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Original scientific paper

ANATOMY OF THE MULTIFLORAL SAGE *SALVIA OFFICINALIS* L. SUBSP. *MULTIFLORA* GAJIĆ, (*LAMIACEAE*)

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Stojanović Danilo, Branislava Lakušić, Violeta Slavkovska and Radiša Jančić (2000): *Anatomy of the multifloral sage Salvia officinalis l. subsp. multiflora Gajić (Lamiaceae).* - Ekologija, Vol. 35, No. 2, 97-103, Belgrade.

The multifloral sage (*Salvia officinalis* L. subsp. *multiflora* Gajić), the subspecies of the common sage, grows only at termophylus limestone in Southeast of Serbia (GAJIĆ, 1973). According to its endemic character, it has being included in the group of Yugoslav taxa of international significance (STEVANOVIĆ *et al.* 1995). The research of the structural attributes of leaves and steams of multifloral sage was performed on material collected from Sićevo gorge in S.E. Serbia. The stem and leaves are covered with the dense indumentum, comprised of different, specifically located glandular and non-glandular trichomes. The anatomic structure of the stem and leaves, along with the specific indumentum features indicate xero-mesomorphic nature of multifloral sage.

Key words: Salvia officinalis, multifloral sage, anatomy, leaf, stem, trichomes, indumentum, glandular trichomes, structural adaptations

INTRODUCTION

Salvia is the most numerous genus of Lamiaceae, subfamily Nepetoidae (CANTINO, 1992) comprising about 700 to 1050 species (WU, LI 1982). *Salvia*

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species are widespread in tropical and subtropical regions of both hemispheres, but the significant number grow in Mediterranean area. There are 36 species registered in "Flora Europaea" (HEDGE, 1972) and 14 of them grow in Serbia (DIKLIĆ, 1974).

Salvia officinalis L. is extremely rare species in Serbia. It occurs only in Sićevo gorge in Southeast of Serbia (GAJIĆ, 1973). These isolated populations of an autohtonous plant, distant from the main range of distribution, seems to be relict (KOJIC, 1973).

GAJIĆ (1973) emphasized the morphological differences of sage from Sićevo gorge, and described it as S. *officinalis* L. subsp. *multiflora* GAJIĆ - the multifloral sage, according to the number of flowers in the verticillasterts (8 - 22) and the length of pedicels (6 - 11mm).

Referring to the current data, this taxon incorporates the most continental populations of *Salvia officinalis* and it has being included in the group of Yugoslav taxa of international significance, according to its endemic character (STEVANOVIĆ *et al.* 1995).

The major goal of this study was to identify eco-anatomical adaptations of leaves, stem and particularly indumentum, responsible, in one hand, for medicinal properties of this famous medicinal plant, and specific phytogeographical distribution on another.

MATERIALS AND METHODS

The investigation of the structural attributes of leaves and steams of *S. officinalis* subsp. *multiflora* was performed on exsiccates and fixated material from the collection of Department of Botany at Faculty of Pharmacy in Belgrade.

Plant material was collected in the flowering phase.

In Sićevo gorge, multifloral sage grows at termophylus limestone, under the intermediate submediterrenian aegean - subcontinental climate (type IV, according to STEVANOVIĆ, STEVANOVIĆ 1995). It forms numerous well-developed shrubs, in *Artemisio - Salvietum officinalis* (Grebenščikov) Nikolić, Diklić plant community.

The plant material was fixated on field in 60% ethanole. The cross sections were obtained by sliding microtome and stained with safranine and alcian - blue. Light microscopy slides were investigated for leaf blade thickness, upper and lower epidermal height, thickness of palisade and spongy parenchyma and the number of their strata. Epidermal structures were investigated as well: the trichome morphology and the occurence and the location of stomata. Indumentum features were observed also by JOEL - 35 SEM, at the Biology faculty in Novi Sad.

RESULTS

The leaf blade is dorsiventral. Lamina is wrinkled, with convex and groove area (at large veins), alternating, at the section. Larger veins form the edge of the recess at the lower side. The thickness of the lamina varies from 100 to 200 μm,

but the wrinkled lamina and dense indumentum multiplies the overall thickness (3 - 4 times). Lower side recesses are up to 500 m m deep. (Fig. 1)

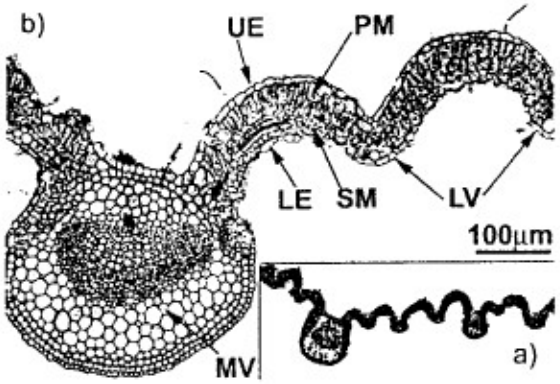

Fig. 1 - Cross section of the leaf blade. a) Wrincled lamina b) Major vein and lamina with convex and groove area; UE...Upper epidermis, PM...Palisade mesophyll, SM...Spongy mesophyll, LE...Lower epidermis, MV...Major vein, LV....Larg veins.

Dense indumentum is built of intertwined nonglandular and glandular trichomes, greyish-green at the upper side and white, denser at the lower . (Fig. 2)

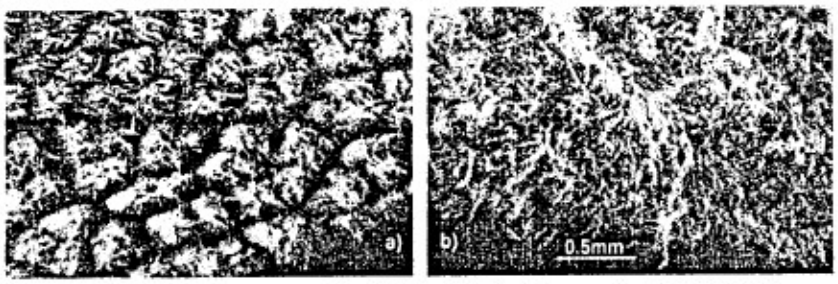

Fig. 2 - Indumentum; a) upper side of the leaf blade, b) lower side of the leaf blade

Nonglandular trichomes are:

A. Unicellular, short, with expanded base and slightly bent, sharp apex, and warty surface (Fig. 3a)

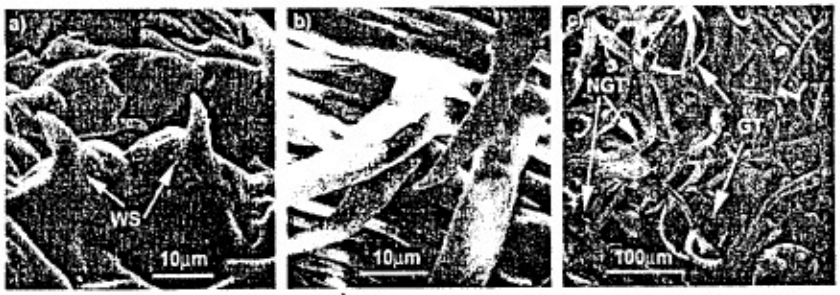

Fig. 3 - Trichomes (SEM); a)Unicelular nonglandular trichome, WS...Warty surface, b)Multicelular uniseriate nonglandular trichome, c) Intertwined trichomes, NGT...Non glandular trichomes GT...Glandular trichomes

B. Multicellular, uniseriate, unbranched, of two or more cells, with apparent joint between first and second. (Fig. 3b). It is not possible to determinate the number of the cells of long intertwined trichomes. (Fig. 3c).

The apex is straight or bent at all types of trichomes, and the surface is warty. Glandular trichomes are:

A. Short capitate, with unicellular stalk and secretory head of one or two cells (Fig. 4a, 4b, 4c)

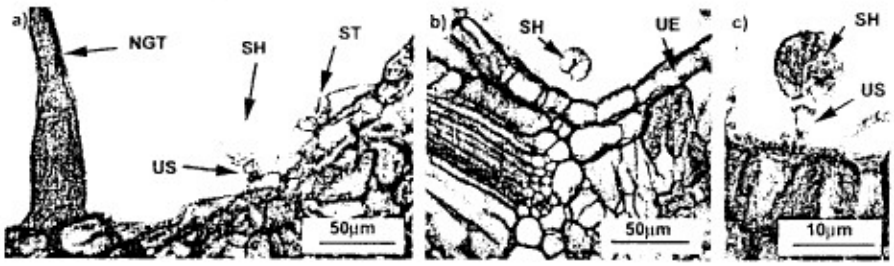

Fig. 4 - Short Capitate Trichomes; a) Short capitate trichome with unicelular secretory head US...Unicelular stalk, SH...Secretory head, ST...stomata, b), c) Short capitate trichome with bicelular secretory head

B. Capitate, with multicellular stalk (2 - 3 cells) and unicellular head. (Fig 5a and 5b).

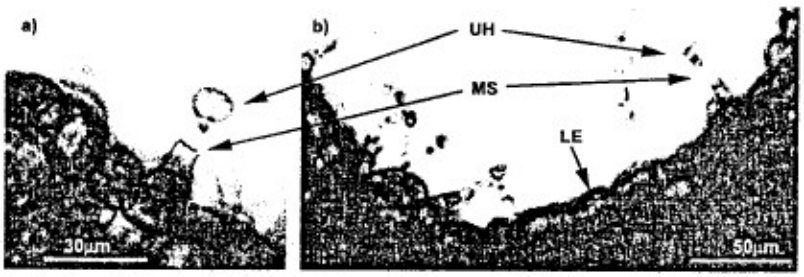

Fig. 5 - Capitate Trichomes With Multicelular Stalk: a) Trichome with untouched unicelular secretory head, b) Trichomes after secretory head rupture, UH...unicelular head

C. Peltate, with unicellular stalk and 8 - 12 cells in secretory head, with large subcuticular region. (Fig 6, 7c).

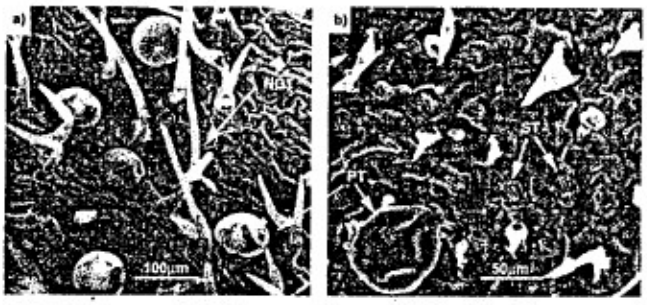

Fig. 6 - Peltate Trichomes; a) Trichomes with untouched multicelular secretory head, b) Trichomes after secretory head cuticle rupture, PT...Peltate trichome,

Heads of pelatate trichomes are above the epidermal layer at S. *officinalis* subsp. *multiflora,* while most of aromatic taxa of *Lamiaceae* have. pelatate trichomes inserted. and their heads aligned with epidermis. (JANCIC, 1984, TODOROVIĆ, 1994, LAKUŠIĆ, 1996).

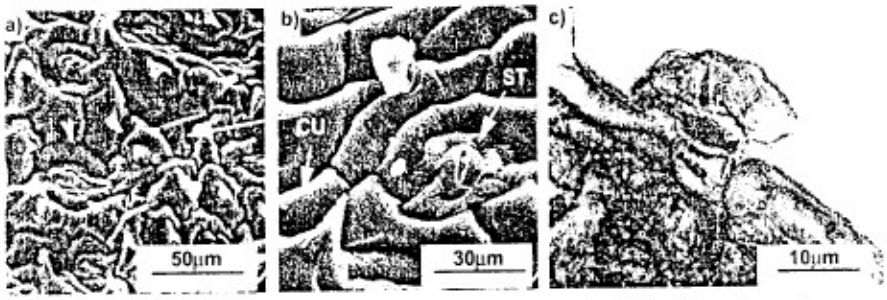

Fig. 7 - Cuticle with paralel striae. a) Striae of the cuticle. b) Parallel cuticle striae and stoma with uplifted guarding cells. c) Peltate trichome without the cuticle.. CU...Cuticle

The size of upper and lower epidermal cells is nearly the same. Epidermal cell walls are thick, particularly the outer one. The walls are the thickest at the middle vein area. The cuticle is thin, with parallel striae (Fig.7a, 7b). In middle vein area the cuticle is thicker, with the waxy particles. Diacytic stomata are present at both side, but more numerous at lower, covered with trichomes in cavities, slightly risen from the epidermis (Fig. 7b, 8a, 8b).

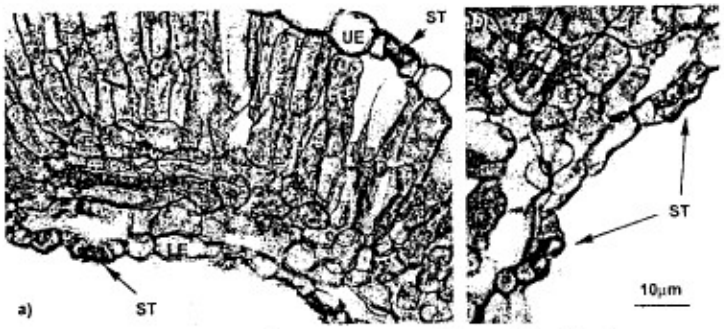

Fig. 8 - Stomata, a) Stomata at upper and lower side of lamina.
b) Uplifted guarding cells at the lower side

Palisade mesophyll is compact, in 2 - 3 strata, and the lower is intermediate with spongy mesophyll, also in 2 - 3 strata, made of isodiametric cells, with small intercellular spaces. Palisade is dominant to spongy , 1.8 : 1.

In the middle vein area, strongly convex at lower side, there are 2 - 3 strata of collenhyma, under the epidermis. Cell walls of dense parenchyma are thickened. Vascular parenchyma surrounds the vascular bundles at all larger veins, and reaches epidermis at both sides. There are 1 - 2 tiny bundles in mesophyll too. (Fig. 1,8).

Thickness of the lamina tissues of S. officinalis subsp. multiflora (μm)

	min	*max*	*med*
upper epidermis	10,67	25,61	16.92
palisade mesophyll	57,47	107,74	73,03
spongy mesophyll	22,78	52,80	36,65
lower epidermis	8,00	19,23	12,37
total thickness	101,82	198,09	138,96

The stem is quadrangular (Fig. 9a), with flat sides, covered with glandular and nonglandular trichomes of same type as at leaf. Epidermal surface is covered with thick cuticle, with fine micropapilas. Epidermal cells have thickened walls particularly outer. Stomata are aligned with epidermis. Collenchyma is developed in the corner of the stem. There are 3 - 4 strata of chlorenchyma, under the epidermis at the sides. Big tangentially elongated endoderm cells separate chlorenchyma from sclerenchyma, which follows the vascular bundles. Bundles and sclerenchyma are large, facing corners while smaller at the side. There are 2 - 4 small bundles, alternating with medullar rays and wood fibers in rows, at the flat side of stem.

The central, medullar area is filled with large, thin walled parenchyma cells.

(Fig. 9b).

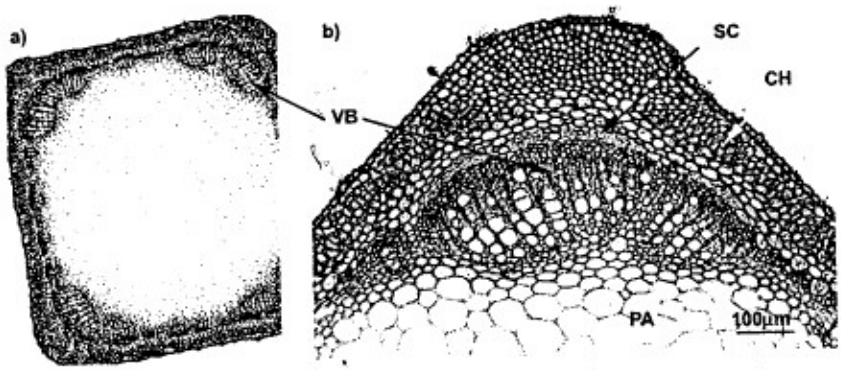

Fig. 9 - Cross section of the Stem: a) Quadrangular stem, b) Detail of the section. CH...Chlorenchyma, SC...Sclerenchyma, PA...Parenchyma, VB...vascular boundles

DISCUSSION

S. officinalis subsp. *multiflora* shows xero - mesomorphic attributes with distinctive heliophytic characteristics of leaves and stem.

The thickness of the leaf, and the dense indumentom at both sides, indicate xerophytic nature of this plant. Specific wrinkled lamina, together with indumentum, increases the overall thickness of the leaf. Unicellular, nonglandular trichomes, and all types of glandular trichomes, are located close to the epidermal surface. Glandular heads of peltate trichomes are large and they cover a significant area. Heads of the capitate trichomes are uplifted, and the trichomes of type C are located mostly in the recesses at the lower side. CHAKALOVA *et al*, (1993.) emphasize that the peltate glandular hairs of *S. officinalis* L. are almost evenly distributed on both surfaces of the leaves, but we find peltate trichomes twice denser at lower surface at *S. officinalis* L. subsp. *multiflora*. Long nonglandular, multicellular trichomes cover the cavities and overlay all the other trichomes.

Well developed indumentum acts as the protection against over-heating and over-transpiration. Volatile fractions of essential oils from capitate glandular trichomes stay close to the surface due to the numerous long, nonglandular trichomes, forming the upper indumentum layer. Capitate trichomes efflux starts at juvenile phase and lasts during the vegetation, (WERKER, 1985), while the peltate trichomes accumulate essential oil in the subcuticle, and releases it after the cuticle rupture. Terpene compounds, with their specific odor and bitter taste are strong ecomones and potential protection against herbivorous animals. (LARCHER, 1995)

Dense mesophyll cells, particularly palisade, act as an adaptation for high radiation and dry habitat conditions. Stomata are mostly covered and protected with glandular and nonglandular trichomes.

Mesomorphic features of this plant are large epidermal cells in one layer and slightly uplifted guarding cells of the stomata..

Xeromorphosys of the stem are represented with the dense indumentum, consist of glandular and nonglandular trichomes of same type as one at the leaf. Epidermal cells are small and the cuticle is well developed.

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ANATOMIJA VIŠECVETNE ŽALFIJE *SALVIA OFFICINALIS* L. SUBSP. *MULTIFLORA* GAJIĆ *(LAMIACEAE)*

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Izvod

Salvia oficinalis L. subsp. *multiflora* Gajić, je endemit jugoistočne Srbije i usled toga uvrštena u listu jugoslovenskih taksona od međunarodnog značaja. Istraživanje strukturnih odlika listova i stabla višecvetne žalfije urađeno je na materijalu prikupljenom sa lokaliteta Sićevačka klisura, odakle je takson i opisan. Stablo i listovi su pokriveni gustim indumentumom, sačinjenim od različitih, specifično raspoređenih mehaničkih i žlezdanih trihoma. Anatomska građa stabla i listova, zajedno sa karakterističnim osobinama indumentuma ukazuju na kseromezomorfnu prirodu višecvetne žalfije.

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