



Histochemical, micromorphology and ultrastructural investigation in glandular trichomes of *Micromeria thymifolia*

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ABSTRACT: Histochemical, micromorphology, ultrastructural and confocal analyses of the trichomes of *Micromeria thymifolia* (Scop.) Fritsch, were carried out using light microscopy, confocal laser scanning electron microscopy, scanning electron and transmission fluorescent microscopy. Three types of glandular trichomes – peltate, capitate and digitiform and non-glandular trichomes are described on the abaxial and adaxial leaf surface. Ultrastructural analyses showed the presence of mitochondria, plastids with lipid globules and proliferations of the granular endoplasmatic reticulum in secretory phases of the heads of glandular trichomes. The results of histochemical tests showed a positive reaction to polysaccharides, lipids and to some secondary compounds such as terpenes and phenols in different types of glandular trichomes. A strong red autofluorescence of the secreted material in glandular trichomes was observed with confocal laser scanning microscopy.

KEY WORDS: *Micromeria thymifolia*, trichomes, histochemistry, micromorphology, ultrastructure

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INTRODUCTION

Micromeria thymifolia (Scop.) Fritsch is an endemic species of the Balkan peninsula (ŠILIC 1979). The genus *Micromeria* Benth. (Lamiaceae, Nepetoideae) as currently understood (HARLEY *et al.* 2004) contains species with a distribution range extending from the Himalayan region to the Macaronesian Archipelago (with Madeira, the Cape Verde and Canary Islands) and from the Mediterranean to South Africa and Madagascar (MORALES 1993; BRAUCHLER *et al.* 2005).

Micromeria thymifolia species have been traditionally used in the Mediterranean area as condiments and medicinal plants (HAMMER *et al.* 2004). Often aromatic, *Micromeria* species are reported to have many pharmacological activities including anaesthetic, antiseptic, antirheumatic and central nervous system stimulant effects (GULLUCE *et al.* 2004). The essential oil of some *Micromeria* species

have significant antioxidant (MEHMET *et al.* 2011), antibacterial and antifungal activities (MEHMET *et al.* 2004). Considering the importance of this species as a medicinal plant, in the present study we carried out micromorphological, ultrastructural and histochemical analyses of glandular trichomes as primary secretory organs of *Micromeria thymifolia* (Scop.) Fritsch. We have also analysed autofluorescence of their secreted products.

MATERIAL AND METHODS

The plant material of *Micromeria thymifolia* (Scop.) Fritsch was collected in Orjenske Lokve (Mt. Orjen, Montenegro), in October 2005. A voucher specimen is kept at the Institute of Botany Herbarium, Faculty of Biology, University of Belgrade.

For the micromorphological study, small segments of leaves were fixed in glutaraldehyde (3% with buffer solution

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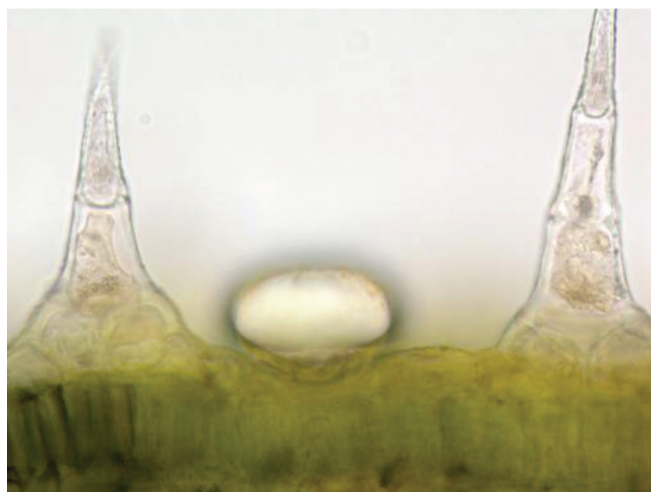


Fig. 1. Hand cross section of fresh leaves of *Micromeria thymifolia* peltate - trichome on adaxial leaf surface 100x.

at pH = 7). The pieces were subsequently dehydrated in a graded ethanol series and critical-point dried, coated with a thin layer of gold (ion sputtering coating) in BALTEC-SCD 005 Sputtering Device, and observations were carried out on a JEOL JSM T 220 15kV SEM.

The leaf samples for ultrastructural examination were sectioned in a drop of 2% glutaraldehyde and put into a fixative solution of 2% glutaraldehyde in phosphate buffer (0.1 M, pH = 7.0), postfixed in 2% OsO₄ and embedded in Araldite. Sections of 1µm thickness were stained with uranyl and lead citrate and examined with a Philips transmission electron microscope 208 S.

Histochemical analyses were performed on hand sections of fresh leaves using the following tests: NADI for terpenes, Sudan IV for lipids, PAS for polysaccharides and FeCl₃ for phenols. The observations were made under a Leitz Dialux light fluorescence microscope HBO 50W block filter A – excitation wavelengths were BP 340 – 380.

Fresh leaves were examined with a CLSM 510 Carl Zeiss with Axioskop FS2mot microscope. A Plan-Apochromat

20x /0.75 objective lens was used, and the excitation wavelengths were 488 and 543nm.

RESULTS AND DISCUSSION

Hand cross sections of fresh leaves (shown *in vivo* in Fig.1) showed that the secretion products accumulated in the subcuticular space of the glandular head of peltate trichomes.

The results of histochemical analysis of the secreted products of the glandular trichomes are presented in Table 1.

With the NADI procedure, the reaction was positive in the peltate trichomes showing an intensive violet color, indicating terpenes (Fig. 2A). Staining with Sudan IV for lipids gave a positive reaction showing a dark orange color in the glandular head of peltate, capitate and digitiform trichomes (Fig. 2B). The FeCl₃ test showed a positive reaction for phenols with a brown staining of the secretory material in the peltate and capitate trichomes (Fig. 2C). Staining with PAS gave a positive reaction for polysaccharides in peltate and capitate trichomes (Fig. 2D).

These results showed a positive reaction to polysaccharides, lipids and to some secondary compounds such as terpenes and phenols. Compared with previously published results on other representatives from the same family (MARIN *et al.* 2008, 2010), the results of our investigation showed qualitative similarities in the content of secretory products in glandular trichomes.

A strong red autofluorescence of the secreted substances was observed with the CLSM in the peltate trichomes (Fig. 3).

Similar to most plants of Lamiaceae species, the adaxial and abaxial surface of *Micromeria thymifolia* leaves carried nonglandular and glandular peltate, capitate and digitiform trichomes (Fig. 4A, Fig. 4B). Peltate trichomes were distributed on both leaf surfaces but more were on the adaxial (Fig. 4A). They consisted of one basal epidermal

Table 1. Histochemistry of secreted material of glandular trichomes of *Micromeria thymifolia*.

Staining Procedure	Target compounds	Peltate trichomes	Capitate trichomes	Digitiform trichomes
NADI	terpenes	+++	-	-
Sudan IV	lipids	+++	++	+
FeCl ₃	phenols	++	+	-
PAS	polysaccharides	++	+	-

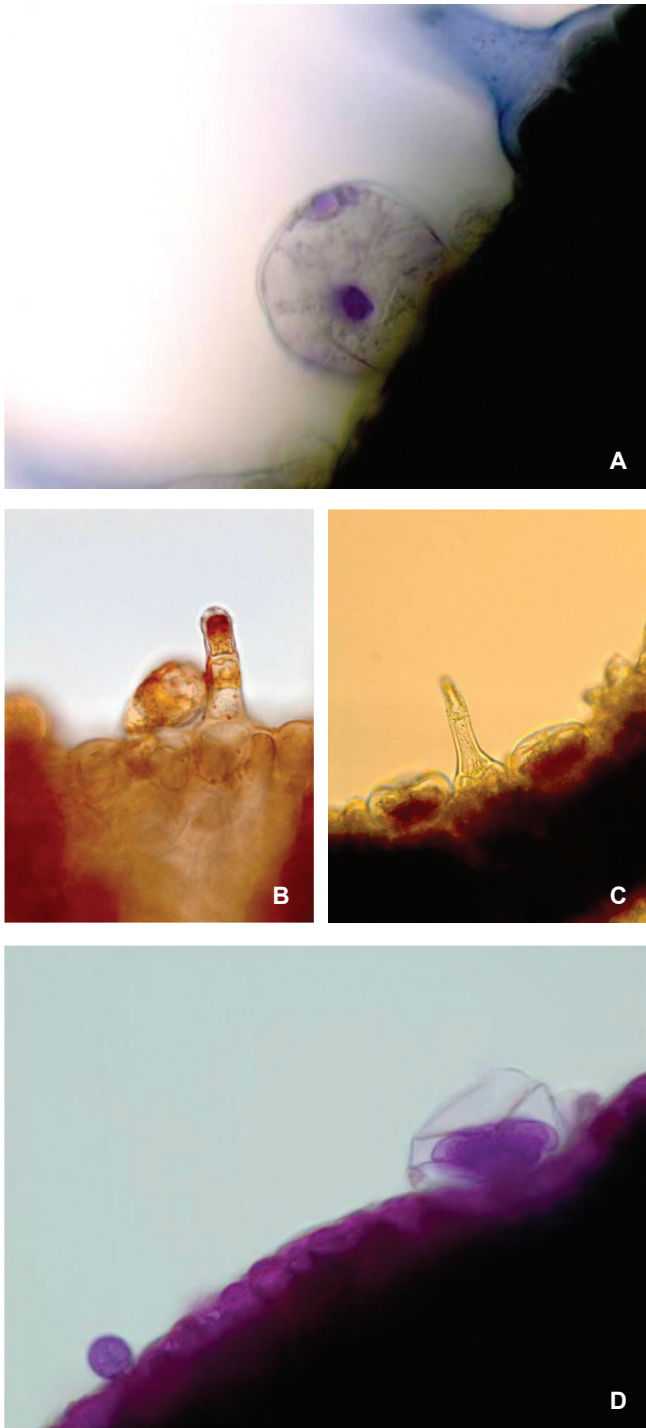


Fig. 2. Histochemical characterization of the secretions of *Micromeria thymifolia* glandular trichomes.

- A. Secretion stained violet in peltate trichome with NADI. 40x
 B. Dark orange staining of secretion with Sudan IV in capitate and digitiform trichomes 100x.
 C. Secretion stained brown in peltate trichomes with FeCl_3 . 40x
 D. Secretion stained dark pink in peltate and capitate trichomes with PAS. 40x

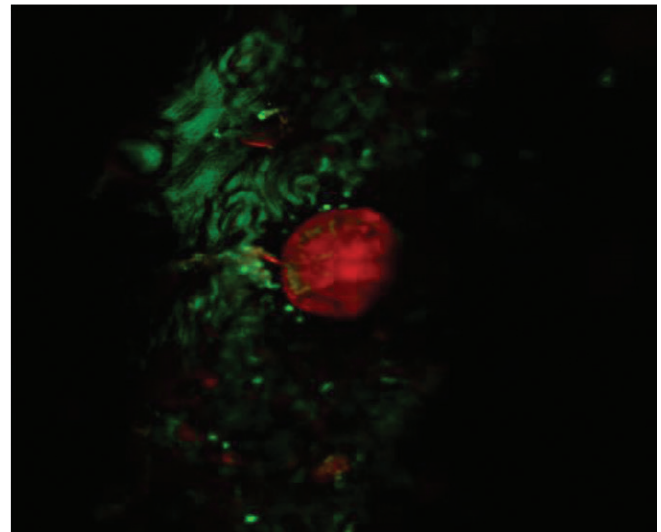


Fig. 3. CLSM micrographs of leaf of *Micromeria thymifolia* 100x

cell, a wide stalk cell and a multicellular head consisting of twelve cells. Capitate trichomes were distributed on both leaf surfaces, and were divided into two types according to the number of stalk cell. Capitate trichomes of type I were composed of one basal cell, one stalk cell and a unicellular head, while capitate trichomes of type II consisted of one basal cell, two stalk cells and a unicellular head. Capitate trichomes were more numerous than peltate and digitiform trichomes on both leaf surfaces (Fig. 4A and 4B). Digitiform trichomes with a less dense distribution were composed of three cells (Fig. 4B). Non-glandular trichomes were densely distributed on the adaxial and abaxial leaf surfaces.

Ultrastructural analyses showed the presence of a dense cytoplasm, plastids with lipid globules and proliferations of the granular endoplasmatic reticulum in secretory phases of the heads of glandular trichomes. The outer cell wall was covered with a thick cuticle (Fig. 5). Ultrastructural investigation is important in trichome research to determine the relations among morphology and cytology, secretion processes and the chemical nature of secreted material.

Using a range of microscopic techniques for observations of trichomes was particularly important, as it provided useful information about the structure and function of these trichomes. The secreted compounds in the glandular trichomes of *M. thymifolia* had a heterogeneous composition, and had biological effects which could be useful in the pharmaceutical and food industry.

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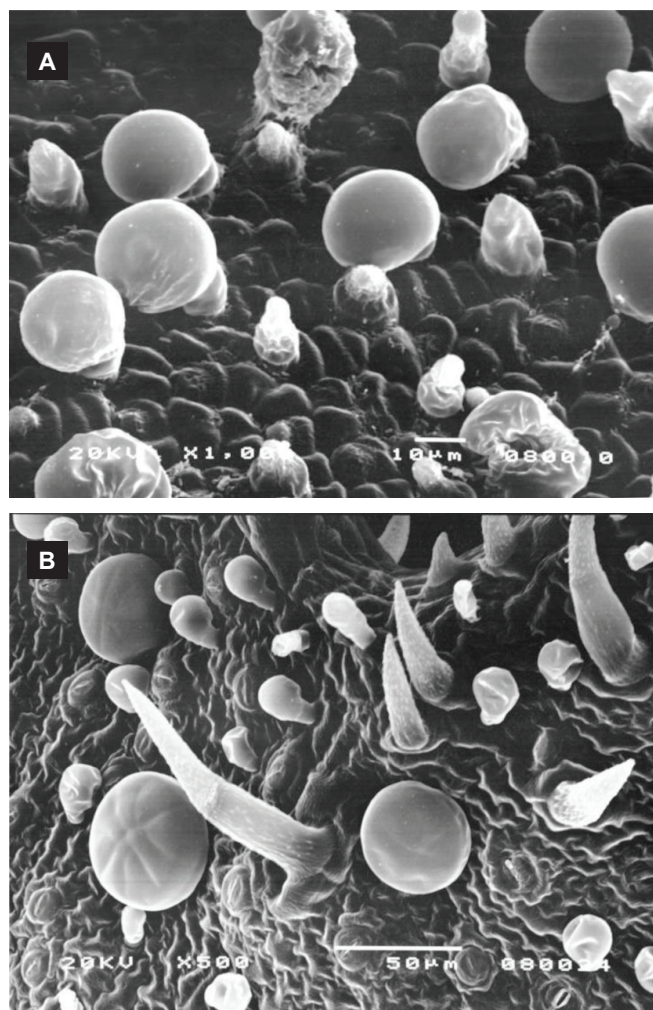


Fig. 4. Scanning electron micrographs of *Micromeria thymifolia* leaves.

A. Peltate, capitate, digitiform and nonglandular trichomes on abaxial leaf surface. Bar = 50µm.

B. Capitate, digitiform and nonglandular trichomes on adaxial leaf surface. Bar = 10µm.

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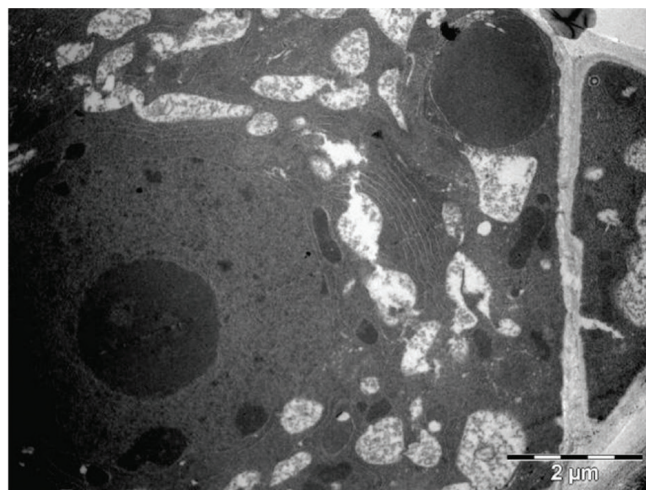


Fig. 5. Transmission electron micrographs of *Micromeria thymifolia* of glandular trichomes Bar 2 µm.

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Botanica SERBICA



REZIME

Histohemijska, mikromorfološka i ultrastrukturalna istraživanja žlezdanih trihoma *Micromeria thymifolia* (Scop.) Fritsch

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Histohemijska, mikromorfološka, ultrastrukturalna i konfokalna istraživanja trihoma *Micromeria thymifolia* (Scop.) Fritsch, sprovedena su korišćenjem svetlosnog mikroskopa, konfokalnog, skening elektronskog i transmissionog elektronskog mikroskopa. Tri tipa žlezdanih trihoma – peltatne, kapitatne i digitiform trihoma, kao i nežlezdane opisane su na licu i naličju lista. Ultrastrukturalna analiza pokazuje prisustvo mitohondrija, plastida sa masnim kapima i proliferaciju granularnog endoplazmatičnog retikuluma u sekretornoj fazi žlezdanih trihoma. Rezultati histohemijskih istraživanja pokazali su pozitivne reakcije na polisaharide, lipide i neke od sekundarnih metabolita kao terpene i fenole u različitim tipovima žlezdanih trihoma. Intezivna crvena autofluorescencija sekretornih produkata žlezdanih trihoma uočena je korišćenjem konfokalnog mikroskopa.

Ključne reči: *Micromeria thymifolia*, trihoma, histohemija, mikromorfologija, ultrastrukturalna

