MICROMORPHOLOGY AND HISTOCHEMISTRY OF LEAF TRICHOMES OF *SALVIA AEGYPTIACA* (LAMIACEAE)

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Abstract: We performed a comprehensive study of trichomes considering the medicinal importance of the essential oils produced in glandular trichomes of *Salvia aegyptiaca* L. and lack of data about leaf trichome characteristics. Micromorphological and histochemical analyses of the trichomes of *S. aegyptiaca* were carried out using light and scanning electron microscopy. We report that the leaves contained abundant non-glandular unbranched trichomes and two types of glandular trichomes, peltate and capitate, on both leaf surfaces. The abaxial leaf side was covered with numerous peltate and capitate trichomes, while capitate trichomes were more abundant on the adaxial leaf side, where peltate trichomes were rarely observed. The non-glandular trichomes were unicellular papillae and multicellular, uniseriate, two-to-six-celled, erect or slightly leaning toward the epidermis. Peltate trichomes were composed of a basal cell, a short cylindrical stalk cell and a broad head of eight secretory cells arranged in a single circle. Capitate trichomes consisted of a one-celled glandular head, subtended by a stalk of variable length, and classified into two types: capitate trichomes type I (or short-stalked glandular trichomes) and capitate trichomes type II (or long-stalked glandular trichomes). Histochemical tests showed that the secreted material in all types of *S. aegyptiaca* glandular trichomes was of a complex nature. Positive reactions to lipids for both types of glandular trichomes were obtained, with especially abundant secretion observed in peltate and capitate trichomes type II.

Key words: *Salvia aegyptiaca*; Lamiaceae; trichomes; micromorphology; histochemistry

INTRODUCTION

The genus *Salvia* (sage) belongs to the Lamiaceae family and includes over 900 species distributed worldwide [1]. The genus comprises herbaceous, suffructicous or shrubby perennials, rarely biennial or annual, often strongly aromatic plants. *Salvia* species have been important since ancient times, and are used in traditional medicines around the world. They possess antioxidant, antibacterial, antidiabetic, antitumor, antiplasmodial and anti-inflammatory activities [2-9]. Many *Salvia* species are used as herbal tea and food, in cosmetics, perfume and pharmaceutical industries; in addition, some species are grown as ornamental plants [10-13].

The leaves of many plants are densely covered with glandular and non-glandular trichomes, which originate from the epidermal cells [14] and vary considerably between species and even on the organs of the same individual in morphology, location, ability to secrete, mode of secretion, composition of secreted material, etc. Plant species that develop glandular trichomes generally produce relatively large amounts of bioactive compounds that include highly concentrated phytochemicals possessing biological activity and potential applications in the food or pharmaceutical industries [15-19]. There are a number of studies on the foliar micromorphology of representatives of the genus *Salvia* [3,20-28]. Tissier [29] emphasized that plant glandular trichomes represent a broad area of research, mainly due to interests in elucidating the biosynthetic pathways of industrially relevant compounds, such as essential oils, pharmaceutical ingredients or substances, which may be used in plant defense.

Salvia aegyptiaca L. (Egyptian sage) is a xerophytic perennial branched shrub distributed on the Arabian Peninsula, Egypt, Palestine, Iran, Afghanistan [30], Canary Island, Morocco, Mauritania, Niger, Chad, Sudan, Ethiopia, Pakistan and India [31]. It is commonly used for various medicinal and cosmetic purposes [32]. The seeds are used as a demulcent and for the treatment of diarrhea and hemorrhoids [33]. The whole plant is used against gonorrhea and eye diseases, and as an antiseptic, cicatrizant, antispasmodic and stomachic [34], and has also been reported to be beneficial in cases of nervous disorders, dizziness, trembling and for stopping perspiration [35]. Because of its uses in folk medicine, it has been examined chemically [30,34,36-40].

The micromorphology and histochemistry of *S. aegyptiaca* have not been studied previously. Considering the potential pharmacological value of this species, we present the results of a micromorphological analysis of the different types of glandular trichomes, and a histochemical study of their secreted products. This work was undertaken in order to evaluate the possible functional significance of the various trichomes.

MATERIALS AND METHODS

Plant material

The leaves of *Salvia aegyptiaca* collected from mature plants grown in the greenhouse of the Institute for Biological Research "Siniša Stanković", Belgrade, were used for analyses in this study. The seeds were obtained from the Seed Bank in Tripoli, which were collected in Om-Jersan on Nafusa (Western) Mountain in Libya. Voucher samples are stored in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOU, Voucher No. 16881).

Light microscopy

For light microscopy (LM), leaf sections of *S. aegyptiaca* were fixed with 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for 24 h at 4°C. Subsequently, the material was washed in sodium phosphate buffer 3 times over 2 h and post-fixed in 1% osmium tetroxide in the same buffer for 24 h at 4°C. The fixed material was washed with distilled water, dehydrated in a graded ethanol series and embedded in Araldite resin CY 212 (Agar Scientific Ltd. England). Semi-thin cross sections (1-1.5 μm thick) were cut on a LKB III ultramicrotome and stained with 0.1% methylene blue in 1% borax. Sections were photographed under Zeiss Axiovert microscope (Carl Zeiss GmbH, Göttingen, Germany).

Histochemical analyses

Histochemical characterization of the secreted material was performed on fresh free-hand sections of leaves using the following histochemical tests: Sudan IV and Sudan Black B for total lipids [41]; osmium tetroxide (OsO_4) for unsaturated lipids [41,42]; Nile blue A for neutral and acidic lipids [43]; Nadi reagent for terpenes [44]; ferric trichloride for phenolic compounds [42]; toluidine blue O for tannins [45]; periodic acid-Schiff (PAS) reagent for polysaccharides [41]; ruthenium red for pectins [46]; Sudan red 7B/hematoxylin [47] for lipophilic and hydrophilic secretions, simultaneously.

Fluorescence microscopy examination under ultraviolet light was utilized for phenolic substance detection [48]. For flavonoid detection, induction of fluorescence with fluorochrome aluminum trichloride under ultra violet light was employed [49].

Standard control procedures were carried out simultaneously. All sections were mounted in glycerin under a coverslip, examined and photographed using Zeiss Axiovert microscope (Carl Zeiss GmbH, Göttingen, Germany).

Scanning electron microscopy

For scanning electron microscopy (SEM), adult leaf segments were coated with a thin layer of gold and palladium in a BAL-TEC SCD 005 sputter coater. Both adaxial and abaxial surfaces were examined with a JEOL JSM-6390 LV at an acceleration voltage of 15 kV.

RESULTS

Micromorphology and secretion

The leaves of *S. aegyptiaca* are oblong, crenate to serrate, basal leaves are stalkless, the upper with a short stalk, and covered with an indumentum of non-glandular and glandular trichomes on both surfaces (Figs. 1 and 2). The abaxial leaf side is covered with numerous peltate and capitate trichomes (Fig. 2), while capitate trichomes are more abundant on the adaxial leaf side, where peltate trichomes are rare (Figs. 1 and 2).

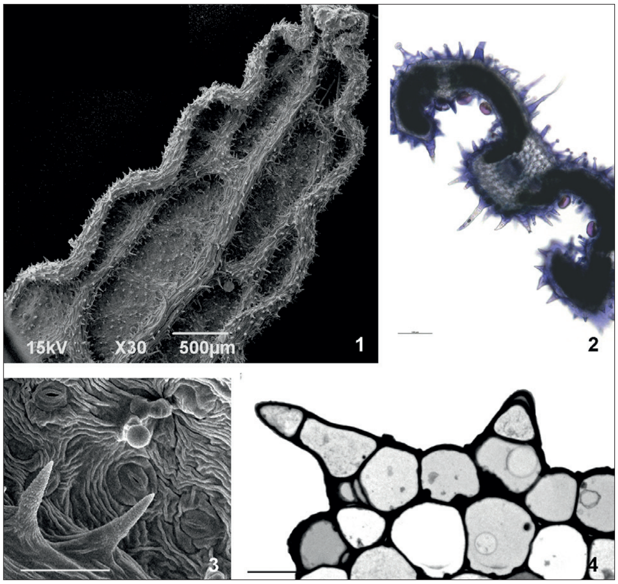
Non-glandular trichomes are of two types: unicellular papillae and multicellular, uniseriate, two-to-six-celled, erect or slightly leaning toward the epidermis. Non-glandular trichomes are characterized by a swollen basal cell, acute apices and thick, warty, cell walls (Figs. 3 and 4). Non-glandular trichomes can be characterized as arrect type according to Payne's classification [50].

The glandular trichomes include capitate and peltate types. Capitate trichomes consist of a one-celled glandular head, subtended by a stalk of variable length, facilitating their classification into two types: capitate trichomes type I or short-stalked glandular trichomes (Figs. 5, 6 and 7) and capitate trichomes type II or long-stalked glandular trichomes (Figs. 8, 9 and 10). The capitate trichomes of both types were observed on both leaf surfaces, but were more abundant on the adaxial epidermis and along the veins. With leaf expansion, the density of the trichomes decreases.

Capitate trichomes type I consist of a cubical or cylindrical basal cell embedded in the epidermis, a unicellular stalk with thick cutinized anticlinal cell walls and a unicellular globoid to ovoid head (Figs. 6 and 7). The cytoplasm of stalk cells is dense and rich in dark-stained organelles and vesicles, and small, translucent vacuoles. The head cell is characterized by dense cytoplasm, dark vesicles and minor vacuoles. The thick cuticle tightly covers the cell of the secretory head in young trichomes, and at maturity a small, often laterally positioned, subcuticular space is present (Figs. 19 and 20). The secreted material, which temporarily accumulates in the subcuticular chamber, probably exudes through the intact cuticle. Following the release of secretion, a shrinking of the head cell and leaning of the trichome toward the epidermis was observed, ending with degradation of the short trichome (Fig. 5).

Capitate trichomes type II are composed of basal vacuolated cubical or cylindrical cell, the stalk of three-four cylindrical cells covered with a thick cuticle and oval secretory head (Figs. 10 and 26). The neck cell (top stalk cell) is rich in cytoplasm and small vacuoles. The secretory head cell is rich in cytoplasm, small vacuoles and dark-stained vesicles. The thick cuticle tightly covers the cell of the secretory head in young trichomes (Figs. 10, 23 and 28). Subsequently, the cuticle detaches from the cell wall, forming the subcuticular space, and secretory product accumulates inside the subcuticular space (Figs. 8, 9 and 27). At the end of the secretory stage, shrinkage of the head cuticle occurs and the secretory head becomes deflated and cup-shaped (Fig. 24).

Peltate trichomes are balloon-shaped and composed of a basal cell, short cylindrical stalk cell and broad head of eight secretory cells arranged in a single circle (Figs. 11, 13 and 16), or 12 secretory cells arranged in two circles (Fig. 12). The basal cell is slightly larger than the other epidermal cells. The most dominant organelle in the basal cell is a large, translucent vacuole. Stalk cells have dense cytoplasm, dark organelles and small, light vacuoles. The head cell volume occupies a large vacuole and osmiophilic dark deposits that are stored in the space between the vacuole and plasma membrane (Fig. 16). The cuticle of young trichomes, in the presecretory stage of development, is closely attached to the secretory cells (Fig. 11). Subsequently, after secretory product starts



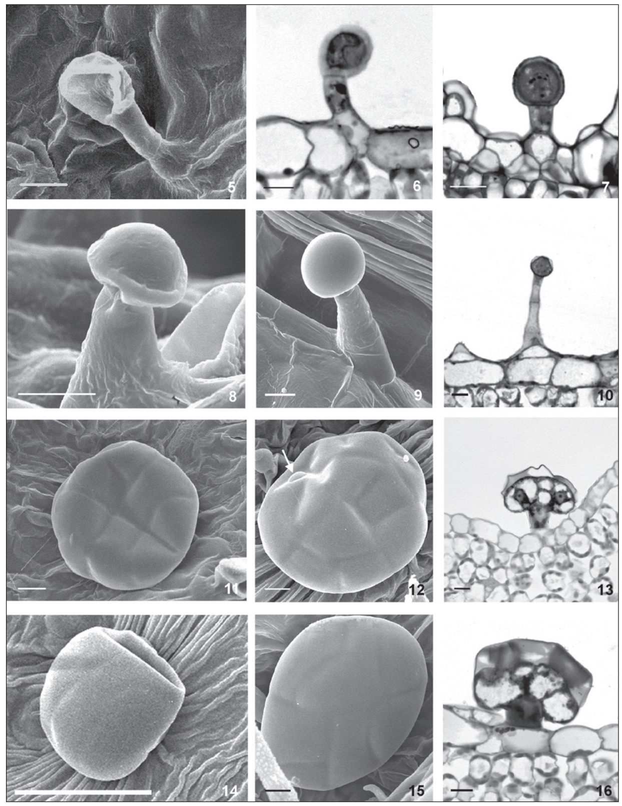
Figs. 1-4. SEM and LM micrographs of *S. aegyptiaca* leaves. **1** – SEM micrograph showing trichome indumentum on abaxial leaf surface; **2** – hand cross-section of a leaf with non-glandular and glandular trichomes; **3** – SEM micrograph showing the morphology of non-glandular and glandular trichomes; **4** – semi-thin section of non-glandular trichomes in the longitudinal direction. Bar = 500 µm (**1**), 100µm (**2**), 50 µm (**3**), 20 µm (**4**).

to accumulate inside the subcuticular space, the cuticle detaches from the secretory cells and becomes wrinkled in appearance (Figs. 12 and 13). At maturity, when the subcuticular space that increased considerably in volume is filled with secreted material, the cuticle becomes smooth (Figs. 14 and 15). SEM images revealed drops of secretory material visible below the cuticle (Fig. 12). LM indicated that cuticle rupture is frequent along the equatorial plane of the head following secretion release and detachment of the cuticular cup (not shown).

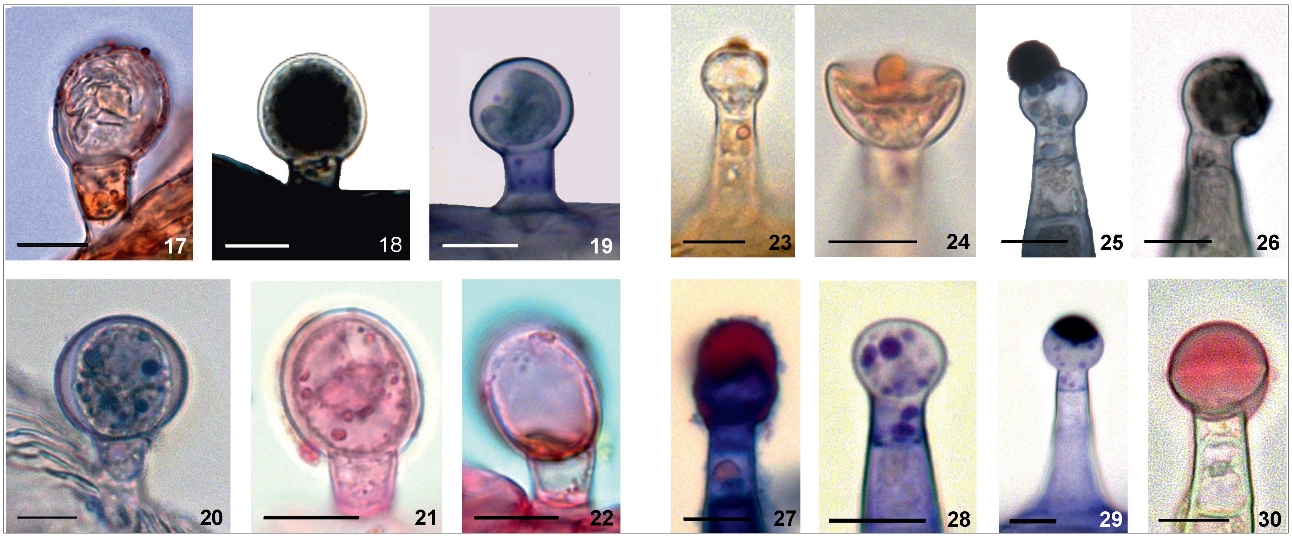
Histochemistry

The results of the histochemical tests carried out to characterize the main classes of compounds contained in the glandular trichomes of *S. aegyptiaca* are summarized in Table 1.

Capitate trichomes type I have a small subcuticular space and secretory material mainly accumulates inside the glandular cell (Figs. 17-22), whereas capitate trichomes type II accumulate secretion inside the head cell and in the subcuticular space (Figs. 23-30). In their large subcuticular sacs, peltate trichomes store abundant secretory product with an emulsion-



Figs. 5-16. SEM and LM micrographs showing types, morphology and structure of glandular trichomes on leaf of *S. aegyptiaca*.; **5** – SEM micrograph of capitate glandular trichome type I; note the shrunken head cell after releasie of the secretion. **6-7** – LM micrographs showing the morphology of capitate trichomes type I; note the thick cuticle; **6** – rich and dense cytoplasm with dark vesicles in secretory head cell; **7-9** – SEM micrographs of mature capitate glandular trichomes type II with a large spherical secretory head; **10** – semi-thin section of capitate glandular trichomes type II showing the secretory head cell with dense cytoplasm and small dark vesicles; **11** – SEM micrograph of juvenile peltate trichome with cuticle closely attached to the secretory head cell walls; **12** – SEM micrograph of peltate trichome with a wrinkled surface; note the decrease in secreted material (↑); **13** – semi-thin longitudinal section of peltate glandular trichome with wrinkled cuticle and small subcuticular space; **14** – SEM micrograph of peltate glandular trichome with the cuticle detached from the secretory head cells; **15** – SEM micrograph of mature, balloon shaped, peltate trichome with smooth cuticle; **16** – semi-thin longitudinal section of mature peltate glandular trichome with osmiophilic secretory product in the subcuticular space. Bar = 10 µm (**5-16**), 50 µm (**14**).

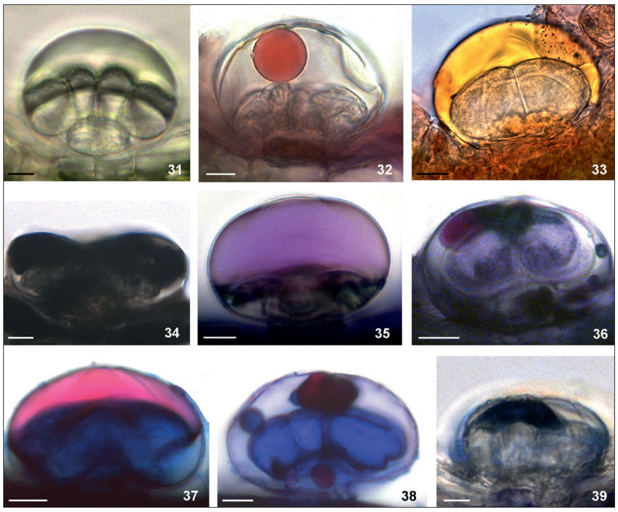


Figs. 17-30. Histochemical characterization of the secretions of capitate trichomes of *S. aegyptiaca*. **17-22** – capitate glandular trichomes type I; **17** – small droplets in the subcuticular space and lipophilic content of the head cell stained orange-brown with Sudan IV; lipophilic, orange, droplets of various sizes are also noted in the neck cell; **18** – OsO_4 test showing black staining in the head cell and neck cell; **19** – violet-blue droplets of essential oils in the subcuticular spaces, head and neck cell can be seen after staining with Nadi reagent; **20** – deep blue droplets in the secretory head indicate the presence of tannins and pink colored droplets at the periphery of cytoplasm signify the presence of pectins after staining with toluidine blue O; **21** – the presence of pink colored droplets in head cell after PAS reaction indicates polysaccharides; **22** – pectins stained pink with Ruthenium red; **23-30** – capitate glandular trichomes type II; **23** – secretory material positively stained with Sudan IV in the subcuticular space, head cell and neck cell; **24** – lipophilic droplet on the deflated cup-shaped head cell; **25** – black-blue colored secretion of lipophilic substances after staining with Sudan black B; **26** – OsO_4 test showing black staining of secretory materials in the subcuticular space and in the head cell; **27** – neutral lipids/essential oils (red color) and acidic lipids/resins (blue color) are evident after staining with Nile blue A; **28-29** – successive stages of terpene secretion; droplets of various sizes in the subcuticular space, head cell and neck cell stained violet-blue after staining with the Nadi reagent; **30** – pectins stained pink with Ruthenium red in the subcuticular space and head cell. Bar = 10 µm (**17-30**).

like appearance (Fig. 31). The secretion stored in the subcuticular space of mature capitate trichomes type II (Figs. 23-29) and peltate trichomes (Figs. 32-39) contained a copious amount of lipophilic substances. Reaction with OsO_4 confirmed the presence of unsaturated lipids in the subcuticular space of capitate trichomes type II (Fig. 26) and peltate trichomes (Fig. 34). In the capitate trichomes type I, a strong osmiophilic reaction was observed in the glandular head (Fig. 18). The presence of essential oil was identified by Nile blue A in secreted material of capitate trichomes type II (Fig. 27) and peltate trichomes (Figs. 37 and 38). Capitate trichomes type I secretion contained only a small amount of terpenoids (Fig. 19), while capitate trichomes type II (Figs. 28 and 29) and peltate trichomes (Figs. 35 and 36) produce a large amount.

Histochemical tests carried out to detect tannins gave positive results in capitate trichomes type I (Fig. 20), and peltate trichomes (Fig. 39). The abundant polysaccharidic compounds other than cellulose were accumulated in the subcuticular space of capitate trichomes type II (Fig. 30) and in the cytoplasm of the head cell of capitate trichomes type I (Figs. 21 and 22) and type II. Polysaccharides were not detected in the secretion of peltate trichomes (Table 1).

The Sudan IV reaction showed that in all trichome types the anticlinal cell walls of stalk cells were impregnated with lipid substances. The reaction with Nile blue and Nadi reagent confirmed the presence of essential oils in the stalk cells of capitate type I (Fig. 19), capitate type II (Figs. 27-29) and peltate trichomes (Figs. 35-38).



Figs. 31-39. Histochemical characterization of the secretions of peltate trichomes of *S. aegyptiaca*. **31** – fully developed peltate trichome *in vivo*; **32-34** – peltate trichomes showing lipophilic secretion (Sudan red 7B/hematoxylin, Sudan IV and OsO_4, respectively); **35-36** – the terpenes within the subcuticular space reacted positively with Nadi reagent (violet-blue secretion); **37-38** – secreted material within the subcuticular space; essential oils stained pink-red, while resins stained blue with Nile blue A; **39** – peltate trichomes showing positive reaction, in the subcuticular space, for tannins with toluidine blue O (deep blue color). Bar = 10 μm (**31-39**).

DISCUSSION

The present study aimed to provide information on the foliar micromorphology and histochemistry of Libyan *S. aegyptiaca*. Comprehensive structural and chemical studies of trichomes could clarify the nature of the secreted material and possible functional significance.

Micromorphology and secretion

Leaves of *S. aegyptiaca* carry both peltate and capitate glandular trichomes, as well as non-glandular, which is characteristic feature for Lamiaceae species [51]. Two types of non-glandular trichomes were observed, unicellular and multicellular. Non-glandular trichomes, by covering the epidermal layer, provide protection to the plant against insect herbivores and airborne propagules of fungi, and they also increase tolerance to freezing, assist in seed dispersal, maintain the water balance in leaves, deflect intense solar radiation, etc. [12,14,52,53]. Since *S. aegyptiaca* grows in arid habitats, the function of these non-glandular trichomes appeared to be protective by covering the layer of epidermis in response to the environmental conditions. It is also believed that the non-glandular trichomes could affect the transpiration process by influencing the water diffusion boundary layer of the transpiring leaf [52]. Non-glandular trichomes of *S. aegyptiaca* were warty, and these structures are believed to arise either from the cell wall or the cuticle [14], which can thicken the walls of the trichomes and contribute to their function.

Glandular trichomes are epidermal structures specialized in particular metabolic functions, such as

Table 1. Results of the histochemical characterization of the main secretory products of *Salvia aegyptiaca* glandular trichomes

Staining procedure	Target compounds	Capitate trichomes type I		Capitate trichomes type II		Peltate trichomes	
		Secretory cell	Subcuticular space	Secretory cell	Subcuticular space	Secretory cell	Subcuticular space
Sudan IV	Total lipids	+	+	+	++	-	+++
Sudan Black B	Total lipids	+	+	+	++	-	+++
OsO_4	Unsaturated lipids	+	-	++	++	++	+++
Nile blue A	Essential oils	-	-	+	+++	-	+++
Nadi reagent	Terpenes	+	+	+++	+++	++	+++
$FeCl_3$	Phenolic compounds	-	-	-	-	-	-
Toluidine blue O	Tannins	++	-	nt	nt	-	++
$AlCl_3$ (UV)	Flavonoids	-	-	-	-	-	-
PAS	Polysaccharides	+	-	nt	nt	+	-
Ruthenium red	Pectins	+	+	+	++	+	-
UV (autofluorescence)	Phenolic compounds	-	-	-	-	-	-

+++ – very strong reaction; ++ – strong reaction; + – moderate reaction; - – negative reaction, nt – not tested the biosynthesis and secretion of particular secretory products performing different functions in plants [54]. Glandular trichomes vary in morphology, structure and also in number per unit area of the epidermis, among species and organs [20,55]. Two main types, peltate with large secretory head and capitate trichomes with stalk and small head, were found in the majority of studied leaves and flowers of different Lamiaceae species. Different variations have been observed in the studied species, in stalk length, head shape and number of cells, release of secretion products, etc.

Peltate trichomes are usually composed of a basal epidermal cell, a short wide stalk cell and a large round head of several secretory cells, which could vary in number among *Salvia* species, from four cells in *S. blepharophylla* [56], *S. divinorum* [23] and *S. verticillata* [57] to six or eight cells in a single disc in *S. aurea* [20], *S. officinalis* [51], or by 12-18 cells arranged in two concentric circles with four central and eight or more peripheral cells [13,21,25,58,59]. The present study showed that *S. aegyptiaca* has peltate glandular trichomes consisting of the basal epidermal cell, short cylindrical stalk cell with cutinized lateral walls and large round head of eight secretory cells arranged in a circle, as observed in the previously studied *S. fruticosa* from Libya [28]. During the secretion phase, peltate trichomes have a characteristic spherically shaped or slightly flattened head due to the development of a large subcuticular space where secretory products accumulate [55,60].

In our research, we noted two main types: short with one-celled cutinized stalk, and long stalked capitate trichomes composed of 3-4 cylindrical cells, with a more or less oval secretory head. Capitate glandular trichomes constitute a significant taxonomic character of the Lamiaceae and form part of the specialized floral properties for pollination [26,61]. Capitate trichomes are very common in many species of *Salvia*, but variability in stalk length and head shape has been noticed [21,23,26,58]. Capitate trichomes generally consist of 1-2 stalk cell(s) and 1-2 cell(s) forming a rounded to pear-shaped secretory head [51]. Considering the variations in capitate trichomes, Werker et al. [51] for the first time classified capitate hairs as type I, II and III according to their morphology and secretion mode. Following Werker et al. [51], type IV capitate hairs were described in *S. officinalis* by Corsi and Bottega [21]. Several researchers divided capitate trichomes into only two types, according to the dimensions of the stalk, the morphology of glandular head and the secretion process [20,55,56]. Serrato-Valenti et al. [20] in *S. aurea* described capitate trichomes type I consisting of a short stalk and bicellular head, and capitate trichomes type II consisting of 1-4 stalk cells, a narrow neck cell and globose unicellular head, and similar types were found in *S. blepharophylla* [56], *S. albicaulis* and *S. dolomitica* [24], etc.

The results obtained in this study present the types of glandular trichomes of *S. aegyptiaca*. The secretions of the glandular trichomes could have different roles, such as chemical defense of plants, guidance for insects, or to act as floral rewards to pollinators, but the specific function of each trichome type is not clear [55]. Detailed studies of the morphology, anatomy and ultrastructure will improve our understanding of their functions. The abundant non-glandular hairs are involved in mechanical defense and protect the plant from excessive transpiration and insolation [21]. Baran et al. [59] assumed that features such as abundance and diversity of glandular trichomes on plant organs, the presence or absence of neck cells, the thickness of their side walls, and stalk length, could show variation according to the xeromorphic character of the plants. The abundant and diverse glandular trichomes and long stalks of the capitate trichomes of *S. aegyptiaca* show the xeromorphic character of the species, which in its native environment grows in dry habitats and is drought-resistant.

In order to clarify the specific secretory products and possible function of each glandular trichome type of *S. aegyptiaca*, further research into histochemical characterization was performed.

Histochemistry

Data obtained from histochemical tests revealed that the secreted material in all types of *S. aegyptiaca* glandular trichomes was of a complex nature. Most of the material was lipophilic, as shown by positive reactions with Sudan dyes. The secreted product also included non-cellulosic polysaccharides in the capitate trichomes as shown using the PAS and ruthenium red tests. Polysaccharides were detected in both types of capitate trichomes, but were not detected in peltate trichomes. According to Werker [62], capitate trichomes are assumed to secrete varying amounts of polysaccharides along with essential oils. It has been suggested that the presence of a viscous secretion might act as a lubricant to facilitate leaf growth, from the origin of the bud to full laminar expansion [63]. The presence of viscous, adhesive polysaccharides, especially in the calyces of blooming flowers and dry calyces, provide good mechanical defense against aphids during flowering and seed development [21].

Comparing the results of histochemical analysis, it can be seen that lipids and terpenes were detected in all types of trichomes. Pectins were detected as a secretory product in both types of capitate trichomes, while in peltate trichomes the reaction was negative in the subcuticular space where secretion is accumulated. Peltate trichomes and capitate trichomes type II are the main structures that secrete the essential oil in *S. aegyptiaca*. Previous findings showed that peltate trichomes produce most of the essential oils in the Lamiaceae [51,62,64-66], although capitate trichomes also produce a few lipophilic compounds [55,67,21]. Comparing other Lamiaceae species, differences in the amounts of secretory products and production sites can be observed [68].

The histochemical study indicates that the secretion of *S. aegyptiaca* peltate trichomes is an oleoresin containing terpenoids (essential oils and resiniferous acids), which was reported in other plants [55,69]. The capitate trichomes contain lipids and polysaccharides. This result is consistent with the phytochemical data available for other species of the Lamiaceae [51,55,67,70]. Positive reactions confirmed that the stalk cells of both trichome types contained lipophilic compounds – essential oils. The synthesis of essential oil in stalk cells was confirmed in our investigation for all trichome types. In most glandular trichomes the stalk cells contain lipophilic substances [21,55,67], but the thick cuticle or cuticular thickenings on the lateral walls of the stalk cells, as well as presence of barrier cells with suberized lateral walls, provide structural support and probably prevent the flow of secreted products into mesophyll tissue and regulate the directional transport of metabolites to the glandular cells above [14,69,71].

The presence of terpenes in the essential oils of the glandular trichomes on *S. aegyptiaca* leaves is probably responsible for the medicinal properties, and suggests a protective function as demonstrated for other members of the Lamiaceae. As the largest class of natural products, terpenes have a variety of roles in mediating antagonistic and beneficial interactions among organisms. They defend many species of plants, animals and microorganisms against predators, pathogens and competitors, having potent antibacterial and antifungal activity, and they are toxic to insects, nematodes, mollusks, etc. [72,73].

Phenolic compounds were not detected by the applied tests, but in subsequent research of *S. aegyptiaca* extracts, phenols and flavonoids were detected using a spectrophotometric method (unpublished data).

The composition of the secreted material of the glandular hairs suggests that these trichomes are involved in chemical defense, because the essential oils are poisonous to most insects; terpenoids are olfactory deterrents for insects, and steroids, flavonoids and especially tannins are very strong insect deterrents [21,74,75].

The secreted material is mostly accumulated in the subcuticular spaces of trichomes, as described for other aromatic Lamiaceae [20,51,64,76,77]. Heterogeneous secreted material was temporarily stored in the subcuticular space in mature peltate trichomes and released by rupture of the cuticle, while in capitate trichomes it was probably released through micropores. It is likely, as proposed previously [78], that under natural conditions, factors such as high temperature and low air humidity can cause the cuticle to burst, releasing the essential oil. Other factors, such as contact with predators, can be involved in the rupture mechanism [62]. When the cuticular sac ruptures due to external pressures, these substances are released to the outside of the cuticular sac and kept on the surface layer of the plant. These substances may provide chemical protection for the plant against various types of herbivores and pathogens by entrapping, deterring and poisoning [79]. The fact that various compounds (terpenoids, phenolics, flavonoids, etc.) are localized in the glandular trichomes that are distributed over much of the plant's exterior suggests that they have a protective function.

CONCLUSIONS

Comparison with previously published results on other investigated species of the family Lamiaceae showed similarities in the morphology, distribution and histochemical contents of glandular trichomes. Our study provides additional information on the micromorphology, distribution and histochemistry of the foliar glandular trichomes of the genus *Salvia*. The secretory product of the glandular trichomes on *S. aegyptiaca* leaves should be further investigated, and complete phytochemical analyses need to be done to identify possible medicinal properties, which could contribute to the search for new natural products for application in pharmaceutical, food and cosmetics industries.

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Authors' contributions: Dušica Janošević and Snežana Budimir designed and carried out the experiments and analyzed and presented the results. Sonja Duletić-Laušević conceived the work, analyzed and interpreted data and wrote the manuscript. Ana Alimpić participated in the experiments and in analyzing and discussing of results. Petar D. Marin discussed the results and revised the manuscript critically. Najat Al Sheef and Abdulhmid Giweli collected plant material used, and participated in the experiments. All the authors read and approved the final version of the manuscript.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest.

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