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TRICHOMES OF *SATUREJA HORVATII* ŠILIĆ (LAMIACEAE) - MICROMORPHOLOGY AND HISTOCHEMISTRY

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Abstract - Considering the importance of *Satureja horvatii* Šilić as an endemic plant, and due to the essential oils produced in its glandular trichomes, we have done a comprehensive study of the micromorphology and a histochemical analysis of the plant's glandular trichomes. This investigation was carried out using light microscopy and scanning electron microscopy. Non-glandular unbranched and three types of glandular trichomes on the leaf surface – peltate, capitate and digitiform – were investigated. The results of histochemical tests showed a positive reaction to polysaccharides, proteins, pectins, lipids and to some secondary compounds such as terpenes, tannins and phenols in different types of glandular trichomes.

Key words: Satureja horvatii, trichomes, micromorphology, histochemistry

INTRODUCTION

Many species of the family Lamiaceae possess essential oils secreted by glandular trichomes distributed on the aerial vegetative and reproductive organs. The glandular trichomes are the primary secretory organs of these plants, and their structure can vary widely among species (Werker, 1993; 2000). The essential oils isolated from various *Satureja* species, which belong to the family Lamiaceae, have been shown to have biological and pharmacological activities, such as antibacterial, antifungal, antiviral, antioxidant (Ciani et al. 2000; Ćavar et al. 2008).

Satureja hortensis and *S. montana* are widely used as a flavoring agent in food products and also as a traditional herbal medicine. The genus *Satureja* L. includes around 200 species of herbs and shrubs, often aromatic, with a center of distribution in the Mediterranean region (Cantino et al. 1992). In the central and western Balkans, nine species of this genus have been registered. *Satureja horvatii* Šilić is an endemic species (Šilić, 1979; Todorović, 1989) of the Orjen-Lovćen mountain massif in Montenegro with a high content of the essential oil (Pavlović et al. 1984, 1987). To the best of our knowledge, published reports on the endemic species of *S. horvatii* only cover the chemical composition of its essential oil (Lakušić et al. 2008, 2011); this paper presents a micromorphological analysis of the different types of glandular trichomes and a histochemical study of their secreted products.

MATERIALS AND METHODS

Plant material

The plant material of *S. horvatii* was collected in Orjenske Lokve (Mt. Orjen, Montenegro), in October 2005. A voucher specimen is kept at the Herbarium of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOU 29697).

After three years of cultivation in the Botanical Garden "Jevremovac" in Belgrade, the micromorphology and histochemistry of *S. horvatii* glandular trichomes were analyzed. Leaves were sectioned and put into a fixative solution of 2% glutaraldehyde in phosphate buffer (0.1 M, pH=7.0), post fixed in 2% OsO4 and embedded in Araldite. Sections of 1 μm thickness were stained in toluidine blue. Investigation was done using an Olympus CX - 41 light microscope.

For the micromorphological study, small segments of leaves were fixed in glutaraldehyde (3% with buffer solution at pH 7-4). 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 a BALTEC-SCD 005 Sputtering Device, and observations were carried out on a JEOL JSM T 220 15kV SEM.

Histochemical analyses were performed on hand sections of fresh leaves using the following tests: PAS for polysaccharides (McManus, 1948), mercury BB for proteins (Mazia, 1953), ruthenium red for pectins (Johansen, 1940), $K_2Cr_2O_7$ for tannins (Gabe, 1968), $FeCl_3$ for phenols (Johansen, 1940), NBA for acid lipids (Cain, 1947), NADI for terpenes (David and Carde, 1964), and Sudan IV for lipids (Pearse, 1985).

Observations were made under a Leitz Dialux light fluorescence microscope HBO 50W block filter A – excitation wavelengths were BP 340 – 380.

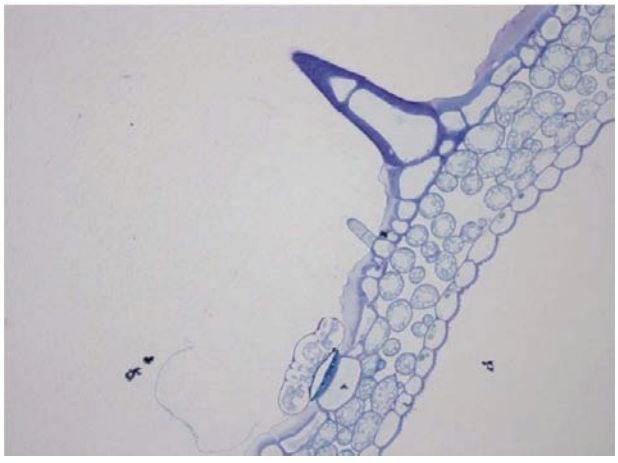


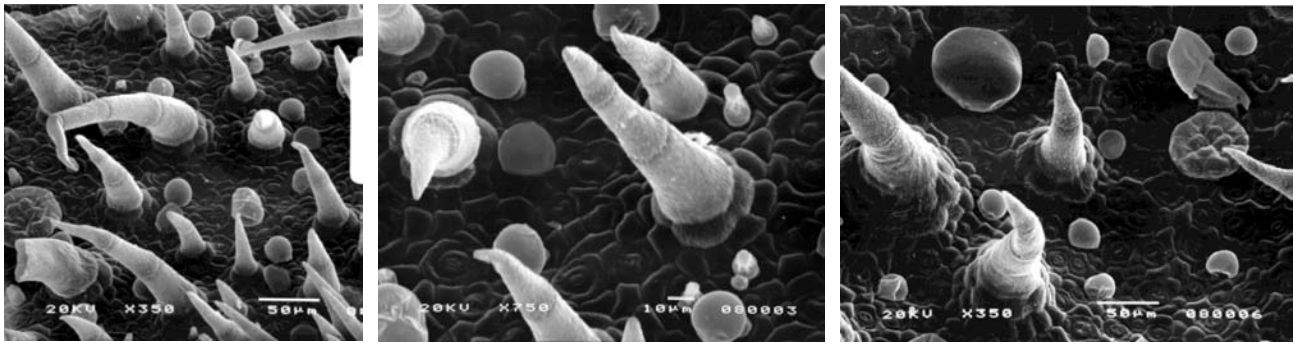
Fig. 1. Light micrograph of *S. horvatii* leaves at 40x magnification.

RESULTS AND DISCUSSION

A leaf cross-section of *Satureja horvatii* (Fig. 1) indicated the presence of several different morphological types of glandular and non-glandular trichomes. The adaxial and abaxial leaf sides of *Satureja horvatii* were covered by an indumentum containing peltate, capitate and digitiform trichomes, as well as unicellular and multicellular unbranched non-glandular trichomes with wart-like cuticular structures (Fig. 2A-2C). An *in vivo* hand cross section of fresh leaves (Fig. 3A, 3B) showed that the peltate trichomes distributed on the adaxial and abaxial leaf sides consisted of one basal epidermal cell, a wide stalk cell and a multicellular head consisting of twelve cells. Capitate trichomes, uniformly distributed on both leaf surfaces, were divided into two types according

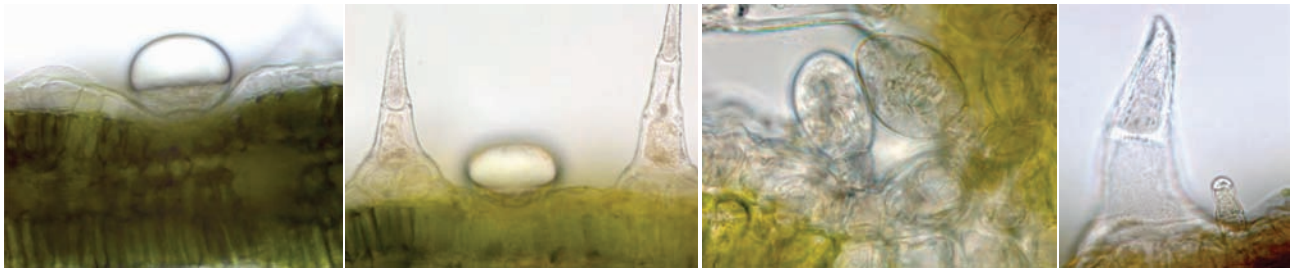
Table 1. Histochemistry of secreted material of glandular trichomes of *S. horvatii.*

Staining procedure	Target compounds	Peltate trichomes	Capitate trichomes	Digitiform trichomes
PAS	polysaccharides	+ +	+	+
Merccuri BB	proteins	+	+	-
Ruthenium red	pectins	+ +	+	+
$K_2Cr_2O_7$	tannins	+ +	+	-
$FeCl_3$	phenols	+ +	+	-
NBA	acid lipids	+ +	-	-
Nadi	terpenes	+ + +	+	-
Sudan IV	lipid	+ + +	+	-



A B C

Fig. 2. Scanning electron micrographs of *S. horvatii* leaves.
A. Adaxial leaf surface of *S. horvatii*. Bar = 50µm.
B. Capitate, digitiform and non-glandular trichomes on adaxial leaf surface. Bar = 10µm.
C. Peltate, capitate and non-glandular trichomes on abaxial leaf surface. Bar = 50µm.



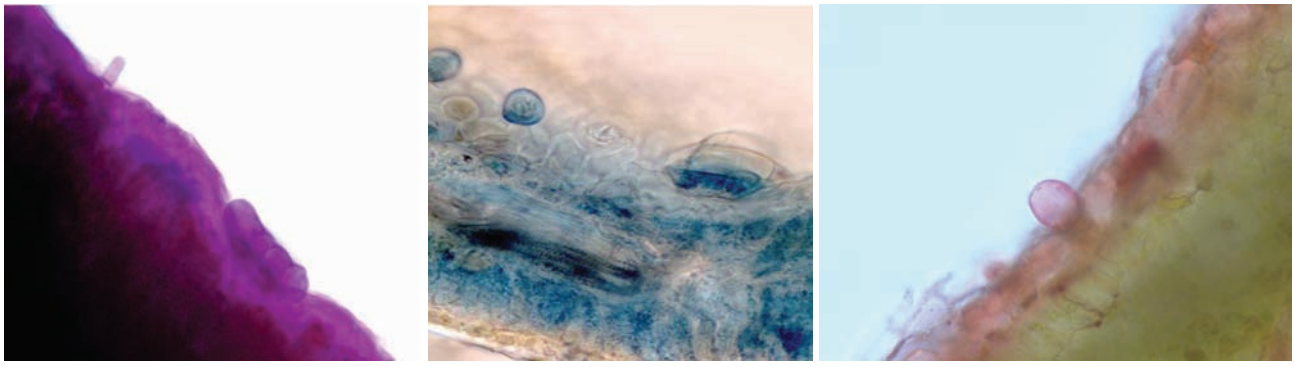
A B C D

Fig. 3. Hand cross section of fresh leaves of *Satureja horvatii.*
A. Peltate trichome on adaxial leaf surface. 40x magnification.
B. Peltate and non-glandular trichomes on adaxial leaf surface. 40x magnification.
C. Capitate trichomes type I and type II on adaxial leaf surface. 100x magnification.
D. Digitiform and non-glandular trichomes on adaxial leaf surface. 100x magnification.

to the shape of the unicellular secretory head. Type I was composed of one basal cell, one stalk cell and spherical unicellular head, while type II was composed of one basal cell, one stalk cell and an ellipsoid unicellular head. Both types of capitate trichomes touched each other with glandular head cells on the margins of the adaxial surface of the leaves (Fig. 3C). Capitate trichomes were more numerous than peltate and digitiform trichomes on the both leaf sides. Digitiform trichomes, which do not show a clear distinction between the apical glandular cell and the subsidiary cells, occurred between the peltate and capitate trichomes with a smaller distribution (Fig. 3D). Non-glandular trichomes were densely distributed on the adaxial and abaxial leaf sides and on the margins of the leaves. Observations of trichomes distributed on vegetative organs using different microscopic techniques provided useful information about their structure.

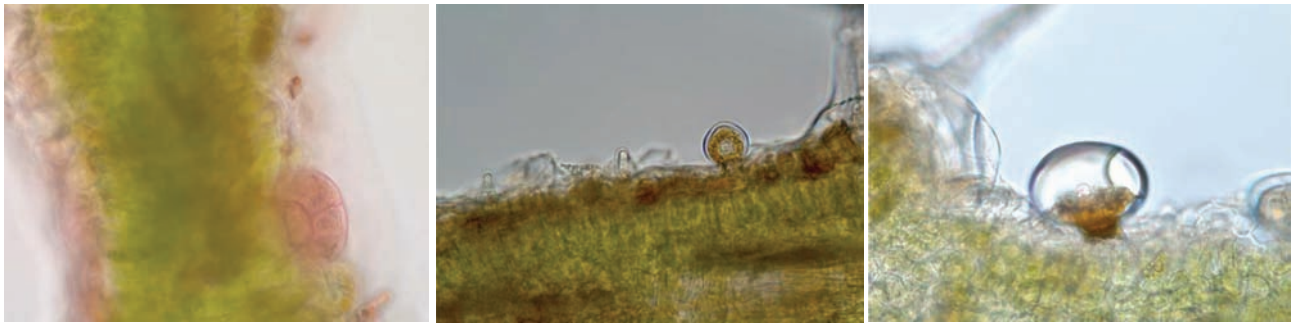
In this study, we focused on the type and distribution of glandular trichomes secreting essential oils.

The results of histochemical analysis of the secreted products of the glandular trichomes are presented in Table 1. These results show a positive reaction to polysaccharides, proteins, pectins, lipids and to some secondary compounds as terpenes, tannins and phenols.



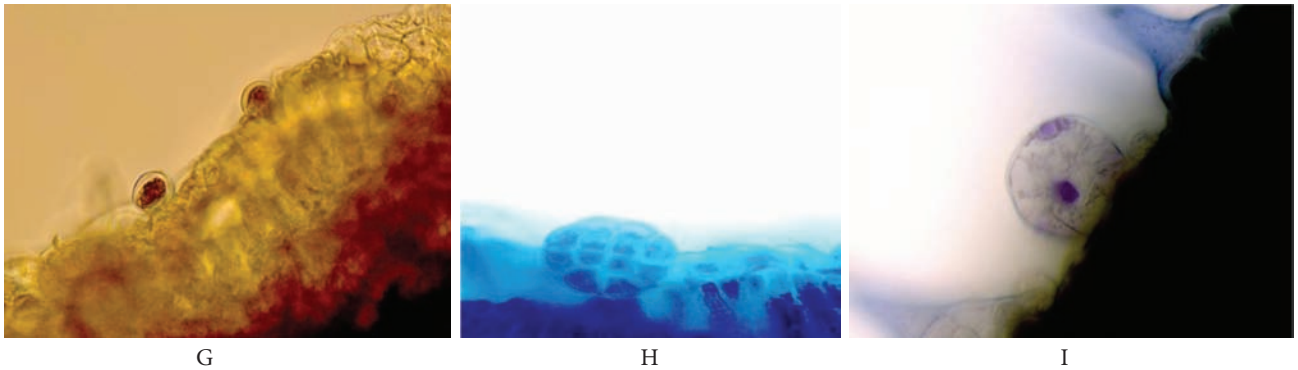
A B C

Fig. 4. Histochemical characterization of the secretions of *Satureja horvatii* glandular trichomes.
A. Secretion staining dark pink in peltate and digitiform trichomes with PAS. 40x magnification.
B. Blue staining of secretion in peltate and capitate trichomes with mercury BB. 40x magnification.
C. Light red staining in capitate trichome with ruthenium red. 40x magnification.



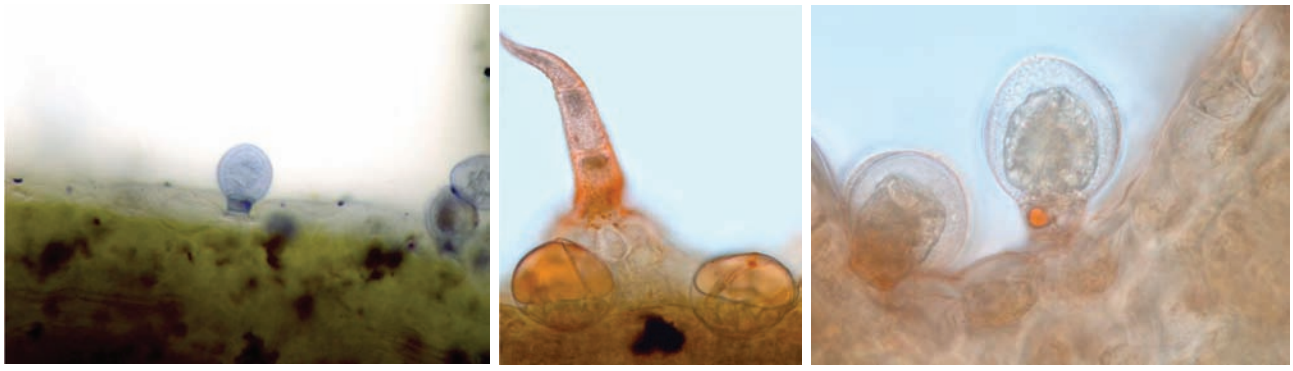
D E F

D. Light red staining in the peltate trichome with ruthenium red. 40x magnification.
E. Secretion staining brown in capitate trichome with $K_2Cr_2O_7$. 40x magnification.
F. Secretion staining light brown in peltate trichomes with $K_2Cr_2O_7$. 40x magnification.



G H I

G. Secretion staining dark brown in capitate trichomes with $FeCl_3$. 40x magnification.
H. Secretion staining dark blue in peltate trichomes with NBA. 40x magnification.
I. Secretion staining violet in peltate trichome with Nadi. 40x magnification.



J K L

J. Secretion staining light violet in capitate trichomes with Nadi. 40x magnification.
K. Dark orange staining of secretion with Sudan IV in peltate trichomes. 40x magnification.
L. Orange staining of secretion with Sudan IV in capitate trichomes.100x magnification.

An intensive positive reaction with PAS for polysaccharides show a pink staining of secretory material in the head of peltate and digitiform trichomes (Fig. 4A), while light red staining with ruthenium red also gave a positive reaction for pectins and can be observed in the head of peltate, capitate and digitiform trichomes (Fig. 4C, 4D). The apical glandular cell of digitiform trichomes developed a small subcuticular space and secreted small amounts of polysaccharides and pectins (Fig. 4A, 4D). Staining with mercury BB for proteins gave a positive reaction, showing a blue color only in the heads of peltate and capitate trichomes (Fig. 4B).

The results of histochemical tests was positive to tannin compounds in the secretory heads of peltate and capitate trichomes with a light brown color (Fig. 4E, 4F), while the $FeCl_3$ test showed a positive reaction for phenols with a dark-brown staining of the secretory products in the peltate and capitate trichomes (Fig. 4G). With the Nadi procedure, the reaction was positive both in the peltate and capitate trichomes, showing a violet color indicating terpene compounds; the reaction was stronger in the peltate trichomes (Fig. 4I, 4J).

Staining with Sudan IV for lipids gave a strong positive reaction in the subcuticular spaces of the peltate and in the stalk cells of capitate trichomes with an intensive orange color (Fig. 4K, 4L). Intensive blue staining with NBA was observed in the secretory heads of peltate and capitate trichomes (Fig. 4H).

The specific compounds 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 (Bezić et al., 2005; Skočibušić et al. 2006; Ćavar et al. 2008). The present study, when compared to previously published results (Marin et al. 2006, 2008, 2010), showed similarities in the content of secretory products and confirmed that the secreted compounds in the glandular trichomes of *S. horvatii* possess a heterogeneous composition, containing polysaccharides, pectins, proteins, phenols, tannins, terpenes and lipids. With the increasing tendency to use volatile oils, our investigation, together with the results of chemical investigations, indicate that *S. horvatii* essential oil from cultivated populations could be applied in the pharmaceutical industries.

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