values are mean ± standard deviation)

CONCLUSIONS

The histo-anatomical investigations on *Perovschia* leaves confirm and complete the existing data in the literature of the field, especially referring to the morphology and distribution of non-glandular and glandular trichomes on leaves as an adaptation to the environmental factors in which plant grows.

Subsessile glandular trichomes with multicellular heads are diagnostic for the genus *Perovskia* (Moon et al., 2009). Therefore, the combination of leaf anatomical and micromorphological characteristics could be helpful in the identification of species.

The volatile oil obtained from *Perovskia* plants has a characteristic biochemical composition, influenced by the moment of determination (the plant ontogenetic stage). The aromatic and therapeutically efficiency of *Perovskia* plant could be indicated by the ontogenetic stage, depending on plant metabolic transformations correlated with the environmental factors.

For the complete characterisation of *Perovskia atriplicifolia* volatile oil, a strict quantitative and qualitative monitoring of this vegetal product, during the biosynthesis stages, may be necessary. The aim of this complex study is to identify the favourable moment of plant harvesting to obtain volatile oils with specifically medicinal and aromatic properties.

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