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THE MICROMORPHOLOGICAL, HISTOCHEMICAL AND CONFOCAL ANALYSIS OF *SATUREJA SUBSPICATA* BARTL. EX VIS. GLANDULAR TRICHOMES

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Abstract - Micromorphology, histochemical and confocal analyses of the trichomes of *Satureja subspicata* (Bartl. ex Vis.) were carried out using light microscopy, confocal laser scanning electron microscopy (CLSM), and scanning electron microscopy. Non-glandular unbranched and two types of glandular trichomes - peltate and capitate - are described. The results of histochemical tests showed a positive reaction to phenolics, tannins, lipids, acid lipids, pectins and polysaccharides in both types of glandular trichomes. A strong red autofluorescence of the lipophilic and hydrophilic secreted material in glandular trichomes was observed with CLSM.

Key words: Satureja subspicata, trichomes, micromorphology, histochemistry, confocal

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INTRODUCTION

Trichomes are hairlike outgrowths of the plant epidermis which are often found on the stems, leaves and reproductive organs of numerous plant species. They possess a variety of characteristics and serve different functions, such as reflecting radiation, lowering plant temperature, reducing water loss (Wagner, 1991), or providing defense against insects. The genus *Satureja* (Lamiaceae) comprises about 200 species of herbs and shrubs, often aromatic, widely distributed in Mediterranean area (Cantino et al. 1992). Due to the presence of secondary metabolites such as flavonoids, steroids and tannins, the *Satureja* species have been known for their healing properties, and the essential oil isolated showed certain biological properties, e.g. antimicrobial activity (Ciani et al. 2000; Skočibušić et al. 2006; Ćavar et al. 2008).

Satureja subspicata Bartl. ex Vis. is a rare, endemic Dinaric species distributed in the eastern Mediterranean area. This plant is a perennial shrub sprouting every spring with new twigs full of linear and leathery leaves and purple flowering during October (Šilić, 2005). The morphology of the hair (glandular and nonglandular) has proved essential in the taxonomic and ecological studies of this family (Dunkić et al. 2001; Werker, 1993; Gersbach, 2002). The glandular trichomes are the primary secretory organs of these plants, and their structure can vary widely among species (Venkatachalam et al. 1984; Werker et al. 1985 a; b).

In this study we investigated the micromorphology and histochemistry of the secretion products of glandular trichomes on the leaves of *S. subspicata*.

MATERIAL AND METHODS

Plant Material

The aerial parts of *S. subspicata* were collected in Virpazar in 2005. Voucher specimens were deposited in the Herbarium of the Institute of Botany, Faculty of Biology, University of Belgrade.

Leaf segments were fixed in 3 % buffered glutaraldehyde (pH 7-4). The pieces were subse-

Fig. 1A

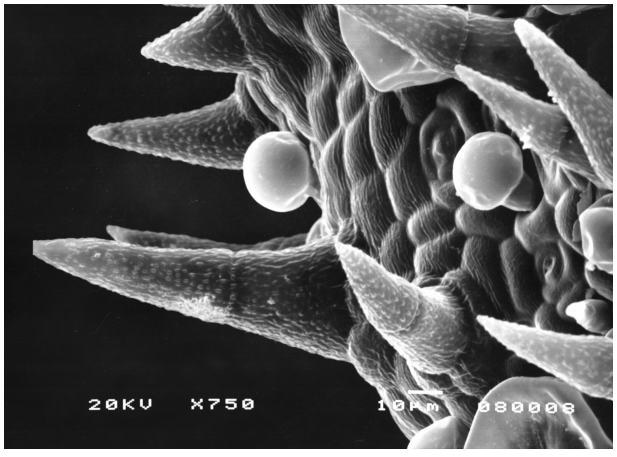

Fig. 1B

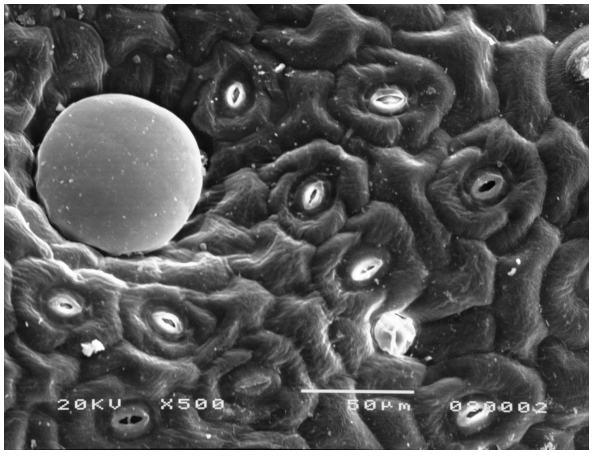

Fig. 1C

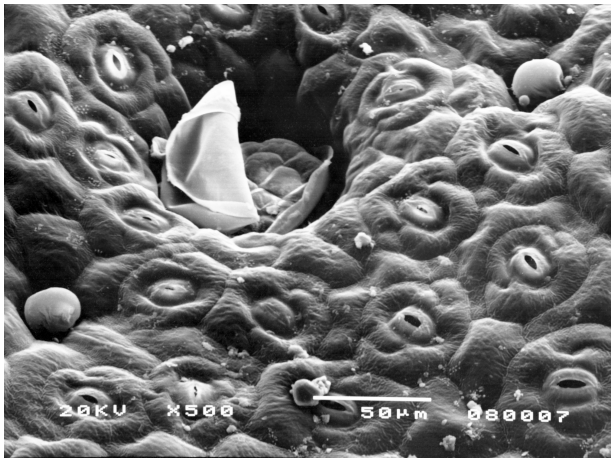

Fig. 1D

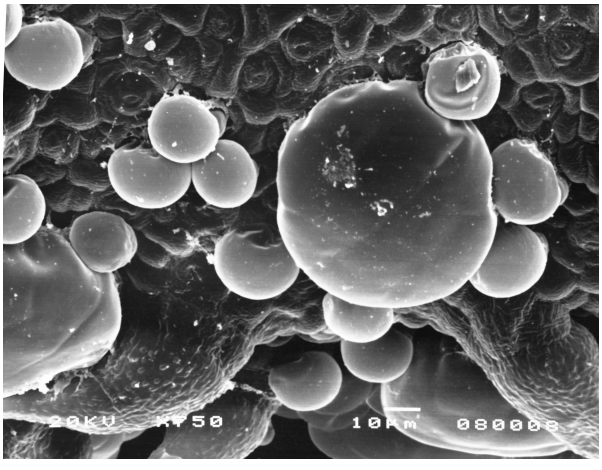

Fig. 1. Scanning electron micrographs of *S. subspicata* – leaves
A. Adaxia leaf surface of *S. subspicata*. Bar = 10µm.
B. Peltate trichome on adaxial leaf surface. Bar = 50µm.
C. Mature peltate trichome on abaxial leaf surface. Bar = 50µm.
D. Capitate glandular trichomes on adaxial leaf surface. Bar = 10µm.

quently dehydrated in a graded ethanol series and critical point dried, coated with a thin layer of gold (ion sputtering coating) in a BALTEC-SCD 005 Sputtering Device, and observations were carried out on a JEOL JSM T 220 15 kV scanning electron microscope.

Histochemical analyses were performed on hand sections of fresh leaves using the following tests: $FeCl_3$ for phenols (Johansen, 1940), $K_2Cr_2O_7$ for tannins (Gabe, 1968), Sudan IV for lipids (Pearse, 1985), NBA for acid lipids (Cain, 1947), Ruthenium red for pectins (Johansen, 1940), PAS for polysaccharides (McManus, 1948). Standard control procedures were carried out simultaneously. The observations were made under a Leitz Dialux light fluorescence microscope HBO 50W block filter A – excitation wavelengths were BP 340 – 380.

Table 1. Histochemistry of secreted material of glandular trichomes of *S. subspicata*

Staining procedure	Target compounds	Peltate trichomes	Capitate trichomes
$FeCl_3$	phenols	++	+
$K_2Cr_2O_7$	tannins	+	+
Sudan IV	lipids	++	+
NBA	acid lipids	+	++
Ruthenium red	pectins	+	++
PAS	polysaccharides	+	++

Fresh leaves were examined with a CLSM 510 Carl Zeiss with Axioskop FS2mot microscope. Plan-Apochromat 20x /0.75 objective lens were used, and the excitation wavelengths were 488 and 543nm.

RESULTS

Besides nonglandular trichomes (Fig. 1.A), which were densely distributed on the margins of the leaves, two types of glandular trichomes - peltate and capitate - were found. The peltate trichomes were distributed on both leaf sides, consisting of one basal epidermal cell, a stalk cell and a head of twelve secretory cells (Fig. 1B). Mature peltate glands are located in epidermal depressions, the cuticular cap was completely detached (Fig. 1C). The capitate glandular trichomes were found on both leaf surfaces. They consisted of one basal epidermal cell, two stalk cells and a round head of one secretory cell (Fig. 1D).

Histochemistry

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

For the histochemical analysis of the secreted material, several staining methods were used. The $FeCl_3$ test showed a positive reaction for phenols with a dark-brown staining of the secretory material in the peltate trichomes and a light-brown staining in the capitate trichomes. (Figs. 2A, 2B). The results of the histochemical tests showed a positive reaction to tannin compounds (Fig. 2C, 2D) in the secretory heads of both types of glandular trichomes, with a brown color. Staining with Sudan IV (Fig. 2E) for lipids gave a positive reaction showing dark red color in the glandular head of peltate and a light red color in the head of capitate trichomes (Fig. 2F).

With the NBA procedure the reaction was positive both in the peltate (Fig. 2G) and capitate trichomes showing a blue color, indicating acid lipid compounds, but the reaction was stronger in the capitate trichomes (Fig. 2H).

An intense positive reaction with ruthenium red for pectins showed a dark red staining of secretory material in the head of capitate trichomes (Fig. 2J); the reaction was also positive in the head of peltate trichomes (Fig. 2I), while pink staining with PAS gave a positive reaction for polysaccharides and was observed in both types of glandular trichomes (Figs. 2K, 2L). The examined stains showed certain differences in color between the peltate and capitate trichomes, but we cannot make firm conclusions about them without detailed chemical analyses.

Strong red autofluorescence of the secreted substances can be seen in both the peltate and capitate trichomes, whereas light green autofluorescence was obtained on the nonglandular trichomes observed with CLSM (Fig. 3).

DISCUSSION

The representatives of the Lamiaceae family are characterized by the presence of peltate and capitate glandular trichomes. The secreted material of heterogeneous composition is temporarily stored in the subcuticular space in mature peltate trichomes and released by rupture of the cuticle, while in the capitate trichomes it is probably released through micropores. Their secretions may be involved in the

Fig. 2A

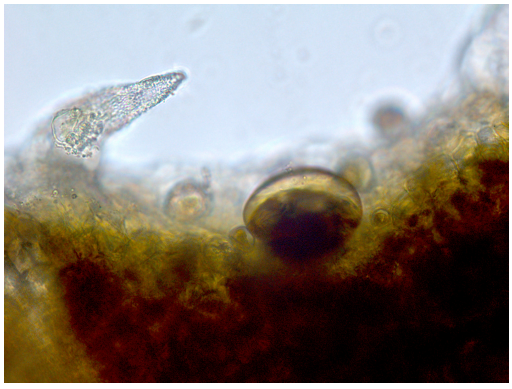

Fig. 2B

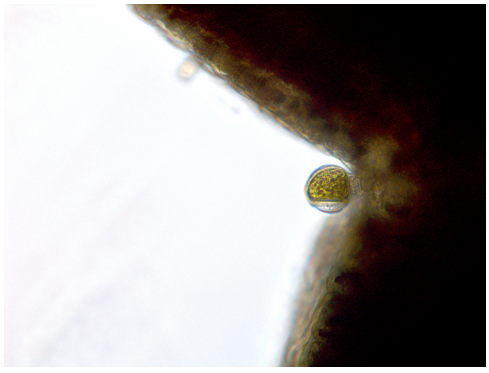

Fig. 2C

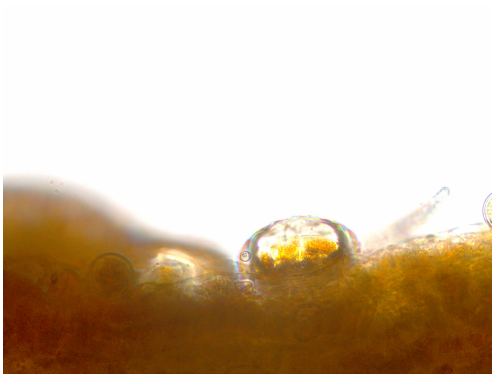

Fig. 2D

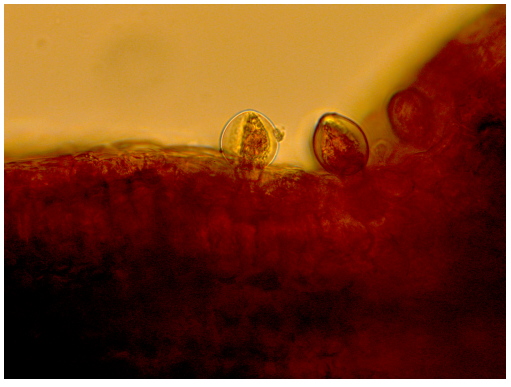

Fig. 2E

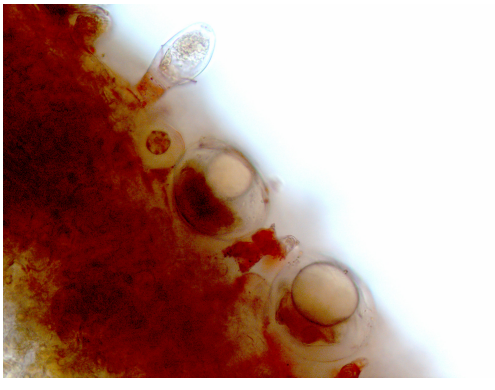

Fig. 2F

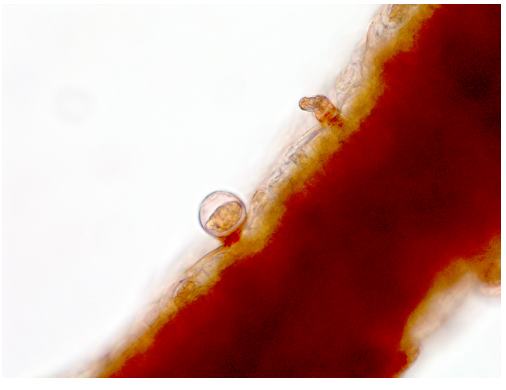

Fig. 2. Histochemical characterization of the secretions of *S. subspicata* glandular trichomes.
Fig. A. - Dark brown staining of secretion with $FeCl_3$ in peltate trichomes; 40x magnification.
Fig. B. - Secretion stained light brown in capitate trichomes with $FeCl_3$; 40x magnification.
Fig. C - Secretion stained brown in peltate trichomes with $K_2Cr_2O_7$; 40x magnification.
Fig. D - Secretion stained light brown in capitate trichomes with $K_2Cr_2O_7$; 40x magnification.
Fig. E. - Red staining of secretion with Sudan IV in peltate trichomes; 40x magnification.
Fig. F. - Light red staining of secretion with Sudan IV in capitate trichomes; 40x magnification.

Fig. 2G

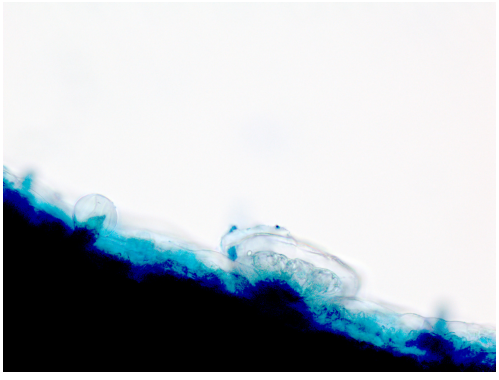

Fig. 2H

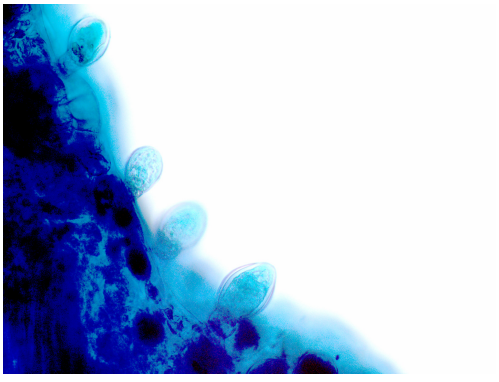

Fig. 2I

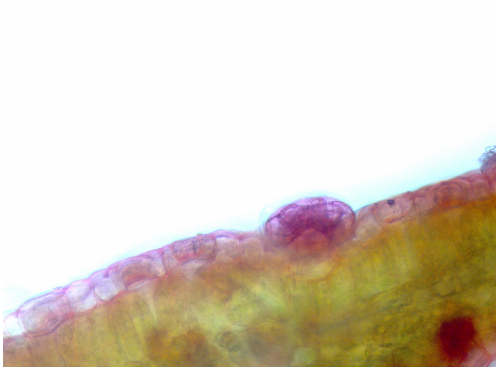

Fig. 2J

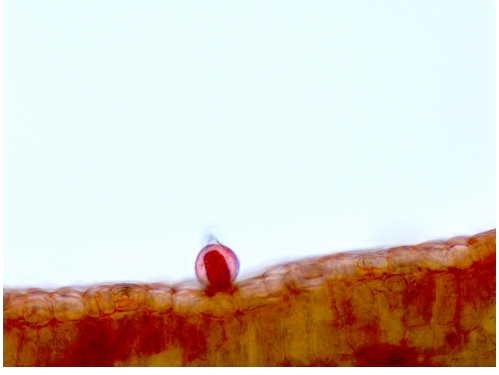

Fig. 2I

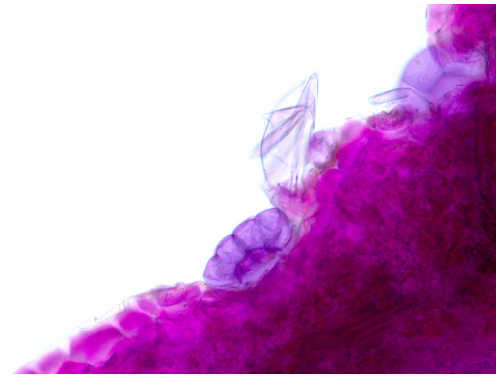

Fig. 2J

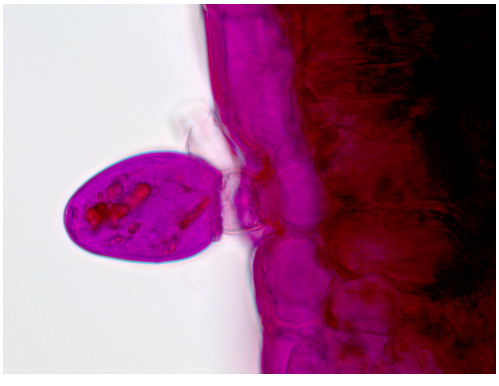

Fig. 2. (continued)
Fig. G. - Secretion stained light blue in peltate trichomes with NBA; 40x magnification.
Fig. H - Secretion stained blue in capitate trichomes with NBA; 400x magnification.
Fig. I - Light red staining in peltate trichome with Ruthenium red; 40x magnification.
Fig. J. - Dark red staining in the capitate trichomes with Ruthenium red; 40x magnification.
Fig. K - Secretion stained pink in peltate trichome with PAS; 40x magnification.
Fig. L - Secretion stained dark pink in capitate trichome with PAS; 100x magnification.

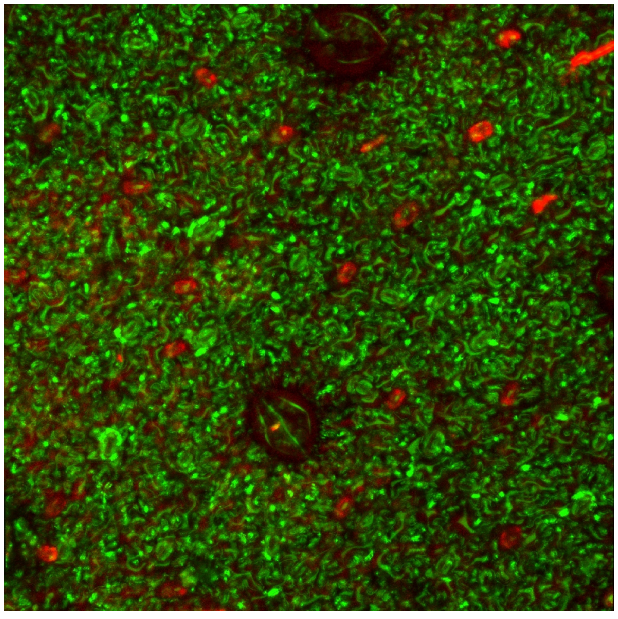

Fig. 3. CLSM Autofluorescence of secreted materials of trichomes of *S. subspicata*

Red autofluorescence in the peltate and strong red in capitate trichomes, and light-green autofluorescence in the nonglandular trichomes; 40x magnification.

chemical defense of plants, or in pollinators' attraction, but the specific composition of the secreted material in many Lamiaceae species have been investigated because of the biological effects of their essential oils, which are widely used in pharmaceutical preparations, perfumery and cosmetics (Werker, 1993; Bakkali et al. 2008). Data from the histochemical tests revealed that the secreted material in the glandular trichomes of *S. subspicata* is of heterogeneous composition, containing phenols, tannins, polysaccharides, pectins and lipids. Our investigation, when compared to previously published results (Marin et al. 2006, 2008), has shown a lot of similarities in the histochemical contents of glandular trichomes with other investigated species belonging to the family Lamiaceae. Histochemical studies together with investigation of trichome distribution could be useful in taxonomical research of the Lamiaceae species. Exact differences could be found using detailed chemical analyses.

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