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Professional paper

MICROMORPHOLOGICAL AND HISTOCHEMICAL EVALUATION OF THE GLANDULAR TRICHOMES OF SALVIA FRUTICOSA MILL. IN DHERMI AREA, IN SOUTH ALBANIA

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Abstract

The species of the genus Salvia are known for their medicinal value due to the presence of chemical compounds or as they are known as secondary metabolites. These compounds are present in the aerial parts of the plant, mainly in their glandular trichomes. The purpose of this study is to evaluate the micromorphological structures of glandular trichomes and identify the secondary metabolites they contain in Salvia fruticosa Mill. in the Dhermi area, in South Albania. The histochemical technique used for the identification of secondary metabolites is the classical technique of staining the freehand sections of fresh plant material, which exploits the properties of certain reagents to form the chemical compounds containing glandular trichomes, stained products visible in the light microscope. Three main types of glandular trichomes were identified: peltate, capitate, and digitiforme. Peltate trichomes possess a basal cell, a short unicellular stalk, and a large secretory head with 8 secretory cells. Four types of capitate trichomes and a type of digitiforme trichome have been found in Salvia fruticosa Mill. Fenoles were detected at a high level at capitate trichomes type I and peltate trichomes. Proteins were detected at a high level at capitate trichomes type III. Alcaloides and tanines were detected at high levels at capitate trichomes type I, II, III and digitiforme trichomes. Tanines were detected at peltate trichomes too. Lipides were detected at high levels at capitate trichomes type II and III. Polisacharides were detected at high levels at capitate trichomes type II, III, IV, digitiforme, and peltate trichomes. Mucilages were detected at high level at capitate trichomes type III and peltate trichomes.

Keywords: secondary metabolites, trichomes, peltate trichomes, capitate trichomes, digitiforme trichomes.

1. Introduction

Salvia (Sage) is the largest genus of Lamiaceae family encompasses about 1,000 species (El-Feky & Aboulthana, 2016) In our country this genus is represented by 16 species (Qosja et al., 1996). Sage is used in traditional medicine in ancient times. Chemical components and the essential oils they contain have antimicrobial, antioxidant, antidiabetic, and anti-inflammatory properties. Some species of Salvia are used as herbal tea and in the food, perfumery, cosmetics, and pharmaceutical industries, and some species are used as ornamental plants (Kahraman et al., 2010). The leaves of Salvia species are covered with nonglandular and glandular trichomes which originate from epidermal cells (Werker, 2000). Salvia is the genus that has the largest number of trichomes in the Lamiaceae family. (Metcalfe and Chalk 1972) For the morphological variety they represent, glandular trichomes are widely used in taxonomy to make comparisons between species within the same genus (Stuessy 2009). *Salvia fruticosa* Mill. (Greek sage) is an aromatic perennial herb or sub-shrub. *S. fruticosa* Mill. is native to the eastern Mediterranean, distributed from Italy, Sicily, and Liberia, to south Balkan and western Syria (Hedge, 1982; Greuter et al., 1986). In our country this species is distributed only in the southwestern part of Albania (Schmiderer et al., 2013). It is one of the most economically important species of Salvia, valued for its beauty, medicinal properties, and culinary use, along with its sweet nectar and pollen, and has a particularly long tradition of application in Greece.

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S. futicosa has more medicinal values than *S. officinalis*. (Mróz & Kusznierewicz, 2023). In different countries, there are different uses of this species. In Turkey, it was used for urinary system aliments. In Lebanon it was used for ulcer pain. (Saab et al., 2021). The essential oils derived from Greek sage were proven to have antioxidant, antibacterial, and anti-inflammatory activities and were used traditionally for treating skin infections (Risaliti et al., 2019; Karadağ et al., 2020). There is not much data on the micromorphology of glandular hairs for Salvia *fruticosa* Mill. Considering that there is no data about the micromorphology of glandular trichomes for this species in our country, the present study was undertaken.

2. Materials and Methods

The materials used for the present study are fresh leaves from *Salvia fruticosa* Mill. collected in Dhermi, in the area called Potami source with geographic coordinates N 40.155336, E 19.639430. For micromorphological investigations of glandular trichomes we used free hand sections of fresh leaves and examined with light microscopy Optika and Olympus at ×40 magnification. For the study of secondary metabolites, the histochemical technique uses the properties of some reagents to form with the chemical compounds that contain trichomes, stained products visible in the light microscope. Photographs were realised with a photocamera (apple phone) directly in the eyepiece and with Microgiciel software. The histochemical reactions that were performed are:

- Staining with Sudan III (Daddi, 1896) for total lipids. Trichomes that have lipid content are colored red.
- Staining with iron (III) chloride (Johansen, 1940) for phenols. Trichomes containing phenols are colored black.
- Staining with potassium bichromate (Gabe, 1968) for tannins. Trichomes containing tannins are colored yellow/orange.
- Periodic acid / Schiff reagent (PAS) (Feder & O'Brien, 1968) for polysaccharides,
- Alcian Blue (Pearse, 1985) for mucopolysaccharides (mucilages). The presence of mucilage gives the blue color of trichomes when stained with alcian blue.
- Wagner reagent (Furr & Mahlberg, 1981) for alkaloids. Alkaloid containing trichomes are colored brown.
- Hager's reagent (picric acid) colors trichomes with protein content in phosphorescent yellow.

For glandular trichome classification we followed (Corsi and Bottega, 1999).

3. Results and Discussion

Micromorphology of glandular trichomes

In *S. fruticosa* leaves both peltate and capitate glandular trichomes were found. peltate trichomes posses a basal cell, a short unicellular stalk, and a large secretory head with 8 secretory cells.

Capitate type I, consists of a short uni or bicellular stalk and a large uni or bicellular head.

Capitate type II, is very small with a short unicellular stalk and an oblong unicellular head. This type of trichome is smaller than other trichomes. In type II we found three subtypes of capitate hair, subtype A with an oblong head, subtype B with cup-shaped head and subtype C with curved head. Capitate type III, is large with a long stalk consisting of one to three cells, a neck cell and a large unicellular head which may be cup-shaped (subtype III A) or round shaped (subtype III B). Capitate type IV, is very large with a very long stalk one to four cells, a neck cell and a unicellular head which may be cup-shaped or round shaped.

The fifth (V) type capitate trichome like finger shaped called digitiform trichome was figured out.

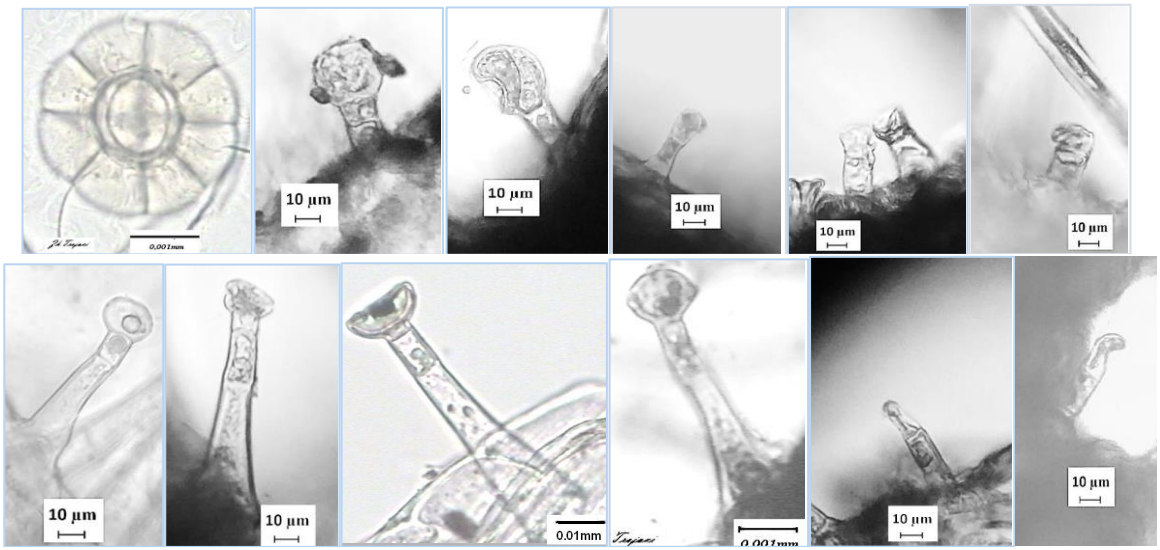

Figure 1. Glandular trichomes in *S. fruticosa* Mill. Dhermi. (1) peltate trichome; (2) and (3) capitate type I capitate trichome; (4), (5), and (6) type II capitate trichome; (7) and (8) capitate type III ; (9) and (10) type IV capitate trichome; (11) and (12) digitiform trichome.

Histochemical analysis for secondary metabolites

The following table shows the results of the histochemical analyses. It is noted that the secondary metabolites that dominate in *S. fruticosa* are alkaloids and tannins. Phenols and proteins were detected in smaller amounts.

Table 1. Results of histochemical reactions in *S. fruticosa* Mill. for capitate trichomes type I-IV

Stain	Detected compound	Control/ Colour	Capitate I		Capitate II		Capitate III		Capitate IV	
			C/S	S/S	S/S	C/S	C/S	S/S	C/S	S/S
FeCl3	Phenoles	black	+++	-	++	++	-	-	++	++
Picric Acid	Proteins	Phosphore-scent yellow	-	-	-	+++	-	+++	?	?
Iodine	Alkaloids	brown	+++	+++	+++	+++	+++	+++	?	?
$K_2Cr_2O_7$	Taninns	Yellow/ orange	+	+++	+++	+++	++	+++	?	?
Sudan III	Lipids	red	+	+	+++	+++	+++	-	-	-
PAS	Polysaccharides	cyclamen	-	+	+++	+++	+++	+++	-	+++
Alcian blue	Mucilages	blue	?	?	++	-	+++	+++	?	?

Table 2. Results of histochemical reactions in *S. fruticosa* Mill. for digitiforme and peltate trichomes.

Stain	Detected compound	Control/ Colour	Digitiforme		Peltate	
			S/S	S/C	C/S	S/S
FeCl3	Phenoles	black	+	-	+++	+++
Picric Acid	Proteins	Phosphore-scent yellow	?	?	-	++
Iodine	Alkaloids	brown	+++	+++	+	-
$K_2Cr_2O_7$	Taninns	Yellow/ orange	++	+++	+++	++
Sudan III	Lipids	red	-	+	+	-
PAS	Polysaccharides	cyclamen	+++	+++	+++	+++
Alcian blue	Mucilages	blue	-	-	+++	+++

Note: S/C: secretory cells, S/S: secretory space
(-) negative, (+) slightly positive, (++) moderately positive, (+++) very positive, (?) no metabolite found

The tables and figures shows histochemical results for secondary metabolites in *S. fruticosa* Mill. trichomes.

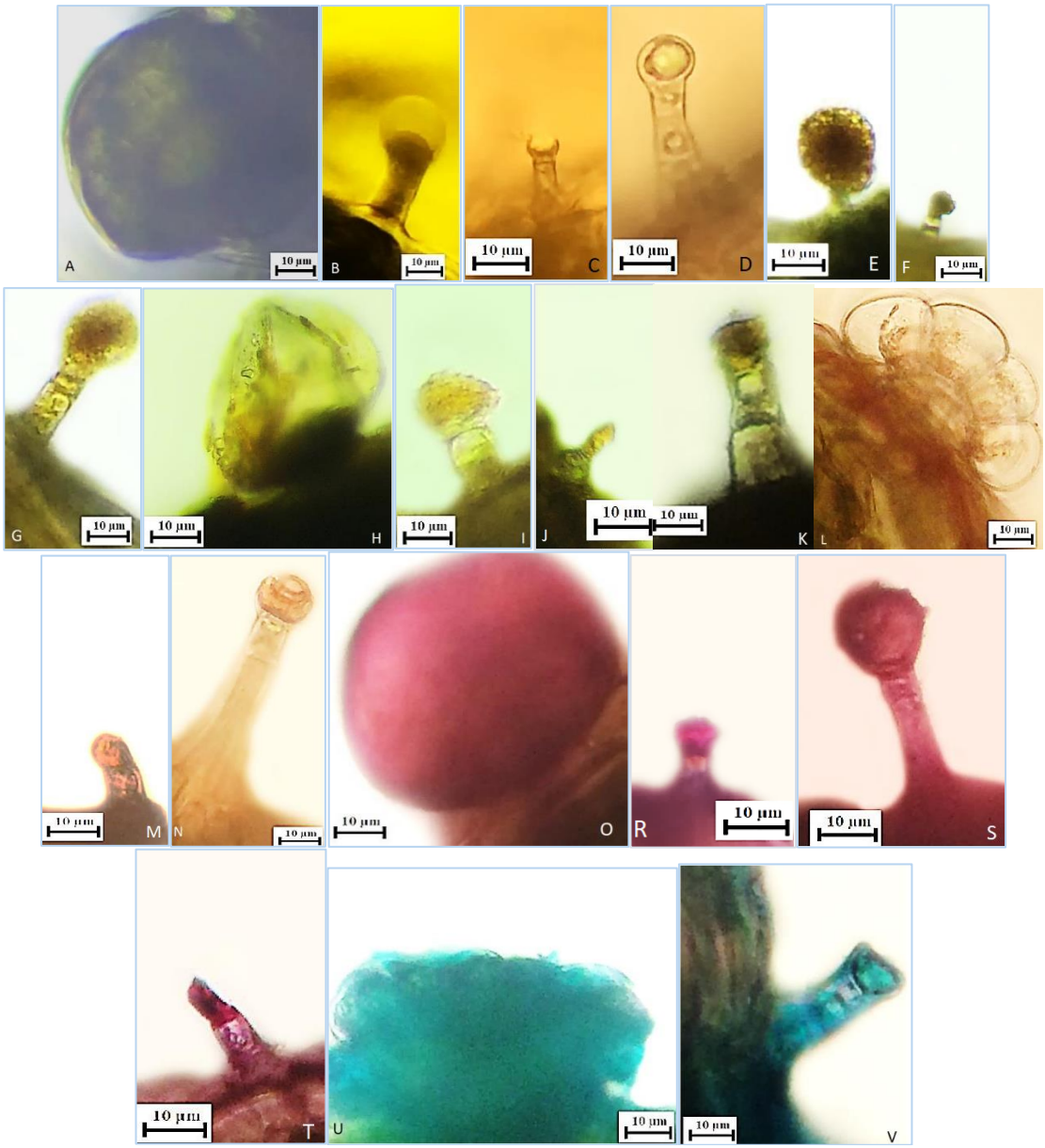

Figure 2. Histochemical results for secondary metabolites in *Salvia fruticosa* Mill. trichomes: A, B – reaction with $FeCl_3$ for phenoles; C, D – reaction with picric acid for proteins; E, F, G – reaction with Wagner reagent for

alkaloids; H, I, J, K – reaction with $K_2Cr_2O_7$ for taninns; L, M, N – reaction with sudan III for lipids; O, R, S, T – reaction with PAS reagent for polysacharides; U, V – reaction with alcian blue for mulicages.

4. Conclusions

The species of the genus Salvia, represented by *S. fruticosa* Mill. in this study, contain in their leaves a variety of trichomes that contain a variety of essential oils and secondary metabolites. In addition to the medicinal, nutritional, aesthetic and pharmaceutical values that this species has, also for the morphological variety that trichomes present and the ease of their study techniques, these structures play an important role in the study of diversity at the intraspecific level (Faust & Jones, 1973 ; Adedeji et al., 2007). So this study can be used as a comparison for other studies that can be done for *S. fruticosa* Mill. in other regions where this species is spread to study the intraspecific diversity at the histological and histochemical level that this species can display. The histochemical analysis shows that the secondary metabolites that have been detected in the highest amount are alkaloids and tannins. Alkaloids isolated from plants serve as antibacterial, antiviral, insecticidal, and antimetastatic effects on various types of cancers both in vitro and in vivo. Plant tannins have been used as additives in animal (specialy in guts) production for many years improving performance or meat quality (Xiao et al., 2018; Shen et al., 2021). The latest studies show that the tannins contained in the plants are used in traditional Chinese medicine as anti-tumor drug (Ding et al., 2023). The presence of alkaloids and tannins increases even more the values of Greek sage to be used as a natural medicine with anti-viral and anti-tumor effects.
It is recommended to do histochemical studies of *S. fruticosa* Mill. in the natural and cultivated habitat and to make comparisons in the amount of secondary metabolites produced by this species depending on the environmental conditions.

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