Morphological and anatomical analysis of the clary sage herbal drug (*Salviae sclareae herba*)

Milica Ranđelović[1,*], Bojan Zlatković[2], Maja Jovanović[2], Bojana Miladinović[1], Milica Milutinović[1], Dragana Pavlović[1], Suzana Branković[3], and Dušanka Kitić[1]

[1] *University of Niš, Faculty of Medicine, Department of Pharmacy, Blvd. Zorana Djindjića 81, 18000 Niš, Serbia*
[2] *University of Niš, Faculty of Sciences and Mathematics, Department of Biology and Ecology, Višegradska 33, 18000 Niš, Serbia*
[3] *University of Niš, Faculty of Medicine, Department of Physiology, Blvd. Zorana Djindjića 81, 18000 Niš, Serbia*
[*] *Corresponding author: milica.randjelovic@medfak.ni.ac.rs*

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This paper presents an analysis of the morphological and anatomical characteristics of the above-ground parts (herba) of clary sage (*Salvia sclarea* L.), with an emphasis on the investigation of its surface structures. After the procedure of the primary processing and drying of the plant material, it was determined that the composition of the crushed herbal drug *Salviae sclareae herba* showed the presence of parts of leaves, bracts, flowers and axis of the inflorescence, stems, and a small amount of ripe nuts. The analysis of the surface structures of the drug parts on a stereomicroscope and then on permanent microscopic slides indicated the presence of two functional types of trichomes (glandular and non-glandular). Non-glandular mechanical trichomes are found in the unicellular and multicellular forms while glandular trichomes are differentiated as capitate, with a greater number of subtypes, and peltate. Peltate trichomes are most common on the calyx and corolla while capitate are most abundant on the surface of the stem and leaf. Further studies should be directed toward the chemical characterization of the essential oil of the herb and individual organs present in the glandular trichomes, along with the investigation of its pharmacological effects.

Key words: *Salvia sclarea*; herb; morphology; anatomy; glandular trichomes; mechanical trichomes

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1. INTRODUCTION

The genus *Salvia* L., within the family Lamiaceae, includes almost a thousand plant species, spread all over the world with a noticeable range of variation. They are especially represented in the tropical and subtropical areas of both hemispheres, and many of them inhabit the Mediterranean area (Diklić, 1974; Kintzios, 2000; Walker et al., 2004). Fourteen species of the genus *Salvia* are represented in the flora of Serbia (Diklić, 1974). Since 2005, the fifteenth, *S. verbenaca*, has been included as a new taxon (Zlatković et al., 2005). These plant species are annual or perennial, herbaceous, semi-herbaceous, shrubby, and aromatic due to the presence of essential oil. *Salvia sclarea* L. (clary sage) is a perennial, rarely biennial, balsam-scented plant. It grows on dry, warm, often stony slopes, in vineyards, and along roads. It is widespread in the Mediterranean area, from Transcaucasia, Iran, and Syria to southern France and northern Africa. In Europe, it occurs in the Mediterranean and sub-Mediterranean areas, and it is cultivated as an ornamental plant species, as well (Diklić, 1974; Sarić, 1989).

Various pharmacological properties are attributed to *Salvia* species: antioxidant, antimicrobial, insecticide, cytotoxic, neuroprotective, hypoglycemic, gastroprotective, etc. (Alimpić, 2016; Aradski-Alimpić et al., 2021). The best-known and most studied species within this genus is *S. officinalis*. It has been used in the treatment of various diseases mostly related to gastrointestinal complaints, but also for excessive sweating, cognitive disorders, inflammations of the skin, oral cavity and throat, etc. (Aćimović et al., 2019). The medicinal properties of *S. sclarea* have been also known to many peoples since ancient times. The old Jamaicans found *S. sclarea* to be exceptional for treating skin ulcers and eye inflammation. Today, it is used most often in its dry or fresh form as a stomachic for digestive problems thanks to its spasmolytic effect and distinctly aromatic properties. Also, it is known in the treatment of some kidney diseases, as an antiperspirant, antidepressive, hypoglycemic, hemostatic, and anticatarrhal agent, and in the treatment of gynecological disorders, gingivitis, stomatitis, aphthae, cough, and colds (Díaz Dellavalle et al., 2011; Dweck, 2000; Guarrera, 2005; Koutsaviti et al., 2016; Leporatti et al., 1985; Peana and Moretti, 2002). According to the instructions of Hager's manual (Hansel et al., 1994), *Salviae sclareae herba*

is used per os for digestive and menstrual problems, general weakness, catarrh, headache, spasms and kidney problems, and topically for inflammatory wounds (Hansel et al., 1994). The essential oil of this plant species, *Salviae sclareae aetheroelum*, finds an important place in aromatherapy. It is most often obtained by hydrodistillation from the dried herbaceous aerial parts of the flower (Jančić et al., 1995). According to the definition from the monograph of the 10th European Pharmacopoeia (Ph.Eur. 10., 2020), this oil is obtained by the process of hydrodistillation of fresh or dried stems in bloom characterized by a colorless, brownish-yellow, or light-yellow liquid with a characteristic odor (Ph.Eur. 10., 2020). This oil reduces the feeling of fear and acts as an anxiolytic to relieve paranoia and delusion and it can also be very helpful for depression, convulsions, and insomnia problems (Dweck, 2000; Imanshahidi and Hosseinzadeh, 2006).

Fig. 1. The elements of the drug *Salviae sclareae herba*; (A) crushed above-ground parts;(B) 1 - calyx, 2 - the fragment of the leaf, 3 - the fragment of the stem, 4 - corolla, 5 - nutlet, 6 - bract

In the aromatic plant species of the Lamiaceae family, essential oil is present on the surface of the organs within the glands (peltate and capitate trichomes). Peltate trichomes consist of a basal cell in the epidermis, short stem cells, and broad heads made of a certain number of secretory cells arranged in one layer, in one or more circles (Alimpić, 2016). Capitate trichomes consist of one basal cell, one to several stalk cells, and a secretory head consisting of one to two cells. In addition, the Lamiaceae species are abundant in mechanical, non-glandular trichomes, which vary in shape, length, number of cells, number of rows of cells, and surface (Alimpić, 2016). Trichomes have great taxonomic and phylogenetic significance, so the type and distribution of trichomes, especially glandular, can be used to differentiate related taxa (Krstic et al., 2006; Stojičić et al., 2016). Knowledge of the characteristics and distribution of trichomes is especially important when identifying the fragmented parts of herbal drugs (Chakalova et al., 1993), to which special attention is paid in this study.

Therefore, this paper aims to evaluate the morphological and anatomical characteristics of the herbal drug *Salviae sclareae herba* (leaf, stem, flower and inflorescence axis), with the emphasis on the analysis of epidermal structures.

2. MATERIALS AND METHODS

2.1. Plant material

The above-ground parts of *S. sclarea*, stems with leaves and flowers (herba), were collected in Malča, near Niš (GPS coordinates: N 43°33′5.27″, E 22°3′6.26″ at about 334 m a.s.l.), in July in full bloom. Dry, grassy and to some extent ruderal habitats in the immediate vicinity of the settlement inhabit the mentioned locality of the species. The plant material was identified by professor Dr. Bojan Zlatković, from the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, while the herbarium specimen was deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" of the Faculty of Biology, University of Belgrade (Voucher No. 17077). The material was dried in a dark, cool, and well-ventilated place, and then stored in paper bags until analysis.

2.2. Morphological and anatomical analysis

Morphological and anatomical characteristics of the herbal drug *Salviae sclareae herba* were studied in the Laboratory for Plant Systematics and Ecology of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš.

A Leica MZ16A stereomicroscope with a Leica DFC320 digital camera was used for a detailed morphological analysis of the drug parts (leaf, stem, axis of the inflorescence, bract, flower, nut) and observation of their surface structures (Leica Microsystem©, Wetzlar, Germany). The anatomical characteristics of the individual organs that make up the drug were monitored on permanent histological slides, with an emphasis on the analysis of the characteristics of the plant's indumentum. Cross-sections of the leaf and stem, cut by hand, were analyzed as well as the parts of the flower (corolla and calyx) and bract which were observed in toto. The preparations were decolorized with chloral hydrate, fixed with gelatin solution, and then observed and imaged with a Leica DFC290 digital camera on a Leica DM 1000 light microscope (Leica Microsystem©, Wetzlar, Germany).

3. RESULTS

3.1. Morphological characteristics of the drug

After the procedure of the primary processing and drying of the plant material, the crushed herbal drug *Salviae sclareae herba* showed the presence of parts of leaves, bracts, flowers, parts of stems and inflorescence axes, as well as a small amount of ripe fruits – nutlets. The calyx is very easily separated from the corolla after drying, so they are mostly found separately in the plant material. The surface structures of the drug parts were investigated in more detail by observing the crushed material using a stereomicroscope. The above-ground parts of *S. sclarea* are densely overgrown with trichomes. Namely, glandular and non-glandular trichomes are observed on all investigated dried parts of the drug; however, their distribution among plant organs is not equal, from the bract which is almost bare to the stems and leaves that are densely overgrown with trichomes. Figure 1. shows the dried and crushed above-ground parts of *S. sclarea*. Figure 2. shows the parts of the drug observed through the stereomicroscope: bracts, flower (calyx and corolla) and inflorescence axis, parts of leaves, stems, and fruit. The dried bract is fragile in structure, pale pink in color, heart-shaped with a pointed tip with the entire rim (Figure 2B). It is almost glabrous on its surface with a significantly lower presence of trichomes compared to other parts of the drug. Stereomicroscopy detected non-glandular and globular peltate trichomes of yellow-orange color, while capitate ones were more difficult to detect.

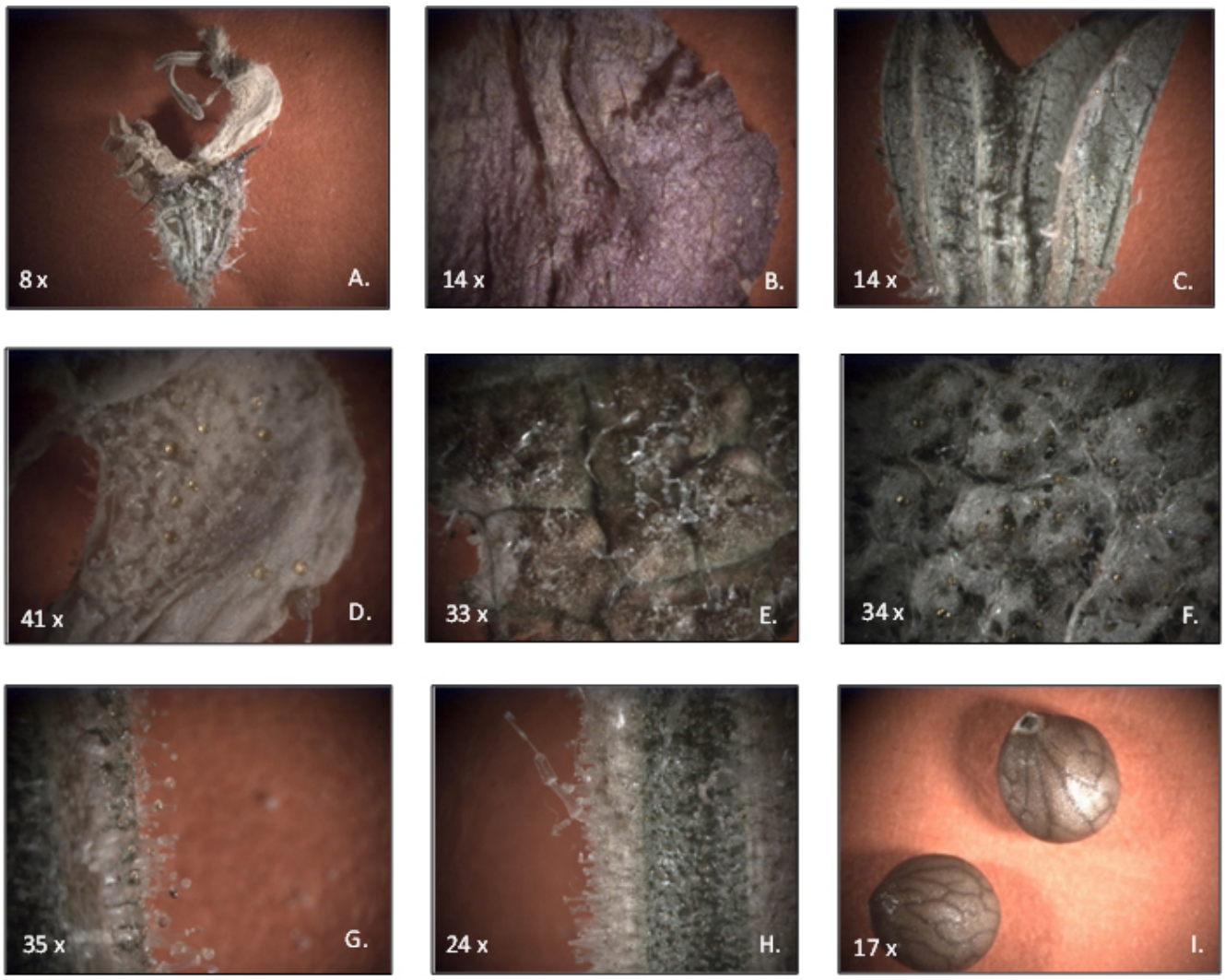

Fig. 2. The fragments of the *Salvia sclarea* L. flower, bract, leaf, inflorescence axis, stem, and nutlets from the dried and crushed plant material; A – flower; B – bract; C – calyx; D – corolla; E – adaxial surface of the leaf; F – abaxial surface of the leaf; G – inflorescence axis; H – stem; I – nutlets; magnification is shown in each image separately.

The two-lipped calyx is greenish-gray in color with prominent ribs and teeth. Sharp mechanical and glandular, peltate and capitate trichomes are visible on the surface (Figure 2A and C). The corolla is collapsed with the retained shape and structure of a fresh two-lipped corolla, pale pink and with long anthers. Numerous mechanical and peltate trichomes with yellow-orange content can be observed on the surface of the corolla using stereomicroscopy, while capitate ones were harder to detect (Figure 2A and D).

The dried leaf is greenish-gray, lighter on the abaxial than the adaxial surface, shallowly serrated along the rim (Figure 2E and F). Both sides of the leaf are densely overgrown with long, multicellular mechanical hairs that intertwine and give the leaf a grayish note. The nerve is densely reticulate, more prominent on the abaxial side. On the adaxial surface of the dried leaf, the nerves have the appearance of shallow incisions, while on the abaxial they are very convex, so "folds" are formed between the nerves. On both surfaces of the leaf, and especially the abaxial one, numerous ball-shaped peltate glands with yellow-orange content are visible, as well as capitate ones with transparent content, and very long, often collapsed, mechanical trichomes.

The inflorescence axis and the stem are quadrangular in cross-section, green-gray in color, and very densely overgrown with long and short mechanical trichomes. Unlike other parts of the drug, long capitate trichomes with transparent content, as well as less present peltate trichomes are very common and noticeable (Figure 2G and H).

Although the plant material was collected during the period of the full flowering, nutlets are present in a smaller percentage in the observed drug sample, which indicates that some individuals from the collected plant material have already formed fruits. The nuts are small, ball-shaped, brown in color, shiny, and bare. Characteristic ornamentation in the form of mesh folds can be seen on the surface (Figure 2I).

3.2. Anatomical characteristics of the drug - analysis of the surface epidermal structures

Light microscopy and the analysis of micrographs of permanent slides determined the presence of non-glandular, mechanical trichomes that are unbranched and uniseriate with one or more cells, as well as glandular ones, differentiated into peltate and capitate types in which the essential oil is located. The smallest distribution of all types of trichomes was observed on the surface of the bracts (Figure 3). Non-glandular, mechanical trichomes are especially present on the nerves and the rim of the bracts (Figure 3A, B, D-H). Short one- to three-cell trichomes with a pointed terminal cell can be seen along the nerves (Figure 3H), while longer ones, with over five cells (Figure 3. D, E, F) are observed on the periphery of the bract. The presence of a distinctly elongated pointed terminal cell was also noticed in some trichomes (Figure 3D).

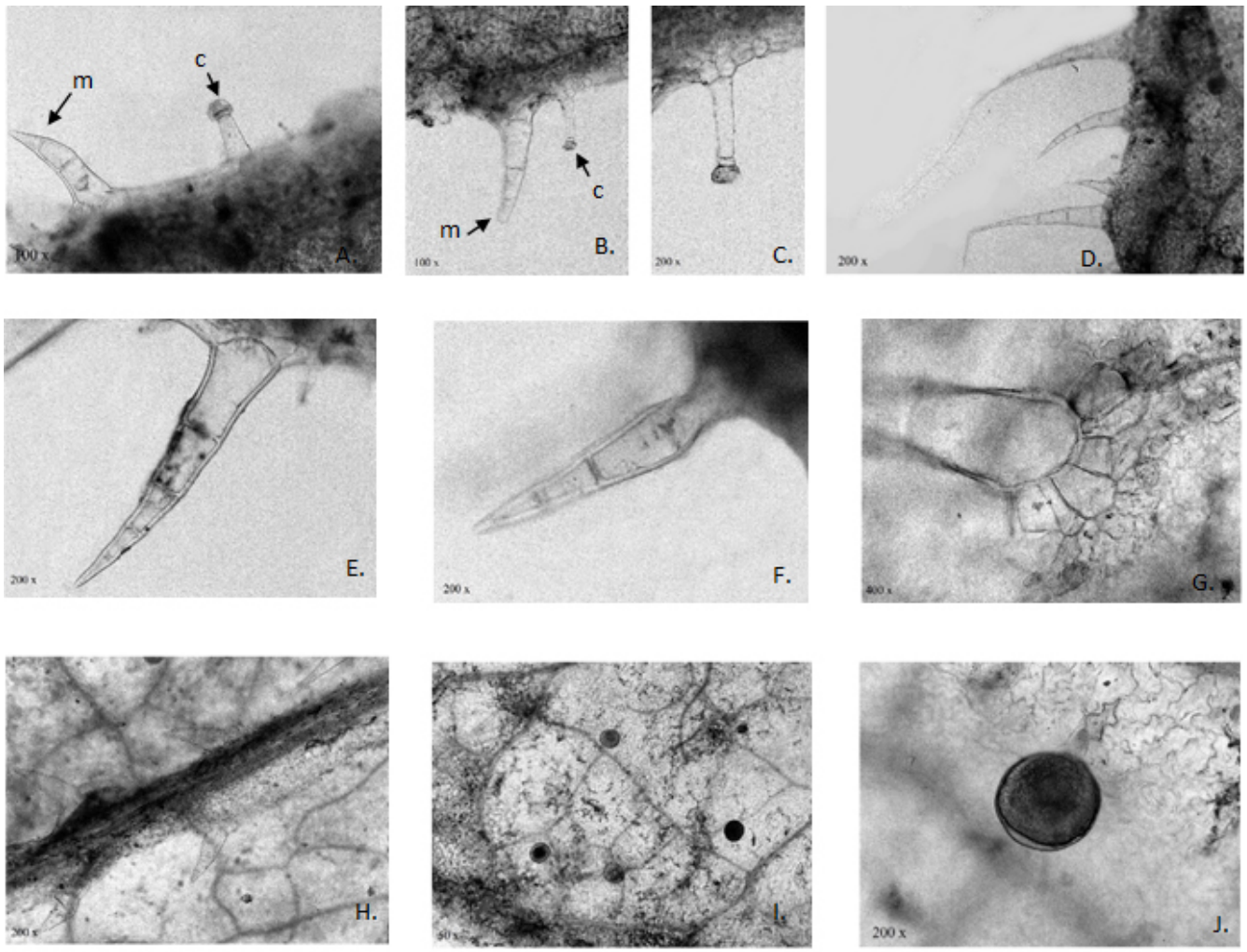

Fig. 3. Trichomes on the surface of a *Salvia sclarea* L. bract; A, B (100×) – mechanical (m) and capitate (c), C (200×) – capitate, D, E, F (200×) – mechanical, G (400×) – mechanical with multicellular base, H (200×) – mechanical distributed on a nerve, I (50×) – peltate and J (200×) – peltate.

The multicellular, mechanical trichomes with a multicellular base were also registered on the surface of the bract (Figure 3G). Peltate glandular trichomes are characterized by dark content and a spherical appearance consisting of a multicellular head with a cutinized wall and a basal cell implanted in the epidermis (Figure 3I and J). The second type of secretory glands are capitate glandular trichomes which are characterized by the presence of a single-celled head (capitulum) and a single-celled stalk (Figure 3A and B). The presence of all types of trichomes on the surface of the flower, calyx, and corolla is significantly higher compared to the bracts (Figure 4 and 5). Non-glandular trichomes are short and consist of up to four cells, with a terminal tapered cell (Figure 4E-G and Figure 5B). Peltate trichomes are distributed over the entire surface of the flower parts (Figure 4A-C, H and Figure 5D, E, F). The surface and nerves of the calyx exhibited the presence of shorter capitate trichomes, often with a flattened and wrinkled unicellular head, although individual trichomes with a longer stalk could be observed (Figure 4A-D and Figure 5A, C). Pollen grains were also registered on permanent corolla slides (Figure 5H).

The leaf surface is rich in capitate trichomes, while peltate and non-glandular ones are less represented (Figure 6). Two types of capitate trichomes were noticed in the slides. One type of glandular capitate trichomes is characterized by a very short unicellular stalk and a larger unicellular head (Figure 6G-K), and the other by an elongated unicellular stalk and a small unicellular head, often flattened (Figure 6H, I, and M). Peltate trichomes were not noticed in significant numbers (Figure 6L). From the group of mechanical trichomes, multicellular ones are found on the leaf surface, where some of them are conspicuously collapsed with multiple bends with a pointed terminal cell (Figure 6A-D, and G), as well as single-celled wedge trichomes (Figure 6E, F, H, and I).

Trichomes are also observed on the epidermis of the cross-section of the stem (Figure 7), built in the form of a continuous layer of oval cells. The indumentum of the stem consists in the largest percentage of glandular capitate trichomes, followed by somewhat less represented peltatate and non-glandular, mechanical - multicellular, and wedge-shaped trichomes. Capitate trichomes occur in two forms, with a short unicellular stalk and a larger single-celled head (Figure 7K) and, more predominantly, ones with a longer unicellular stalk and a smaller single-celled head (Figure 7A-E). Peltate trichomes, which occur much rarer than capitate ones (Figure 7J), were noticed on the stem's epidermis. Mechanical trichomes are long, multicellular, often collapsed and relatively rare (Figure 7 F, G), while wedge-shaped, unicellular ones, are more common (Figure 7C, H, I). Types, subtypes, as well as the distribution of trichomes on the examined above-ground organs of *S. sclarea* are presented in Table 1.

4. DISCUSSION

A stereomicroscope was used to monitor the surface structures of the drug *Salviae sclareae herba*, which were subsequently,

Table 1. Types, subtypes and distribution of the trichomes on the above-ground organs of *Salvia sclarea* L

Type	Subtype	Distribution
Non-glandular	wedge-shaped, unicellular	bract, calyx, leaf, stem
	multicellular	bract, calyx, corolla, leaf, stem, inflorescence axis
	multicellular with a distinctly elongated, pointed terminal cell	bract
Glandular	capitate with a long stalk	bract, calyx, corolla
	capitate with a short stalk	bract, calyx, corolla
	capitate with a long stalk and small head	leaf, stem, inflorescence axis
	capitate with short stalk and large head	leaf, stem
	peltate	bract, calyx, corolla, leaf, stem, inflorescence axis

with much more detail, defined by observing permanent microscopic slides. The presence of both functional types of trichomes (glandular and non-glandular), as well as a number of subtypes of trichomes, indicates a high diversity of epidermal structures that participate in the construction of the characteristic indumentum (hairy sheath) of *S. sclarea* (Table 1). The diversity, as well as the functional and morphological specificity of the mentioned structures in this plant, open a great possibility of their application in the taxonomy and classification of herbal drugs obtained from above-ground parts of the genus *Salvia*, Lamiaceae plants, and other plants. Trichomes are specialized epidermal cells that not only provide protection to the plant organism, complementing the mechanical and biochemical barrier to pathogenic microorganisms, and herbivores, but also play a role in reducing the intensity of transpiration and drying of plants in warmer habitats (Jančić, 2002). The peculiarity of the anatomical structure of trichomes in plants can sometimes be very diverse and complex, which is certainly the case with many species from the Lamiaceae family. When examining and describing trichomes, the following characteristics should be followed, stated in relation to the potential importance they have for the plant (glandular or non-glandular, unicellular or multicellular, uniseriate and multiseriate, the presence of surface ultrastructures (e.g. warts, papillae, or stripes), voluminosity - collapse or swelling of cells, number and type of cells that build trichomes (base, body, and apical part). Additional features in their characterization that can be observed are differences in wall thickness, presence of lignification, crystals or cystolites, cuticle thickness, calcium carbonate impregnation, and filling of segments with cellular content (Metcalfe and Chalk, 1980). The presence of glandular and mechanical trichomes is a feature of almost all plant species of the Lamiaceae family (Stojičić et al., 2016). Vegetative and reproductive parts of all *Salvia* species are characterized by a richly developed indumentum which is a mixture of non-glandular and glandular trichomes. Eiji and Salmaki (2016) studied trichomes of the Iranian *Salvia* species (calices and leaves) and categorized non-glandular ones as simple trichomes (short, long, and extremely long), and branched trichomes. Glandular trichomes were divided into capitate and peltate. Capitate glandular trichomes were described, in the same study, as sessile or subsessile, with short or long stalks. The common types of trichomes present in most species of the genus *Salvia* are non-glandular, short, simple trichomes followed by short capitate. Among short, simple trichomes, bicellular and multicellular forms are more common than unicellular ones. Branched non-glandular trichomes are found only in *S. multicaulis*, while extremely long ones are observed in *S. sclareopsis*, *S. mirzayanii*, *S. syriaca*, *S. leriifolia*, *S. xanthocheila*, *S. verbascifolia*, *S. ceratophylla*, *S. atropatana*, and *S. aethiopis*. The stalk of capitate trichomes can be single-, double- and multicellular, and the head can be single- or multicellular. Peltate glandular trichomes in species of the genus *Salvia* usually consist of a short single-celled stalk and a multicellular head, built of four to eight cells.

Unlike capitate, these glands were not observed in all species of the mentioned genus (e.g. *S. bracteata*, *S. macrochlamys*, *S. sclarea*, *S. aethiopis*, *S. oligophylla*). Eiji and Salmaki (2016) noted the presence of short and long non-glandular trichomes on the surfaces of the calyx and leaf of *S. sclarea* from Iran. These researchers have noted the presence of sessile, subsessile, and long glandular capitate trichomes. Unlike the wide distribution of peltate glands on all analyzed above-ground organs of *S. sclarea* from the area of Serbia, it is interesting that peltate trichomes are not present in the material from Iran. The results of the research of the mentioned authors indicate that the micromorphology of trichomes provides very reliable data for the morphological differentiation of species; however, due to its high variability, this characteristic cannot be used to elucidate phylogenetic relationships within the genus *Salvia* (Eiji and Salmaki, 2016).

Özdemir and Şenel (1999) analyzed *S. sclarea* from the area of Turkey and concluded that peltate glands are found only on the surface of leaves and flowers, but not on the stem. However, in our plant material, peltate trichomes were observed on all above-ground organs, including the stem. The mentioned authors state that three types of capitate trichomes can be found: with a short stalk and a large round head, with a shorter or longer stalk but with a head in which the cuticle fell off, as well as a capitate with a goblet-shaped head (Özdemir and Şenel, 1999). The analysis of glandular structures of *S. sclarea* from Austria confirmed the presence of peltate and several species of capitate trichomes. Namely, Schmiderer et al. (2008) found that the leaves, unlike the material analyzed in this study, are characterized by the presence of almost exclusively peltate trichomes, especially on the abaxial surface. Capitate trichomes type I (very small glands with a narrow head) were rarely present and appeared only on young leaves. On the abaxial surface of the calyx, the capitate trichomes were densely distributed on the nerves, with a smaller number of peltate ones between them. Its adaxial surface was characterized by small capitate glands of type I and capitate ones with a short stalk. The abaxial surface of the corolla had both types of glands while the adaxial side was without trichomes. Peltate glands were accumulated on the lateral edges of the lower lip, while capitate ones are mostly distributed on the edges and top of the upper lip. Capitate oil glands were always transparent and colorless, while peltate glands were

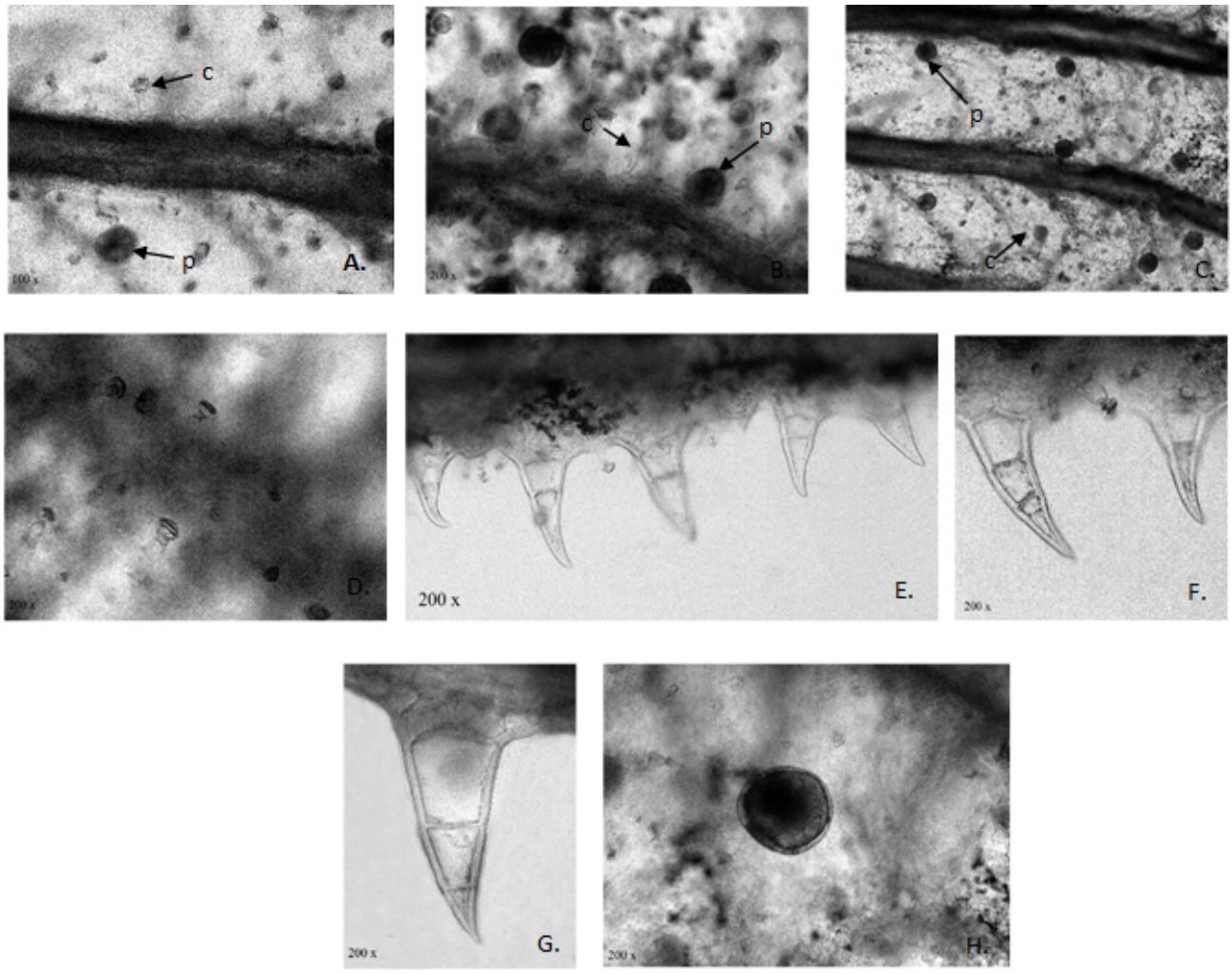

Fig. 4. Trichomes on the surface of a *Salvia sclarea* L. calyx; A (100×), B (200×), C (50×) – peltate (p) and capitate around nerves (c), D (200×) – capitate, E, F, G (200×) – mechanical, H (200×) – peltate.

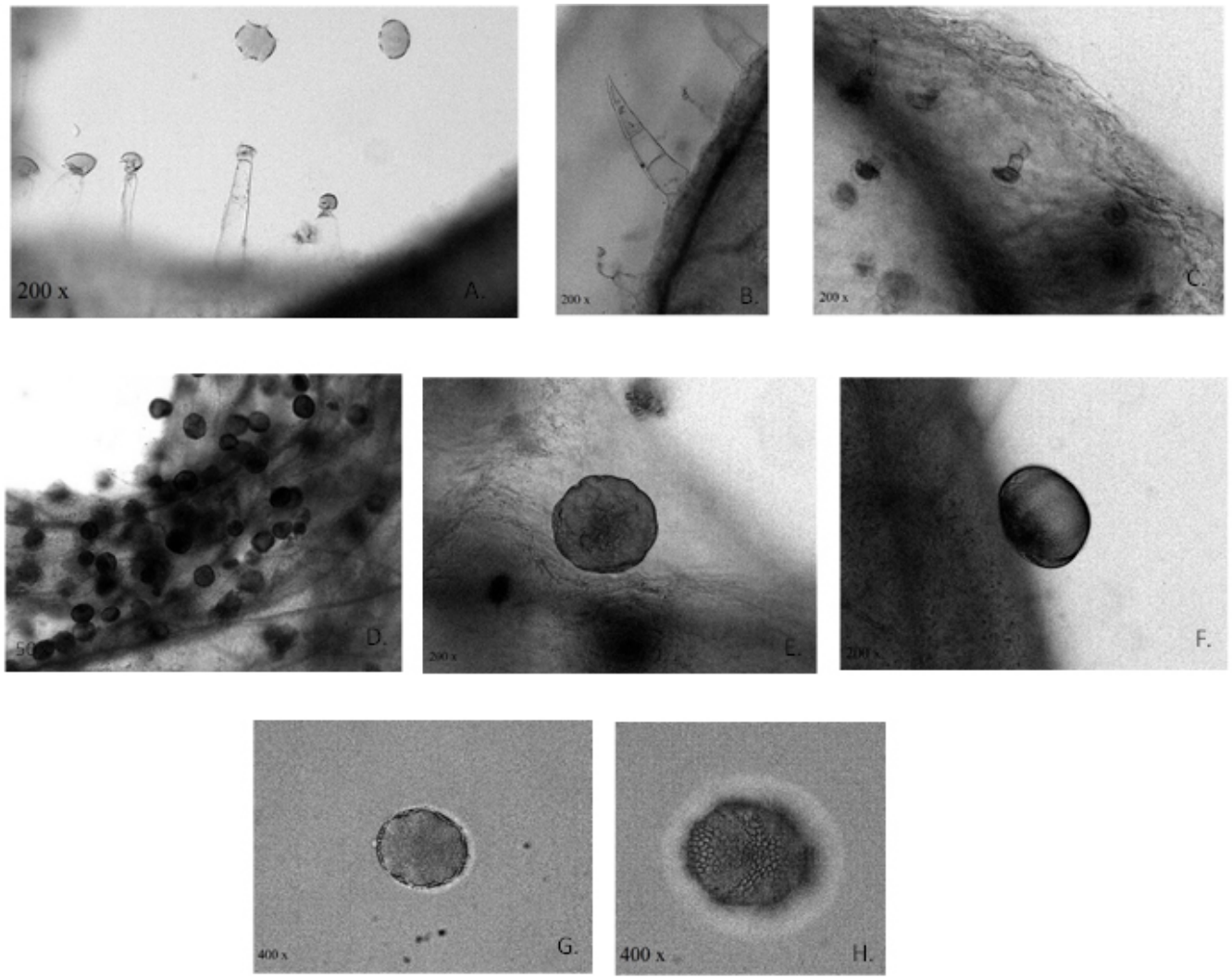

Fig. 5. Trichomes on the surface of a *Salvia sclarea* L. corolla (A (200×) – capitate, B (200×) – mechanical, C (200×) – capitate, D (50×) – peltate, E, F (200×), and G, H (400×) – pollen grain).

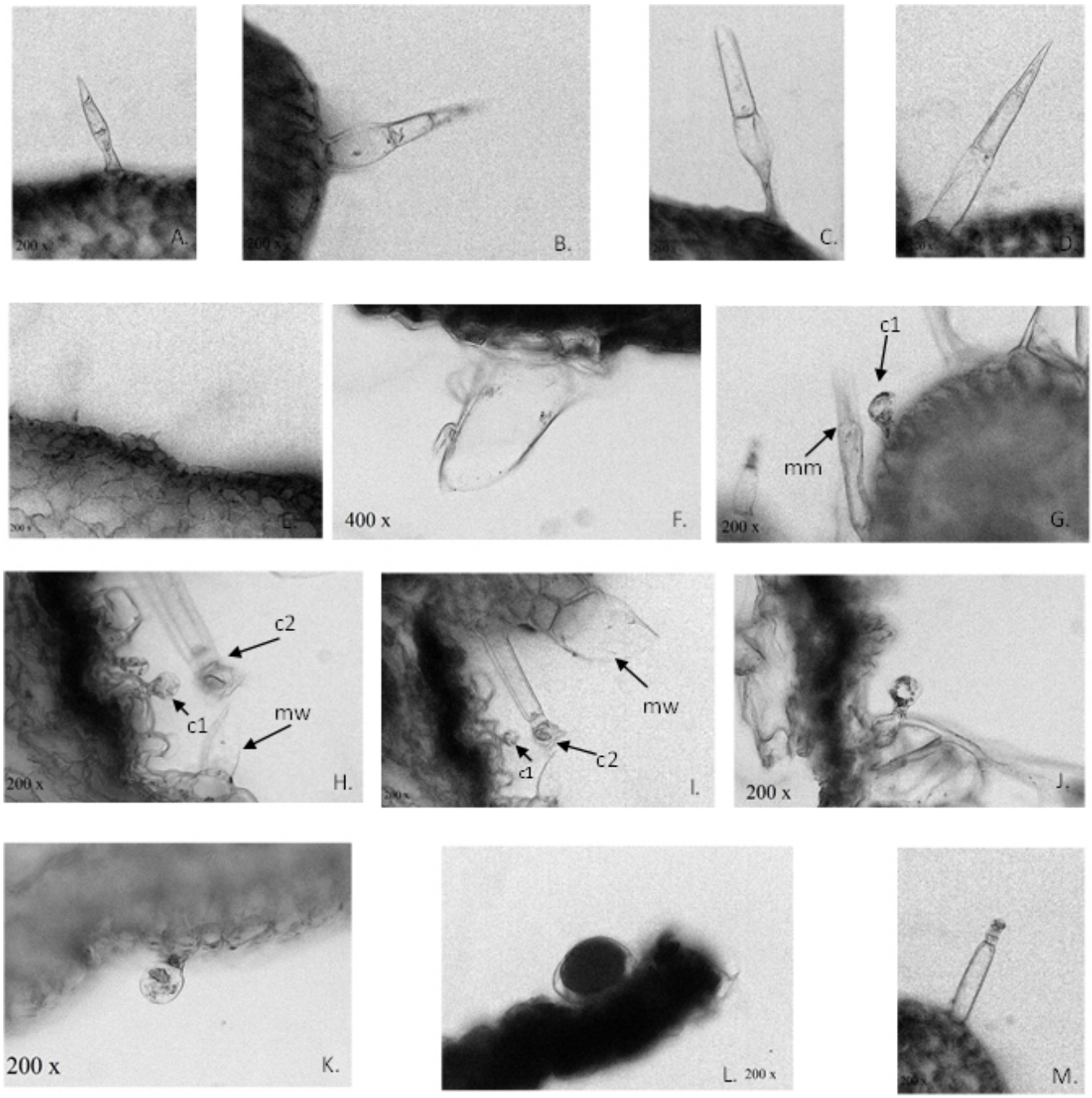

Fig. 6. Trichomes on the surface of a *Salvia sclarea* L. leaf (A, B, C, D (200×) – mechanical multicellular, E (200×), F (400×) – mechanical wedge-shaped, G (200×) – mechanical multicellular (mm) and capitate with short stalk and large head (c1), H, I (200×) – mechanical wedge-shaped (mw), capitate with short stalk and larger head (c1) and capitate with long stalk and small head (c2), J, K (200×) – capitate with short stalk and larger head, L (200×) – peltate, M (200×) – capitate with long stalk and small head)

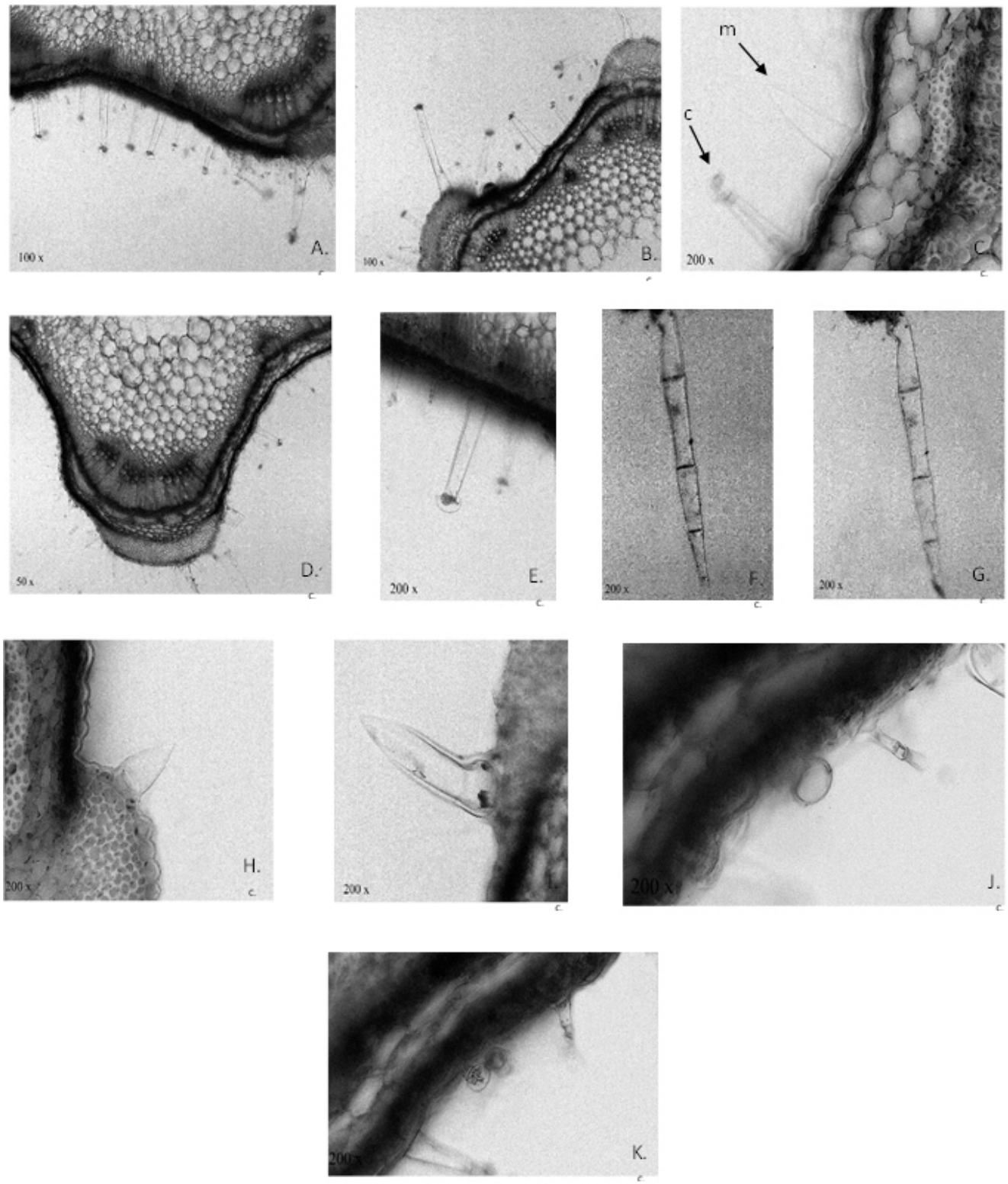

Fig. 7. Trichomes on the surface of a *Salvia sclarea* L. stem; A, B (100×) – capitate with a long stalk and a small head, C (200×) – capitate with long stalk and small head (c) and mechanical wedge-shaped (m), D (50×), E (200×) – capitate with long stalk and small head, F, G (200×) – mechanical multicellular, H, I (200×) – mechanical wedge-shaped, J (200×) – peltate, K (200×) – capitate with a short stalk and large head.

transparent, whitish, opaque, or yellowish on the leaves (Schmiderer et al., 2008), which only to some extent coincides with our samples because peltate trichomes were always, in all parts of the plant, filled with orange-yellow contents.
The morphology, distribution, and type of secretion of glandular trichomes of *S. sclarea* leaves and flowers from the area of Italy were examined by Bini Maleci (1988). Based on histochemical and morphological differences, the author concluded that one type of glandular trichomes was characterized by hydrophilic secretion, and three by lipophilic. Hydrophilic trichomes were capitate and short, characterized by the secretion of mucilaginous content and present on all organs of *S. sclarea*, especially on the adaxial surface of leaves and bracts. Lipophilic trichomes were peltate and capitate containing the essential oil. Capitate oil glands were present on young offshoots, on the abaxial surface of cotyledons and young leaves, remaining until the first year (type I), and on flowers (type II). Peltate trichomes were observed on the abaxial surface of the leaves and the entire flower. The flowers were the richest in essential oil because they contained glandular trichomes with lipophilic secretion (Bini Maleci, 1988). Our research also recorded the highest density of capitate and peltate trichomes in the corolla and calyx parts, with noticeable oil content in the subcuticular space.

CONCLUSION

The analysis of the surface structures of *Salviae sclareae herba* (leaves, bracts, calyx, corolla, axis of the inflorescence, and stem) on a stereo and light microscope showed the presence of two functional types of trichomes: glandular and non-glandular. The non-glandular ones are a mechanical type of trichomes represented in a unicellular or multicellular form, while glandular trichomes are differentiated as capitate, most common on the flower structures, and peltate, most abundant on the surface of the stem and leaf. Glands, within the Lamiaceae aromatic species, are characterized by the presence of essential oils with numerous volatile secondary metabolites, having a significant protective function in the plants. In addition, a previous research of *S. sclarea* flower and leaf glands showed that the one subtype of capitate trichomes produce mucilaginous content. A qualitative and quantitative analysis of the *S. sclarea* essential oil should be further carried out, including the investigation of its pharmacological effects.

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