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Micromorphological and anatomical characteristics of *Salvia amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm. (Lamiaceae)

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In this study, we examined *Salvia amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm.collected in the Republic of North Macedonia, which are for the first time subjected to detailed micromorphological and structural analysis using light and scanning electron microscopy. The nutlets and mucilage were additionally subjected to spectroscopic analysis using Raman and Attenuated Total Reflectance Fourier Transform Infrared (ATR FT-IR) spectroscopy. The anatomical structure of stems and leaves is described and compared. The stems, leaves and calyces bear numerous one- and multi-cellular nonglandular trichomes, and various peltate, capitate and digitiform glandular trichomes. The nutlets differ in size and shape, as well as in myxocarpy. The nutlets predominantly contained α-linolenic and linoleic acid, while the mucilages are primarily formed of polysaccharides. The results obtained in this study confirmed the importance of micromorphological and anatomical analysis of *Salvia* L. spp. plant parts, particularly trichomes and nutlets, thereby contributing to the knowledge about the variety of micromorphological characteristics within the genus *Salvia* L.

Keywords: Salvia amplexicaulis Lam.*;* Salvia jurisicii Košanin*;* Salvia ringens Sibth. & Sm.*;* microscopy*;* trichomes*;* nutlets*;* mucilage

Introduction

The genus *Salvia* L. (Lamiaceae), comprises nearly 1000 species widely distributed (Walker and Sytsma 2007). Various *Salvia* L. species are used in traditional medicine worldwide, for food flavoring, in cosmetics, perfumery and pharmaceutical industry (Dweck 2000), due to the production of the essential oils which are proved to exhibit considerable biological activities (Alimpić et al. 2015a, 2015b).

Salvia L. can be distinguished from the other Lamiaceae genera by unique staminal architecture, having only two stamens which are separated by a significantly elongated connective tissue (Will and Claßen-Bockhoff 2017; Hu et al. 2018; Kriebel et al. 2019). Based on the calyx, corolla and stamen morphology, Bentham first established an infrageneric classification of *Salvia* L. (Hu et al. 2018). The classifications were studied and modified by several authors. Molecular phylogenetic studies have demonstrated that *Salvia* is not monophyletic (Will and Claßen-Bockhoff 2017). Regarding circumscription of the genus *Salvia* L., different opinions are proposed, one option is to treat the five embedded genera as subgenera and maintain *Salvia* in a broad sense (Drew et al. 2017; Kriebel et al. 2019) and the other is to split *Salvia* L. into six smaller genera (Will and Claßen-Bockhoff, 2017). The molecular data obtained on 220 *Salvia* L. species confirm four highly supported clades in *Salvia* L., including four subclades within Clade I (*Salvia* s.s.).

Certain *Salvia* L. species were analyzed for their micromorphological, anatomical, ultrastructural or histochemical features. The studies were mostly focused to the glandular trichomes, which are the sites of the essential oils synthesis (Serrato-Valenti et al. 1997; Bisio et al. 1999; Corsi and Botega 1999; Krstić et al. 2006; Kamatou et al. 2007; Özkan 2008; Anačkov et al. 2009; İlçim et al. 2009; Mayekiso et al. 2009; Baran et al. 2010a, 2010b; Charchari et al. 2010; Kahraman et al. 2009, 2010a, 2010b; Al Sheef et al. 2013; Mousavi et al. 2014; Celep et al. 2015; Polat et al. 2015; Giuliani et al. 2017a, 2017b; Eiji and Salmaki 2016; Janošević et al. 2016; Najar et al. 2018; Gul et al. 2019b). Glandular trichomes, besides representing the most widespread type of surface secretory structures, attract attention of scientists as systematically important characters (Atalay et al. 2016; Eiji and Salmaki 2016; Gul et al. 2019a, 2019b), as well as due to their ability to synthesize various metabolites, applied in plant-plant and plant-environment interactions (Xiao et al. 2017; Tissier 2018).

Nutlet morphology in Lamiaceae Martinov has proved to be useful at different taxonomical levels in several studies (Marin 1996; Oran 1997; Duletić-Laušević and Marin 1999; Kahraman et al. 2009; Kahraman et al. 2011; Polat et al. 2015). The micromorphology, anatomy, mucilaginous reactions of nutlets of various *Salvia* L. species were the subject of several studies (Habibvash et al. 2007; Özkan et al. 2009; Kahraman et al. 2010a; Kahraman and Doğan 2010; Buyukkartal et al. 2011; Salimpour et al. 2014; Ifrim 2012; Mousavi et al. 2013; Salgado-Cruz et al. 2013; Capitani et al. 2013; Celep et al. 2014). The nutlets of *S. hispanica* L. (chia), until now, is the only *Salvia* L. species analyzed by Raman spectroscopy (Salgado-Cruz et al. 2013). In this research Raman and Fourier transform infrared (FT-IR) spectroscopy are included as complementary vibrational spectroscopic techniques and innovative tools for chemical content identification and determination based on monitoring of characteristic vibrations (Vaskova and Buckova 2014).

According to Hedge (1972) Flora of Europe comprises 36 *Salvia* L. species, with 15 species in flora of the Republic of North Macedonia flora (Matevski, in preparation), of which *S. amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm., were selected for current research.

Salvia amplexicaulis Lam. is a perennial herb, reaching a height of 80 cm. Leaves are shortly petiolate or sessile, pubescent below and subglabrous above. Flowers are composed of patient-pubescens calyx and violet corolla and 6-8 are arranged in inflorescences. It is distributed on the Balkan Peninsula (Hedge 1972). *S. amplexicaulis* Lam. was pharmacologically surveyed by Kolak et al. (2001), Ulubelen (2003), Janicsák et al. (2006), Petrović et al. (2009), Veličković et al. (2012), Orhan et al. (2012) and Alimpić et al. (2015a, 2017a), who analyzed the extracts and essential oil composition, as well as their bioactivities.

Salvia jurisicii Košanin is a rare and endemic species of the central part of the Republic of North Macedonia. It is a perennial herb inhabiting arid habitats. Stems, reaching height of 60 cm, are diffusely branched with dense and elongated non-glandular hairs. Leaves are pinnate composed of 4-

6 pairs of narrow linear segments. Inflorescences are composed of loose 4-6 flowers. Corolla is violet-blue, resupinate, calyces are shortly villous and glandular - punctate (Hedge 1972). It is an attractive floricultural plant. *S. jurisicii* Košanin herb has been the object of a few pharmacological studies for its medicinal properties, performed by Janicsák et al. (2006, 2010), Abreu et al. (2008) and Alimpić et al. (2015a, 2017b).

Salvia ringens Sibth. & Sm. is hardy herbaceous perennial herb, about 60 cm in height, with erect and scarcely branched stems. Leaves are pinnatisect or pinnate with 3-6 pairs of small lateral segments, petiolate, rugose and hairy. Verticillasters are made of 2-4 wide open two-lipped flowers with violet-blue colored corolla. It inhabits dry stony and grass-covered places of south and eastern Balkan Peninsula (Hedge 1972). It is highly valued as ornamental and melliferous plant due to beautiful purple flowers and pleasant fragrance. Several studies have dealt with the composition and biological activities of *S. ringens* Sibth. & Sm.essential oil and/or extracts (Tzakou et al. 2001; Šavikin et al. 2008; Nikolova 2011; Coisin et al. 2012; Georgiev et al. 2013; Tusevski et al. 2014; Janicsák et al. 2007, 2011; Alimpić et al. 2015b).

Stamen and corolla morphology of *S. amplexicaulis* Lam. and *S. jurisicii* Košanin (subclade I-C) clearly differ from *S. ringens* (subclade I-D). Species of subclade I-C have reduced lower lever arms lacking fertile thecae and falcate corollas, while representatives of subclade I-D are characterized by fertile, at least subfertile, thecae on the lower lever arms. In comparison with subclade I-C, the corolla of subclade I-D *Salvia* species is comparatively large, and the upper lip is rather straight (Will and Claßen-Bockhof 2017).

In view of the lack of information concerning the microscopical characteristics of *S. amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm., the aim of the present study was to examine the anatomical structure and surface features of the stems, leaves, flower parts and nutlets using light and scanning electron microscopy. Additionally, the chemical composition of the nutlets and their mucilage was identified using Raman and FT-IR spectroscopy.

Material and methods

Plant material

Aerial parts and nutlets of *S. amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm. were collected at the end of the flowering phase from natural populations in the Republic of North Macedonia in July 2011. Voucher samples are deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (Voucher No. 16673, 16774 and 16671, respectively).

Microscopic techniques

Stereomicroscopic analyses. Leaves, stems, calyces, corollas and nutlets of investigated species are examined and photographed using stepeomicroscope Nikon SMZ18 (Tokyo, Japan) without any prior preparation. Further, length and width of nutlets were measured using DIGIMIZER 4.3.4. Intensity of myxocarpy was monitored during 8 hours after wetting and nutlets were photographed using LEICA DMLS.

Light microscopy (LM) analyses. Small leaf and stem sections of the *Salvia* L. species were fixed in FAA (formalin–acetic acid–ethanol 10:5:85), dehydrated by increasing ethanol series (10-100%) and embedded in paraffin wax at 58°C. Cross sections (8 μm thick) were stained with hematoxylin and photographed using Zeiss Axiovert microscope (Carl Zeiss GmbH, Göttingen, Germany). Simultaneously, small leaf and stem sections of examined *Salvia* L. species were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), post-fixed in 1% osmium tetroxide in

same buffer, 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 stained with 0.1% methylene blue and photographed using Zeiss Axiovert microscope (Carl Zeiss GmbH, Göttingen, Germany).

The whole nutlets were fixed in 50% ethanol, dehydrated through a gradual series of ethanol and embedded in paraffin (Histowax, 56-58°C). Sections of 8-12 µm thickness were stained with safranin and alcian blue (Ruzin, 1999) and examined by a light microscope Leica DM2000 microscope equipped with a digital camera (Leica DFC320) and Leica IM1000 software.

Scanning electron microscopy (SEM) analysis. For SEM analysis, small sections of leaf, stem, calyx and corolla and whole and cut nutlets of tested species were coated with a thin layer of gold using BALTEC SCD 005 sputter coater (100 seconds, 30 mA), and subsequently examined and photographed using JEOL JSM-6390W.

Raman and FT-IR spectroscopy analyses of nutlets and mucilage. Raman spectroscopy of the nutlets at longitudinal section was performed using a XploRA Raman spectrometer (Horiba Jobin Yvon). Raman scattering was excited by a frequency-doubled Nd/YAG laser at a wavelength of 785 nm (maximum output power of 20-25 mW) equipped with a 1200 lines/mm grating, spectra were recorded by applying exposure time 5 s and accumulated from 10 time scans, using 100% filter. Spectral resolution was about 1 cm^{-1} and calibration was checked by 520.47 cm^{-1} line of silicon. In order to take possible sample inhomogeneity into account, for each sample at least ten Raman spectra were recorded, and then the average spectrum was used for a representative spectrum for each sample. For mucilage analysis the Attenuated Total Reflectance Fourier transform Infrared (ATR FT-IR) spectroscopy was applied, since this method does not require intense sample preparation, except that nutlets were soaked in water at room temperature for maximum 45 min in order to develop mucilage. The mucilage was prepared according to the procedure described by Salgado-Cruz et al. (2013), and characterized by ATR FT-IR using a spectrophotometer (IRAffinity-1, Shimadzu, Japan), operating with wavenumbers between 600 and 4000 cm^{-1}, with a resolution of 4 cm^{-1} and 100 scans. Raman and FT-IR spectra were analyzed by Origine Pro 8.6 software (OriginLab, Northampton, MA, USA) and were smoothed by using Savitzky–Golay filters, based on 10 points. Characteristic bands of specific functional groups were obtained from literature records.

Results and Discussion

Morpho-anatomical charasteristics

Leaves of *S. amplexicaulis* Lam. were simple, crenate, with clearly visible midrib (Fig. 1-2), those of *S. jurisicii* Košanin were intensively green and pinnate, deeply incised forming several narrow linear segments (Fig. 7), while *S. ringens* Sibth. & Sm. leaves were large, rough and pinnate (Fig. 12). Leaf blades (especially abaxial side) and petioles of examined species were densely covered by long, whitish non-glandular trichomes and yellowish peltate trichomes (Figs. 2, 3, 8, 13). In the studies of Pakistan Lamiaceae Martinov species, Gul et al. (2019a, 2019b) also identified two main types of epidermal appendages, glandular and non-glandular trichomes with several subtypes. In spite of the morphological differences, anatomical structure of leaves of examined species was quite similar. Leaves were bifacial as it was previously observed in many other *Salvia* L. species (Özdemir and Şenel 1999; Baran et al. 2008; Aktaş et al. 2009; Kahraman et al. 2009; Özdemir et al. 2009; Dereboylu et al. 2010; Celep et al. 2014), with exception of some equifacial (Kahraman and Doğan, 2010; Bercu et al. 2012). Epidermal cells were oval to rectangular and covered with cuticle, which was especially thick on *S. jurisicii* Košanin leaves. Epidermal cells of Pakistan Lamiaceae Martinov species were found to be irregular, isodiametric, and rectangular (Gul et al. 2019a, 2019b). The cells of the adaxial epidermis were significanly larger than abaxial ones as previously reported by Özkan and Soy (2007), Aktaş et al. (2009), Anačkov et al. (2009), Kahraman and Doğan, (2010),

Kahraman et al. (2010b), Dereboylu et al. (2010), Shirsat et al. (2012), Celep et al. (2014) and Atalay et al. (2016). Similar to the other representatives of the tribe Mentheae Dumort., *Salvia* L. species possess amphi- and hypostomatic leaves (Moon et al. 2009a). Additionaly, Gul et al. (2019b) found epistomatic leaves in *S. moorcroftiana*. In *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm. hypostomatic leaves were found, and in *S. amplexicaulis* Lam. amphistomatic ones with paracytic stomata in all of examined species (Figs. 4, 9, 17). Salimpour et al. (2012) found paracytic and diacytic stomata on leaves of eight Iranian *Salvia* L. species, while several Turkish (Özdemir and Şenel 1999; Baran et al. 2008; Kahraman and Doğan 2010; Dereboylu et al. 2010), Romanian (Bercu et al. 2012) and Iranian (Bagheri et al. 2016) *Salvia* L. taxa possess diacytic stomata. The Lamiaceae Martinov species from Pakistan examined by Gul et al. (2019a; 2019b) had amphi-, hypo- and epistomatic leaves, while the most common type of stomata were anisocytic and diacytic. Chlorenchyma consisted of compact palisade parenchyma on the adaxial side and spongy parenchyma with large intercellulars on the abaxial side (Figs. 18-20, 22-23, 25, 28-29). Similar to the other *Salvia* L. species (Baran et al. 2008; Aktaş et al. 2009; Özdemir et al. 2009; Dereboylu et al. 2010; Bercu et al. 2012; Celep et al. 2014; Bagheri et al. 2016) and other representatives of Lamiaceae Martinov (Atalay et al. 2016), leaves of examined species contained 1-3 layers of compact palisade parenchyma oriented to adaxial side and 3-4 layers of spongy parenchyma with many intercellular spaces oriented to the abaxial leaf side. In the midrib, one large closed collateral vascular bundle was present surrounded with multilayer parenchyma as previously shown for several *Lamium* L. species (Atalay et al. 2016); a small subepidermal group of sclerenchymatous cells could be observed in the adaxial side at the midrib level (Figs. 19, 22, 28-29), which is considered as an adaptation to an arid environment (Moon et al. 2009a). Also, sclerenchyma fibers were visible along with phloem on the abaxial side of examined leaves as it was previously established by Özdemir et al. (2009) and Dereboylu et al. (2010).

The stems of *S. amplexicaulis* Lam. and *S. jurisicii* Košanin were quadrangular and densely covered by whitish non-glandular trichomes (Figs. 31-32, 35), while stem of *S. ringens* Sibth. & Sm. was round-shaped in cross section and hairless (Figs. 38-39). Although quadrangular stems are typical for *Salvia* L. species (Baran et al. 2008; Özdemir et al. 2009; Dereboylu et al. 2010; Salimpour et al. 2012), some species possess round-shaped (Aktaş et al. 2009) or rectangular stems (Celep et al. 2014). In general, stems of examined *Salvia* L. species showed similar structure. Epidermis was single layered and covered with cuticle. Cortex was composed from subepidermally distributed 1-2 layered collenchyma (multilayered in the corners of quadriangular stems) and several layers of parenchyma (Figs. 34, 36-37, 40). Previous studies showed that collenchyma was multilayered in stem corners of *Salvia* L. species, making them typically quadrangular (Özdemir and Şenel 1999; Baran et al. 2008; Özkan and Soy 2007; Özdemir et al. 2009; Kahraman and Doğan 2010; Salimpour et al. 2012). Similar stem structure was previously reported for Turkish *Lamium* L. species (Atalay et al. 2016). Sclerenchyma cells were visible along with phloem (Figs. 34, 36-37, 40), similar to other *Salvia* L. species (Özdemir and Şenel, 1999; Baran et al. 2008; Özkan and Soy 2007; Özdemir et al. 2009; Kahraman and Doğan 2010; Kahraman et al. 2010a, 2010b; Salimpour et al. 2012). In the xylem, large vessels and smaller tracheids were observed in cross sections of all examined species. One to multilayered medullary rays in stems of *S. amplexicaulis* Lam. and *S. jurisicii* Košanin (Figs. 34, 37) were observed. Cambium was not well distinguished (Figs. 34, 36-37, 40). In the *S. ringens* Sibth. & Sm. stem centre, parenchymatous cells were broken forming medulla (Fig. 38). Many of previously examined *Salvia* L. species have quite uniform stem anatomy (Özdemir and Senel 1999; Baran et al. 2008; Özkan and Soy 2007; Aktaş et al. 2009; Kahraman 2009, 2010a, 2010b; Koyuncu et al. 2009; Özdemir et al. 2009; Kahraman and Doğan 2010; Salimpour et al. 2012; Shirsat et al. 2012; Celep et al. 2014).

Calyces of *S. amplexicaulis* Lam. and *S. jurisicii* Košanin were campanulate (Figs. 42, 45), and *S. ringens* Sibth. & Sm.had tubular-campanulate (Fig. 50), two-lipped calyx in all of examined species (tridentate upper and bidentate lower lip). The outer side had clearly expressed veins, densely covered with whitish non-glandular trichomes and peltate trichomes (Figs. 42, 44-45, 47, 50).

Corolla of *S. ringens* Sibth. & Sm. was blue-violet coloured, two-lipped (Figs. 48-49); two epipetalous stamens and a long pistil with two-lobed stigma in the end are visible (Fig. 49). The current findings are consistent with descriptions found in Flora Europaea (Hedge 1972) and with reports on *S. sclarea* L. (Özdemir and Şenel 1999), *S. tchihatcheffii* (Fisch. & Mey.) Boiss. (Aktaş et al. 2009), *S. marashica* A. İlçim, F. Celep & Doğan (İlçim et al. 2009), *S. ekimiana* Celep & Doğan (Celep and Doğan 2010) and *S. hasankeyfense* Dirmenci, Celep & O. Guner (Celep et al. 2015).

Trichomes features

The indumentum of different organs (leaves, stems, flower parts) of analyzed *Salvia* L. species was composed of non-glandular and glandular trichomes, which presence is characteristic of the family Lamiaceae Martinov (Werker et al. 1985; Baran et al. 2010b; Atalay et al. 2016; Gul et al. 2019a, 2019b). Trihomes have often been used in plant classification (Wagner 1991; Atalay et al. 2016; Gul et al. 2019a, 2019b). Plant species that contain glandular trichomes produce relatively large amounts of bioactive compounds which include highly concentrated phytochemicals with biological activities of interest to many industries (Dyubeni and Buwa, 2011). In the recent decades, many researchers were focused on the micromorphological characterization of trichomes of *Salvia* L. species (Bisio et al. 1999; Corsi and Bottega 1999; Özdemir and Şenel 1999; Krstić et al. 2006; Özkan and Soy 2007; Özkan 2008; Özkan et al. 2008; Schrimiderer et al. 2008; Aktaş et al. 2009; Anačkov et al. 2009; İlçim et al. 2009;Mayekiso et al. 2009; Moon et al. 2009a; Baran et al. 2010a, 2010b; Kahraman and Doğan 2010; Kahraman et al. 2010a, 2010b; Bercu et al. 2012; Dyubeni and Buwa 2012; Salimpour et al. 2012; Shirsat et al. 2012; Al Sheef et al. 2013; Celep et al. 2014; Celep et al. 2015; Janošević et al. 2016; Gul et al. 2019b).

Non-glandular trichomes. Leaves of all examined species were covered with non-glandular trichomes variable in morphology and length. According to Werker (2000) they could be classified as unicellular (often triangular, erect) (Figs. 5, 9-10, 18) and multicellular (variable in length and/or shape, hornlike or flagelliform) (Figs. 4, 10, 14-15, 18). Long, whitish, multicellular trichomes were observed on the stems of *S. amplexicaulis* Lam. and *S. jurisicii* Košanin (Figs. 31-32, 35) and also in the calyx indumetum of all examined species (Figs. 42-43, 45-46, 50). Other *Salvia* L. species showed similar diversity of non-glandular trichomes on different plant parts of *S. sclarea* L. (Özdemir and Şenel 1999), *S. verticillata* L. (Krstić et al. 2006), *S. recognita* Fisch. & Mey. (Özkan 2008), *S. cadmica* Boiss. (Özkan et al. 2008), *S. repens* Burch. ex Benth. (Mayekiso et al. 2009), *S. chrysophylla* Stapf (Kahraman et al. 2010b), *S. fruticosa* Mill. (Al Sheef et al. 2013), *S. quezelii* Hedge & Afzal-Rafii (Celep et al. 2014), *S. aegyptiaca* L. (Janošević et al. 2016). SEM micrographs showed papillae on non-glandular trichomes (Figs. 5, 9, 15, 17), as previously observed on *S. verticillata* L. (Krstić et al. 2006), *S. fruticosa* Mill. (Al Sheef et al. 2013) and on trichomes of *Lamium* L. representatives (Atalay et al. 2016). In the study of Lamiaceae Martinov representatives from Pakistan, Gul et al. (2019a) divided non-glandular trichomes into subtypes: dendritic, stellate, conical, falcate, simple and 1–6 cells long having granulate and smooth surface ornamentation. Variation in cell number and morphology of non-glandular trichomes can be considered as a potential taxonomic trait (Gul et al. 2019b).

Non-glandular trichomes, especially in plants which grow under arid conditions, may serve as a mechanical barrier against many external factors, such as herbivores and pathogens, extensive light, extreme temperatures, excessive water loss, against competitor plants, etc. (Werker 2000).

Glandular trichomes. They are the primary secretory structures of Lamiaceae Martinov plants, showing variability among species (Serrato-Valenti et al. 1997; Gul et al. 2019a, 2019b). In many Lamiaceae Martinov, two main types of glandular trichomes are found - capitate and peltate, named according to the shape of their secretory head (Werker 2000; Atalay et al. 2016). Gul et al. (2019b) classified glandular trichomes observed on 22 Lamiaceae Martinov taxa into seven different types: capitate, subsessile capitate, sessile capitate, sunken, barrel, peltate, and clavate. In our

research, peltate and capitate trichomes were found on vegetative and reproductive organs of analyzed *Salvia* L. species, while digitiform trichomes were found only in *S. jurisicii* Košanin.

Peltate trichomes (subsessile trichomes) are common in the tribe Mentheae Dumort. (Moon et al. 2009a) and their secretion product is released to the outside only when the hair is touched ("long-term glandular hairs") (Bisio et al. 1999). Peltate trichomes were found in all three analyzed species; they were visible as yellowish, balloon-shaped structures (Figs. 3, 8, 13) and consisted from basal cell in the epidermis, short stalk and broad secretory head composed from variable number of cells arranged in 1-2 circles. Secretory heads of peltate trichomes of *S. jurisicii* Košanin had 8 cells in one circle (Figs. 10-11, 24), while those in *S. ringens* Sibth. & Sm. had 12 cells (4 in central and 8 in peripheral circle) (Figs. 17). The number of secretory head cells of *S. amplexicaulis* Lam. was not determined, because they were examined in the post-secretory phase (Fig. 19). The number of secretory head cells varied among species of the genus *Salvia* L., from four cells in *S. blepharophylla* Brandegee ex Epling (Bisio et al. 1999), *S. verticillata* L. (Krstić et al. 2006), *S. repens* Burch. ex Benth. (Mayekiso et al. 2009) to 6-8 cells in single circle in *S. aurea* L. (Serrato-Valenti et al. 1997), *S. officinalis* L. (Werker et al. 1985), *S. fruticosa* Mill. (Werker et al. 1985; Al Sheef et al. 2013) and 8-16 cells arranged in two concentric circles (usually 1-4 cells in central and 4 or more in the peripheral circle) (Corsi and Bottega 1999; Kamatou et al. 2007; Özkan 2008; Schrimiderer et al. 2008; Anačkov et al. 2009; Baran et al. 2010a,2010b; Kahraman et al. 2010b; Celep et al. 2014; Janošević et al. 2016).

Capitate trichomes are commonly composed of basal cell, 1-2 stalk cells and 1-2 cells forming a round or pear-shaped secretory head (Werker et al. 1985; Fahn 1988). Secretory material of capitate trichomes is extruded to the outside soon after their production ("short term glandular hairs") (Bisio et al. 1999). In *Salvia* L. species examined in present work, two main types of capitate trichomes were recognized: type I (short capitate) and type II (long capitates trichomes). Moon et al. (2009a) used different terminology; they differ capitate glandular trichomes (the head cell was attached to a single cell stalk) and pilate glandular trichomes (the stalk consisted of more than one cell).

Type I was present in all of examined species and varied broadly in morphology, although it was generally composed from cubic or cylindrical basal cell, short stalk, and round or oval single-celled secretory head, with or without neck cell. In *S. amplexicaulis* Lam., three morphological subtypes of type I were found (IA, IB, IC), while in the other two species only subtype IA was present. Subtype IA was composed of basal cell, **a** broad and short stalk cell which may be absent, and a globular or ovoid unicellular head (Figs. 6, 10, 15, 16, 20, 27, 30, 41). Subtype IB was composed of a basal cell, a short stalk cell, unicellular head and a broad and short neck cell inserted between head and stalk (Fig. 21). Subtype IC possessed an ovoid basal cell, unicellular head and an elongated stalk cell expanded in the lower part (Figs. 20, 33). On the *S. amplexicaulis* Lam. stem, only subtype IC of capitate trichomes was observed (Figs. 33). In *S. jurisicii* Košanin leaves and in *S. ringens* Sibth. & Sm.leaves and stems only subtype IA was present with rounded, significantly cutinized unicellular head (Figs. 27, 30, 41).

Type II was found on the flower parts of *S. ringens* Sibth. & Sm. and consisted of a basal cell, 1-3 stalk cells and unicellular head, with or without neck cell (Figs. 51-55) and was separated into five morphological subtypes (IIA-E). Subtype IIA was found on pedicels; stalk is wide and expanded in the basal part; short neck cell is inserted between stalk and head; cuticle is shrinking on the top of head forming cup-shaped gap (Figs. 51). Subtype IIB is found in pedicel; those trichomes have cylindrical stalk (Fig. 52). Subtype IIC is found in pedicel, calyx and corolla; stalk is cylindrical and composed of three cells (Figs. 53). Subtype IID is found on the corolla; stalk is flattened, wide and connected to head by extremely thinner neck cell (Figs. 54). Subtype IIE is found on corolla; stalk is massive and trilateral, extremely expanded in the lower part (Fig. 55).

Broad variations of capitate trichomes were observed throughout the genus *Salvia* L., so that certain researchers classified capitate trichomes into two main types based on their stalk length, morphology of secretory head and mode of secretion, presence/absence of neck cell (Serrato-Valenti et al. 1997; Ascensão et al. 1999; Bisio et al. 1999; Baran et al. 2010a, 2010b). The neck cell was present in some types of capitate trichomes, which has an important role especially for xeromorfic plants, acting to prevent the backflow of secreted substance through the apoplast (Baran et al. 2010b). Some species bear only short capitate trichomes, such as *S. albicaulis* Benth. and *S. dolomitica* Codd (Kamatou et al. 2007) and *S. greggii* A. Gray (Dyubeni and Buwa 2012), while *S. marashica* A. İlçim, F. Celep & Doğan flowers are covered entirely by multicellular black-headed glandular hairs (İlçim et al. 2009). Serrato-Valenti et al. (1997) described two types of capitate trichomes in *S. aurea*, and similar types were recognized in *S. blepharophylla* Brandegee ex Epling (Bisio et al. 1999) and *S. aegyptiaca* L. (Janošević et al. 2016). Other authors found three types in some sage species, such as *S. argentea* L. (Özdemir and Şenel 1999; Schrimiderer et al. 2008; Baran at al. 2010a), *S. verticillata* L. (Krstić et al. 2006), *S. recognita* Fisch. & Mey. (Özkan 2008), *S. smyrnea* L. (Baran et al. 2010b), four types in *S. officinalis* L. (Corsi and Bottega 1999) and *S. chrysophylla* Stapf (Kahraman et al. 2010b), while in *S. fruticosa* Mill. (Al Sheef et al. 2013) and *S. quezelii* Hedge & Afzal-Rafii (Celep et al. 2014) five types of capitate trichomes were found.

Digitiform trichomes were present only on *S. jurisicii* Košanin leaves and were composed of wide and short basal cell, three stalk cells and one apical cell (Figs. 23, 26). Similar digitiform trichomes were found on leaf indumentums of *S. fruticosa* Mill. (Al Sheef et al. 2013) and in the other Lamiaceae Martinov representatives, such as *Plectranthus ornatus* Codd (Ascensão et al. 1999), *Pogostemon cablin* Benth. (Rusydi et al. 2013), *Thymus quinquecostatus* Celak (Jia et al. 2013) and *Melissa officinalis* L. (Chwil et al. 2016). They usually consisted of 3-4 cells of approximately the same width arranged in the same line, without clear distinction between apical and other cells (Ascensão et al. 1999; Al Sheef et al. 2013; Rusydi et al. 2013).

Morpho-anatomical and chemical features of nutlets and mucilage

Nutlet anatomy and micromorphology. Studies on nutlet (mericarp) micromorphology and pericarp anatomy in some Lamiaceae Martinov genera have proved to be useful in variable degrees at different taxonomic levels (Marin, 1996; Duletić-Laušević and Marin 1999; Büyükkartal et al. 2011; Kahraman et al. 2011). Previous studies of the genus *Salvia* L. showed that the most important taxonomic characters of nutlets were shape, size, shape of basal and apical parts, shape and position of the abscission scar, surface ornamentation, width of individual pericarp layers, presence of myxocarpy and mucilage properties (Marin 1996; Duletić-Laušević and Marin 1999; Habibvash et al. 2007; Özkan et al. 2009; Büyükkartal et al. 2011; Kahraman et al. 2011).

Nutlets of *Salvia* L. species examined in this study were brown, 1.97-3.08 mm in length and 1.05-2.27 mm in width, with length/width ratio from 1.34 to 1.88, rounded on the top and narrowed in the base (Figs. 56-58, 62-64, 68-70; Tab. I). These findings are consistent with those obtained for other *Salvia* L. spp. by Özkan et al. (2008, 2009) and Kahraman et al. (2010a, 2010b, 2011) . The size of nutlets in Mentheae Dumort. varies from 0.6-4.3 mm in length (Moon et al. 2009b). Nutlet shape is characterized as spheroidal for *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm.and prolate-spheroidal for *S. amplexicaulis* Lam., following the classification given by Özkan et al. (2009) for 12 taxa from the section *Salvia* (Sect. *Eusphace* Bentham). These two shapes were the most common in Turkish *Salvia* L. species (Özkan et al. 2009; Kahraman et al. 2011). The ventral side of examined *Salvia* L. nutlets were flattened, with more or less distinguished ventral rib and with the round to triangle abscission scar, while dorsal side was gently convex (Figs. 56-58, 62-64, 68-70). Moon et al. (2009b) reported that nutlets of Salviinae (Dumort.) Endl. have an areolate/round abscission scar without an extended area, while an extended abscission scar U or V-shaped area commonly occurs in both Menthinae (Dumort.) Endl. and Nepetinae (Dumort.) Coss. & Germ. Similar morphological properties of nutlets were found in other analyzed *Salvia* L. species from Turkey (Özkan et al. 2008,

2009; Özkan & Soy 2010;Celep and Doğan 2010; Kahraman and Doğan 2010; Kahraman et al. 2010b, 2011; Büyükkartal et al. 2011), Iran (Salimpour et al. 2014; Mousavi et al. 2013) and Romania (Ifrim 2012).

Surfaces of examined nutlets were rough, with more or less expressed irregular sculptures (reticulate ornamentation) (Figs. 59, 65, 71; Tab. I). The ornamentation is formed by outer cell walls of exocarp cells (Özkan et al. 2009). Marin (1996) described irregular sculptures in *S. ringens* Sibth. & Sm.nutlets surface although other members of section *Salvia* (Sect. *Eusphace* Bentham) showed more regular surface pattern. Ifrim (2012) described ornamentation of *S. ringens* Sibth. & Sm. nutlets as verrucate, although in the surface are not visible distinct hills characteristic for this type of ornamentation. Nutlets of *S. jurisicii* Košanin showed similar sculpturing pattern as other members of section *Plethiosphace* Benth. (*S. nemorosa*, *S. pratensis*, *S. austriaca*, *S. nutans* and *S. verbenaca*) (Marin 1996). Literature data on *S. amplexicaulis* Lam. nutlets are not available till now. Özkan et al. (2008) found reticulate ornamentation in *S. cadmica*, while Özkan et al. (2009) reported on three ornamentation patterns of 12 *Salvia* L. taxa from Turkey, i.e. foveate, reticulate and verrucate. Büyükkartal et al. (2011) highlighted that three endemic *Salvia* L. species (*S. hedgeana, S. huberi* and *S. rosifolia*) had a fourth type of ornamentation - coliculate. Kahraman et al. (2011) found all four types in section *Hymenosphace*, while species from section *Aethiopis* (*S. limbata* and *S. palaestina*) had smooth to rugose nutlet surfaces (Kahraman and Doğan, 2010).

Oran (1997) and Hassan and Al Thobaiti (2014) examined the cross sections of nutlets and reported three pericarp parts, i.e. exocarp (epicarp), mesocarp and endocarp, while Duletić-Laušević and Marin (1999), Habibvash et al. (2007), Ryding (2010) and Kahraman et al. (2018) additionally defined schlerenchyma region between mesocarp and endocarp. The exocarp cells are mostly differentiated into colourless mucilaginous cells and brownish non-mucilaginous cells. The variation in thickness of the exocarp seems to be correlated to the amount of mucilage produced by the mucilaginous cells (Ryding 2010). The cells of mesocarp were parenchymatous and brownish. The sclerenchyma region comprises a single layer of vertically arranged brownish “bone cells”. The single layer of endocarp cells was colourless or sometimes faintly brownish. Findings of present study (Figs. 74-85) confirmed previous reports on *Salvia* L. species showing considerable uniformity of pericarp structure of *Salvia* L. species from anatomical point of view (Oran 1997; Duletić-Laušević & Marin, 1999; Habibvash et al. 2007; Hassan and Al Thobaiti 2014; Kahraman et al., 2018).

Myxocarpy. The occurrence of myxocarpy, the production of mucilage by wetted mericarps, is widespread in the subfamily Nepetoideae (Dumort.) Caruel (Duletić-Laušević and Marin, 1999; Büyükkartal et al. 2011). Mucilage producing cells are distributed in exocarp and their cell walls contain hygroscopic spiral fibrils (Duletić-Laušević and Marin, 1999; Ryding 2010). Width of this layer varied from 0.25 to 4.24 µm among 13 Iranian *Salvia* L. species (Habibvash et al. 2007). Nutlets of three examined species produced yellowish, transparent mucilage (Figs. 60, 66, 72; Tab. 1), with clearly visible fibrils (Figs. 61, 67), except in *S. ringens* Sibth. & Sm.mucilage (Fig. 73). The period of mucilage formation was shorter (15 min) in the smaller nutlets of *S. amplexicaulis* Lam. and *S. jurisicii* Košanin (sect. *Plethiosphace* Benth.) and longer (45 min) in the larger nutlets of *S. ringens* Sibth. & Sm. belonging to sect. *Salvia* (sect. *Eusphace* Bentham). The ecological role of mucilage production has been considered along with its importance as taxonomical character, as it was earlier proven in *Salvia* L. (Hedge 1970; Duletić-Laušević and Marin 1999). In some species from section *Salvia* (sect. *Eusphace* Bentham) (*S. glutinosa* L. and *S. officinalis* L.) myxocarpy was completely absent (Ifrim 2012). Smaller nutlets have ability of faster production of greater mucilage amount comparing with larger ones (Duletić-Laušević and Marin 1999). Ifrim (2012) obtained different results for relatively large nutlets of *S. viridis* L. which formed mucilage extremely fast (about 2 min after wetting). Hedge (1970) grouped *Salvia* L. species into four groups according to mucilage properties, with transparent, translucent, milky opaque and brownish opaque. In the studies of Büyükkartal et al. (2011) and Ifrim (2012), *Salvia* L. species varied in the rate, amount and

properties of produced mucilage. Celep et al. (2014) found that myxocarpy was present in *S. quezelii* Hedge & Afzal-Rafii and absent in *S. pinnata* L., which could be useful for distinction those morphologically similar species.

Characterisation of nutlets and mucilage by Raman and FT-IR spectroscopy. In the previous studies, which employed conventional methods, the nutlets of *Salvia verbenaca* L., *S. officinalis* L., and *S. aegyptiaca* L. showed a higher content of polyunsaturated fatty acids, primarily α-linolenic and linoleic acids (up to 70%), oleic and palmitic acids (up to 30%) (Azcan et al. 2004; Bagci et al. 2004; Kiliç et al. 2005, Ben Taarit et al. 2010; Ben Farhat et al. 2015). The Raman spectra of surveyed *Salvia* L. nutlets (Fig. 86) contained predominant bands of unsaturated fatty acids. The molecular formulas of α-linolenic and linoleic fatty acids are extremely simple containing only CH_2, CH_3, and COOH as functional groups and C-C, C=C, C-H, O=C, C-O, and O-H as chemical bonds, in a case of α-linolenic and linoleic acids there are three or two C=C bonds, respectively (Lv et al. 2016). Because these acids differ manly in the position of the double bonds, their Raman spectra are highly similar (De Gelder et al. 2007). For evaluation and identification of fatty acids in *Salvia* L. nutlets (Fig. 86) there is so called a fingerprint region (800-1800 cm^{-1}) well known for characterization of the unsaturation level of the fatty acid chain (Farhad et al. 2009).

These unsaturated acids are fluid, so their Raman spectra show broad bands (De Gelder et al. 2007). The most characteristic band and intense signal in examined *Salvia* L. nutlets (Fig. 86), occurs at 1660 cm^{-1} indicating a C=C stretching mode of the cis unsaturated fatty acid part. Furthermore, there are strong vibrations at 1266 cm^{-1} assigned to C-H bending deformation. The bands at 1305 and 1443 cm^{-1} attributed to CH_2 deformations and the CH_2 scissoring mode have also a significant intensity, and the bands at 838 and 866 cm^{-1} were assigned to C-C stretching (Machado et al. 2012; Lv et al. 2016), all above mentioned responsible for α-linolenic and linoleic fatty acids. Composition of fatty acids in nutlets indicated the presence of linoleic, but also α-linolenic acid with specific band at 1006 cm^{-1} (Fig. 86), which could be connected with unsaponifiable matter from oil fraction. The bands at 1006 and 1036 cm^{-1} could also indicate the presence of globulins and phenylalanine in nutlets (Li-Chan 1996; Ma et al. 2003).

The *Salvia* L. nutlets produce a large amount of mucilaginous polysaccharides on the surface, mainly composed of hydroxyl and carbonyl functional groups of carboxylates and carboxylic acid, present in polysaccharide complexes, eg. D-xylose and D-mannose, D-arabinose, D-glucose, galacturonic and glucuronic acids (Goh et al., 2016; Julio et al. 2016; Timilsena et al. 2016). Fig. 87 shows the characterization of analyzed *Salvia* L. mucilage provided by ATR FT-IR spectroscopy. FT-IR spectra of mucilage show bands in three spectral regions: around 3300, 2920 and 900–1650 cm^{-1}, commonly recognized as polysaccharides and representing O-H, C-H bonds of CH_2 groups, and C=O, C-O-C, C-O-H, respectively (Muñoz Hernandez 2012; Goh et al 2016).

The mucilage spectra (Fig. 87) showed a broad band at approximately 3350 cm^{-1} corresponding to O-H stretching vibration. The low intensity and broad bands at 2920 and 898 cm^{-1} were assigned to C-H stretching vibration of the aromatic rings and CH_2 groups, respectively. The region between 1400 and 1800 cm^{-1} is typically used to detect presence of carboxylic groups (Singh and Bothara 2014). The presence of uronic acid was confirmed based on the results obtained by FT-IR spectra according to band at 1421 cm^{-1}, which represents the symmetric stretching vibration of carboxylate group (C-O-O-H) of uronic acid (Muñoz Hernandez 2012). Uronic acid is the main component of two major polysaccharides, glucuronoarabinoxylan and galactosyluronic acid (Timilsena et al., 2016), which is commonly found in gums (Singh and Bothara 2014), especially in polysaccharide fraction of mucilage of *S. hispanica* L. (Goh et al. 2016). The band at 1622 cm^{-1} was previously described and assigned to mannose ring stretching, also observed for chia mucilage (Muñoz Hernandez 2012; Goh et al. 2016). Low intensity band at 1735cm^{-1} is attributed to C=O stretching vibration of carboxylic acid (Muñoz Hernandez 2012), recorded only in mucilage of *S. amplexicaulis* Lam. (Fig. 87). The strong band at 1047 cm^{-1} is assigned to 1-4 glycosidic linkages

(C-O-C) (Wang and Somasundaran 2007; Timilsena et al. 2016). The xyloglucan from polysaccharide fraction of mucilage could be indicated by the presence of low intensity bands at 1150cm^{-1} and 1317, 1371 cm^{-1}, attributed to glycosidic C-O-C stretching and CH_2 bending vibration, respectively (Szymanska-Chargot and Zdunek 2013; Bashir et al. 2016).

These results support the assumption that Raman and FT-IR spectroscopy can rapidly provide information on the chemical profile of *Salvia* L. nutlets to be used as a fingerprint for different species. Raman spectroscopy results confirm that the *Salvia* L. nutlets are predominantly contain α-linolenic and linoleic acids. The results obtained from ATR FT-IR spectroscopy proved that *Salvia* L. mucilages are primarily consist of polysaccharides.

Conclusion

The analyzed *Salvia* L. species showed quite similar leaf and stem anatomy. In the indumentum of vegetative and generative organs of investigated species, different non-glandular trichomes, as well as peltate, several subtypes of capitate and digitiform glandular trichomes were found. The microscopical investigation of nutlets showed differences in size, shape and myxocarpy and similar pattern of ornamentation. The nutlets of all three analyzed species predominantly contained α-linolenic and linoleic acid, while the mucilages are primarily formed of polysaccharides. The obtained results confirmed the importance of microscopical analysis of sage organs, especially trichomes and nutlets, as a contribution to the knowledge about the diversity of the micromorphological features among *Salvia* L. representatives. In this study the Raman spectroscopy of nutlets and FT-IR analysis of mucilage were employed for the first time for *Salvia* L. species, except for *S. hispanica* L. (chia) mucilage. These results could be a reliable basis for more detailed analysis and identification of potential differences in nutlet fatty acids among *Salvia* L. species, as well as mucilage polysaccharides content using spectroscopic methods.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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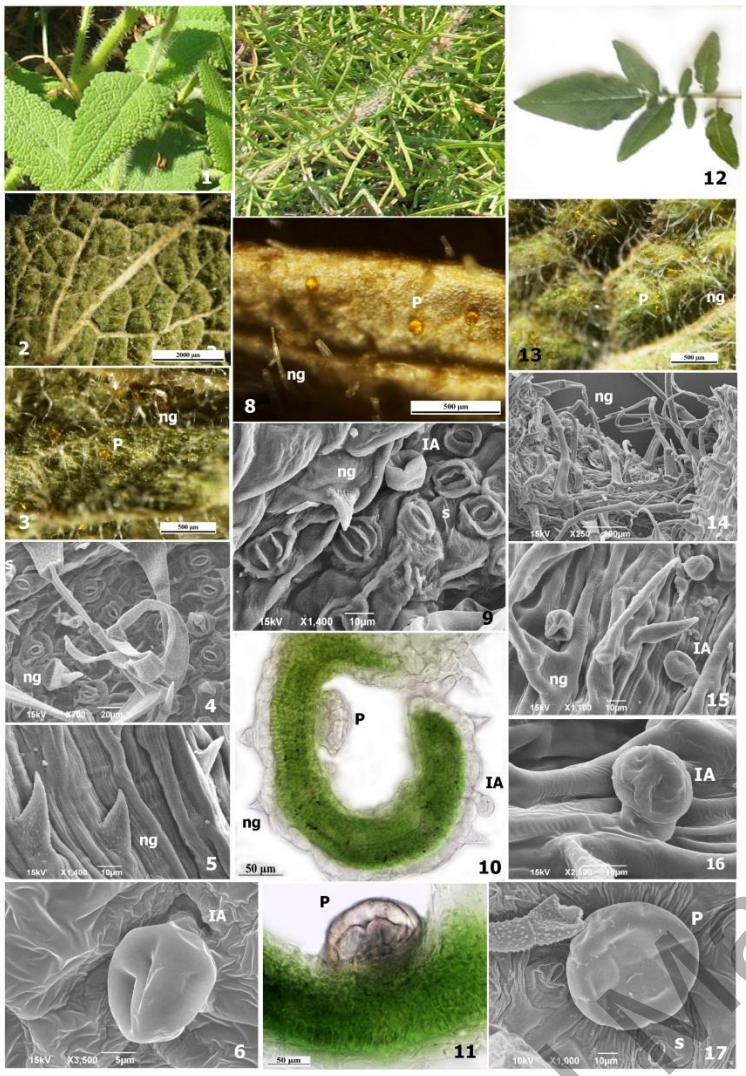
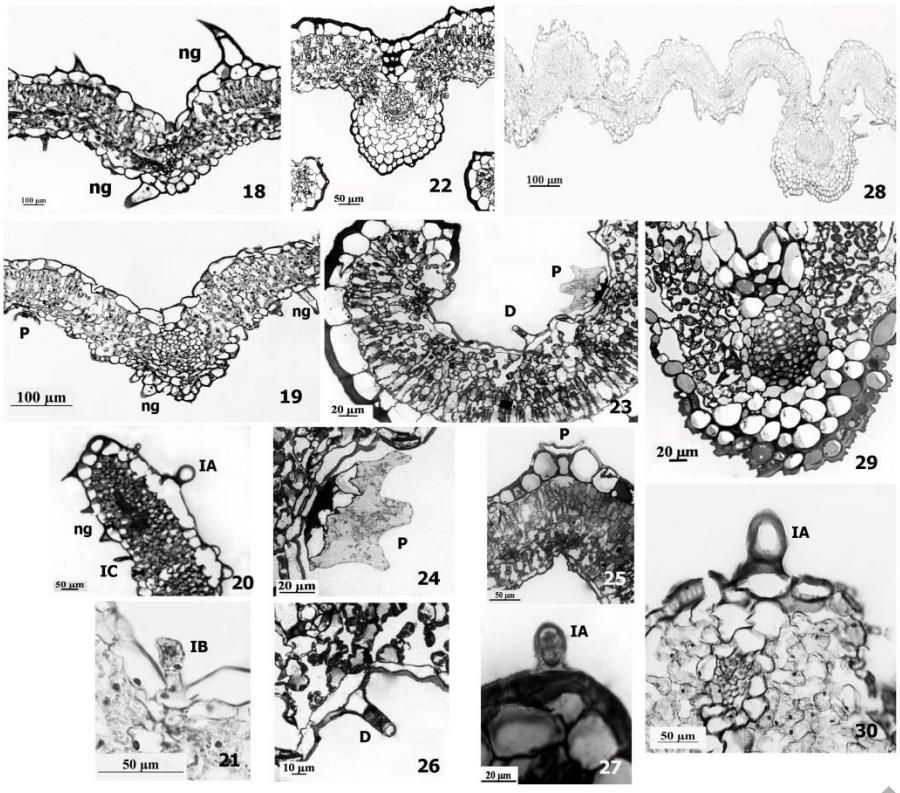
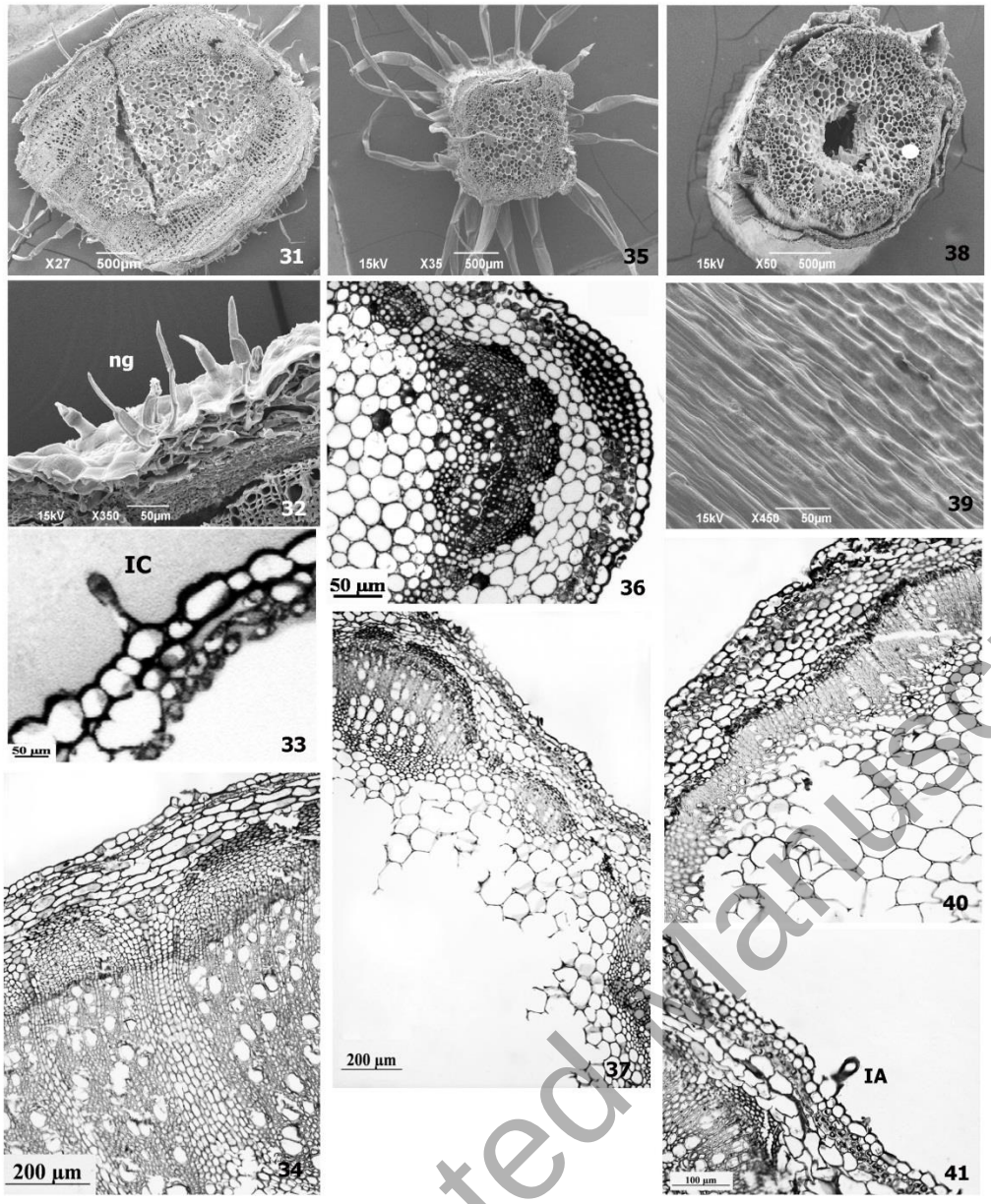


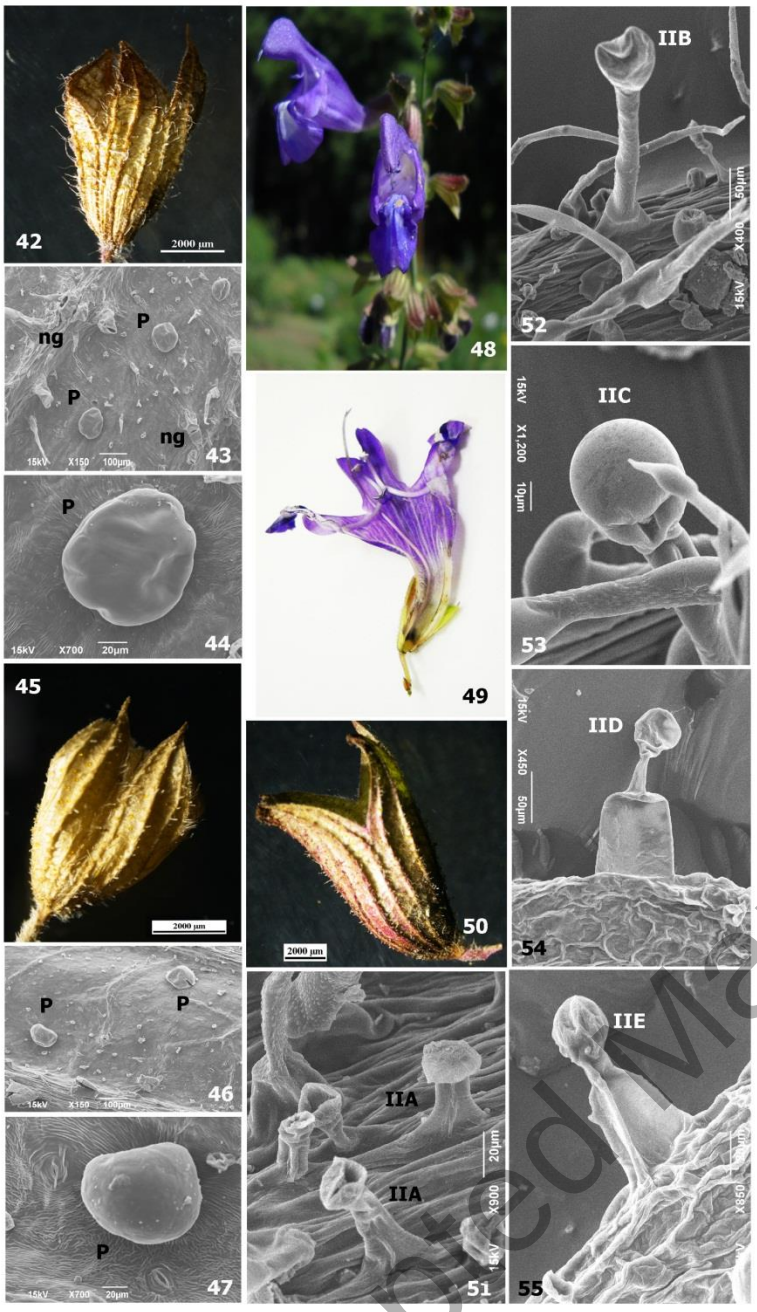
Figures. 1-17. Morphological and micromorphological properties of examined *Salvia* L. species leaves: **Figs. 1-6.** *S. amplexicaulis* Lam. leaf**: 1.** The appearance of *S. amplexicaulis* Lam. leaf; **2.** Abaxial leaf side (stereomicroscope) (20 x) showing reticulate venation; **3.** Abaxial leaf side (stereomicroscope) (60 x) showing non-glandular (ng) and peltate trichomes (P); **4-6.** SEM micrographs showing abaxial leaf surface with numerous stomata (s) (**4.**) and non-glandular trichomes (ng) (**4-5.**) as well as capitate trichomes IA (**6.**); **Figs. 7-11.** *S. jurisicii* Košanin leaf: **7.** The appearance of *S. jurisicii* Košanin leaf; **8.** Petiole appearance (stereomicroscope) (70 x); with non-glandular (ng) and peltate trichomes (P); **9.** SEM micrograph of abaxial leaf surface showing numerous stomata (s), non-glandular (ng) and capitate trichomes IA; **10-11.** Cross section of leaf *in vivo*; leaves bear non-glandular (**10.**), capitate trichomes IA (**10**.) and peltate trichomes (P) (**10-11.**); **Figs. 12-17.** *S. ringens* Sibth. & Sm. leaf: **12.** Appereance of *S. ringens* Sibth. & Sm. leaf; **13.** Abaxial leaf side (stereomicroscope) (50 x) showing non-glandular (ng) and peltate trichomes (P); **14-17.** SEM micrographs showing abaxial leaf side with non-glandular (ng) (14-15.), capitate trichomes IA (**15-16.**), peltate trichomes (P) and stomata (s) (**17.**).



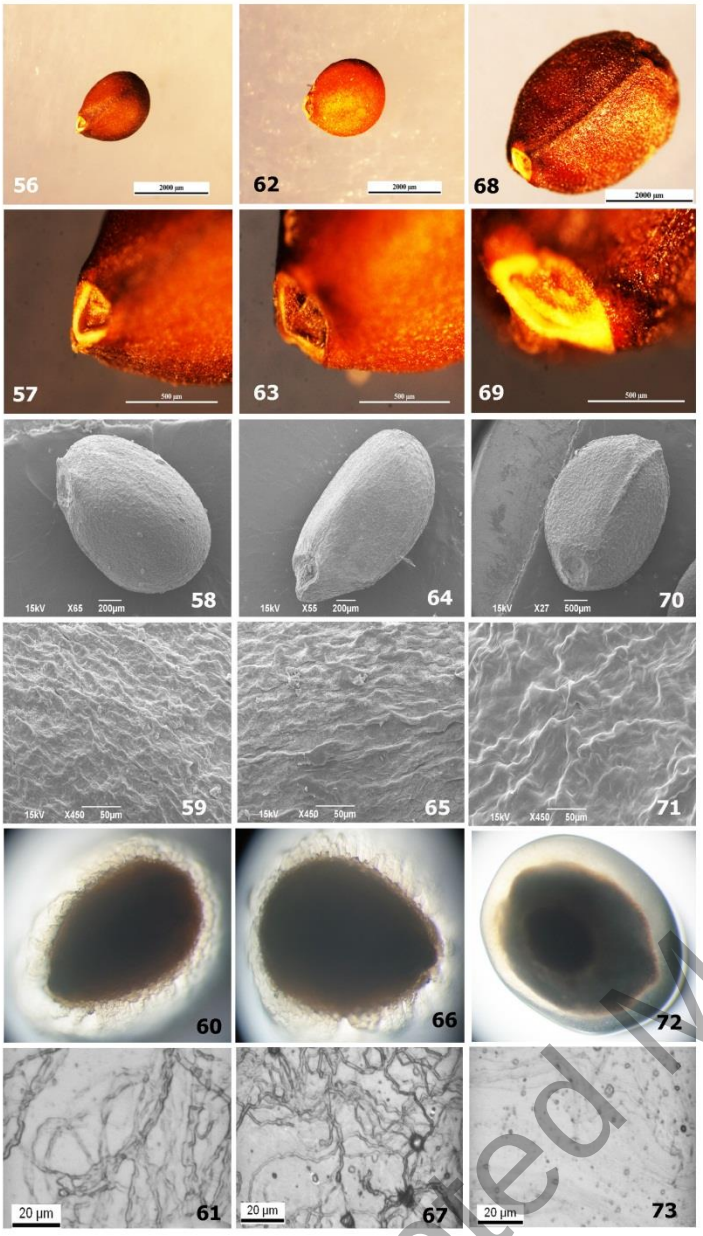
Figures. **18-30.** Anatomical properties of three *Salvia* L. species leaves (LM): **Figs. 18-21.** *S. amplexicaulis* Lam. leaf cross sections showing glandular and non-glandular (ng) trichomes on both leaf surfaces. Peltate trichome in the post-secretory phase can be observed on the abaxial side (**19.**). Capitate trichomes of type I are visible **(20-21.)** with three different subtypes: IA is noticed on adaxial side **(20.),** while IB **(21.)** and IC **(20.)** are observed on abaxial leaf side. **Figs**. **22-27.** Cross sections of *S. jurisicii* Košanin leaf**.** Midrib is clearly expressed with sclerenchyma fibers on adaxial side. Peltate trichomes were present in both leaf surfaces **(23-25).** Digitiform trichomes were present on the abaxial side **(23, 26.),** while capitate trichomes of type I (subtype IA) were noticed on the adaxial side only **(27.). Figs**. **28-30.** Cross sections of *S. ringens* Sibth. & Sm.leaf showing structure of leaf **(28.)** and midrib **(29.)**, as well as capitate trichomes IA on the abaxial leaf surface **(30.).**



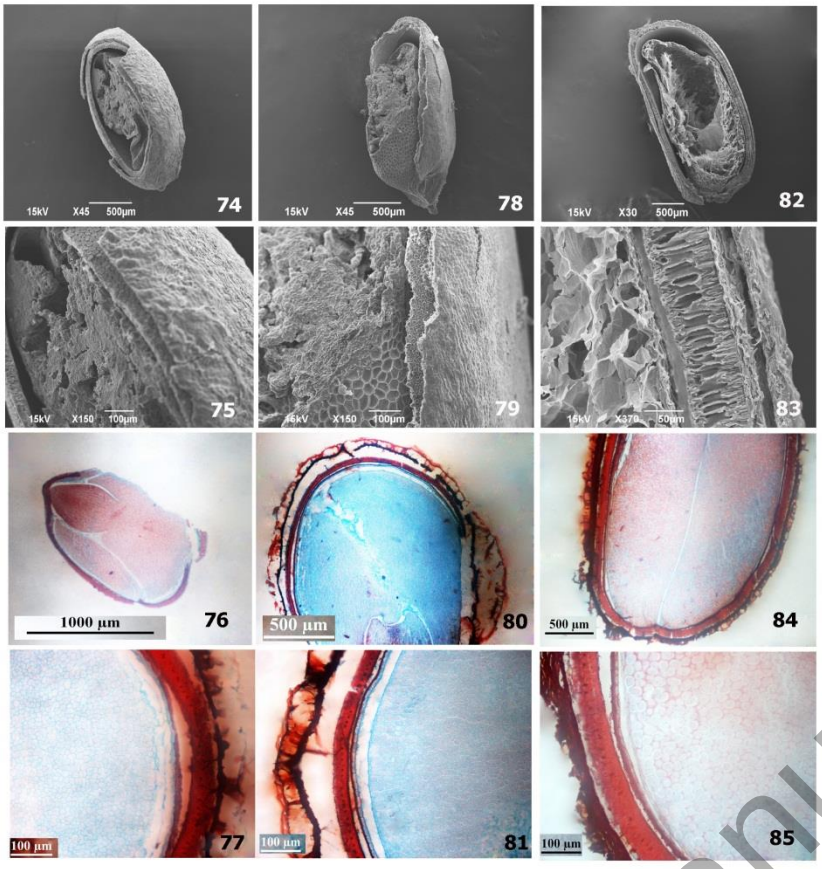
Figures. **31-41.** Morphological, micromorphological and anatomical properties of examined *Salvia* L. species stems. **Figs. 31-34.** *S. amplexicaulis* Lam. stem cross section. SEM micrographs showing quadrangular stem shape and numerous non-glandular (ng) trichomes on stem surface **(31-32.).** Cross sections observed by LM showed the presence of capitate trichomes IC **(33.)** as well **as** stem structure **(34.). Figs**. **35-37.** *S. jurisicii* Košanin stem cross sections showing quadrangular stem shape and the presence of numerous, long non-glandular trichomes **(35.).** Stem structure was observed by LM **(36-37.). Figs**. **38-41.** *S. ringens* Sibth. & Sm.stem cross section. Stems are round and without non-glandular trichomes (SEM) **(38-39.).** Stem structure was observed by LM **(40-41.)** showed the presence of capitate trichomes IA **(41.).**



Figures. **42-55.** Morphological and micromorphological properties of examined *Salvia* L. species floral parts. **Figs. 42-44.** *S. amplexicaulis* Lam. calyx. **42.** Calyx appearance (stereomicroscope) (10x), **43-44.** SEM micrographs showing an adaxial side of the calyx with peltate (P) and non-glandular trichomes (ng); **Figs. 45-47.** *S. jurisicii* Košanin calyx. **42.** Calyx appearance (stereomicroscope) (15x), **46-47.** SEM micrographs showing the adaxial side of the calyx with peltate (P) trichomes; **Figs. 48-55.** *S. ringens* Sibth. & Sm. floral parts. **48-49.** The appearance of intact **(48.)** and opened flower (49**.); 50.** Calyx appearance (stereomicroscope) (7.5x), **51-55.** SEM micrographs showing diferent subtypes of capitate trichomes II on pedicel **(51-52.)** and corolla **(54-55.); 51.** Capitate trichomes IIA, **52.** Capitate trichomes IIB, **53.** Capitate trichomes IIC, **54.** Capitate trichomes IID, **55.** Capitate trichomes IIE.



Figures. **56-73.** Morphological and micromorphological properties of examined *Salvia* L. species nutlets and mucilage. **Figs. 56-61.** *S. amplexicaulis* Lam. nutlets and mucilage. **56-57.** Nutlet appearance (stereomicroscope): **56.** 20x, **57.** 100x; **58-59.** SEM micrographs showing whole nutlet **(58.)** and surface ornamentation **(59.); Fig. 60.** Nutlets forming mucilage (stereomicroscope) (40x); **Fig. 61.** Mucilage appearance with clearly visible fibrils; **Figs. 62-67.** *S. jurisicii* Košanin nutlets and mucilage. **62-63.** Nutlet appearance (stereomicroscope): **62.** 20x, **63.** 100x; **64-65.** SEM micrographs showing whole nutlet **(64.)** and surface ornamentation **(65.); Fig. 66.** Nutlets forming mucilage (stereomicroscope) (40x); **Fig. 67.** Mucilage appearance with clearly visible fibrils. **Figs. 68-73.** *S. ringens* Sibth. & Sm. nutlets and mucilage. **68-69.** Nutlet appearance (stereomicroscope): **68.** 20x, **69.** 100x; **70-71.** SEM micrographs showing whole nutlet **(70.)** and surface ornamentation **(71.); Fig. 72.** Nutlets forming mucilage (stereomicroscope) (20x); **Fig. 73.** Mucilage appearance without fibrils.



Figures. **74-85.** Cross sections of examined *Salvia* L. species nutlets. **Figs. 74-77.** *S. amplexicaulis* Lam. nutlets. **74-75.** SEM micrographs showing manual cross sections of a nutlet; **76-77.** Cross sections observed by LM showing structure of nutlet; **Figs. 78-81.** *S. jurisicii* Košanin nutlets. **78-79.** SEM micrographs showing a manual cross sections of nutlet; **80-81.** Cross sections observed by LM showing structure of nutlet; **Figs. 82-85.** *S. ringens* Sibth. & Sm. nutlets. **82-83.** SEM micrographs showing manual cross section of nutlet; **84-85.** Cross sections observed by LM showing structure of nutlet.

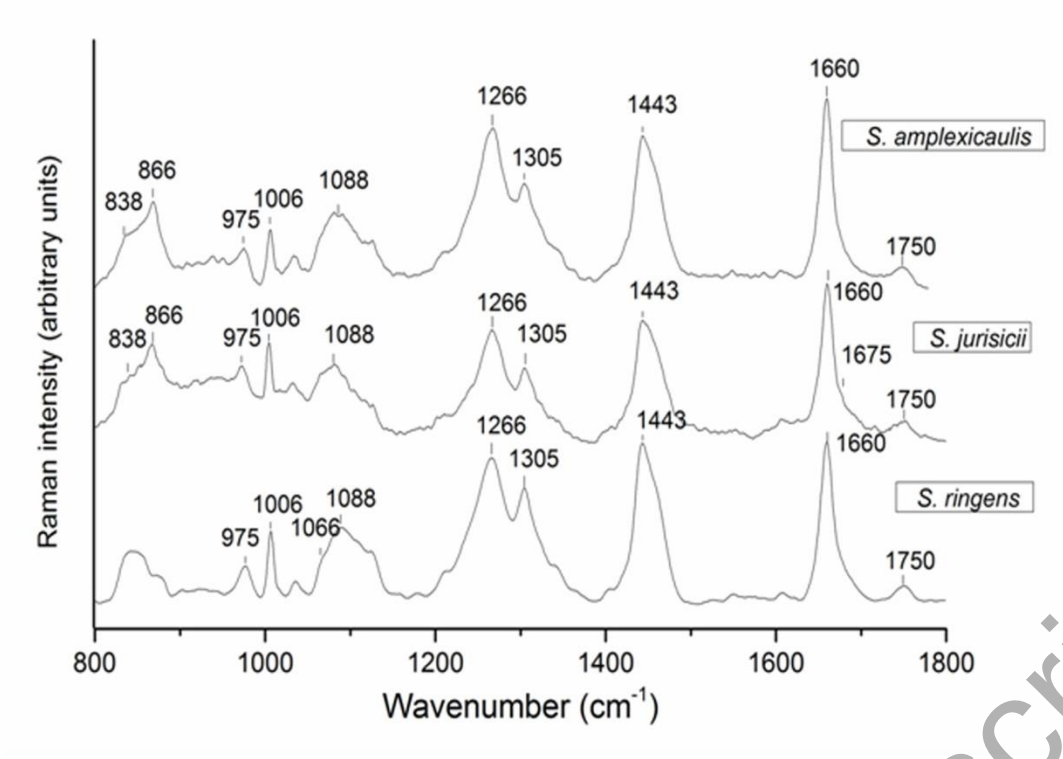


Figure 86. Raman spectra of *S. amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm. nutlets in the spectral range from 800 to 1800 cm^{-1}.

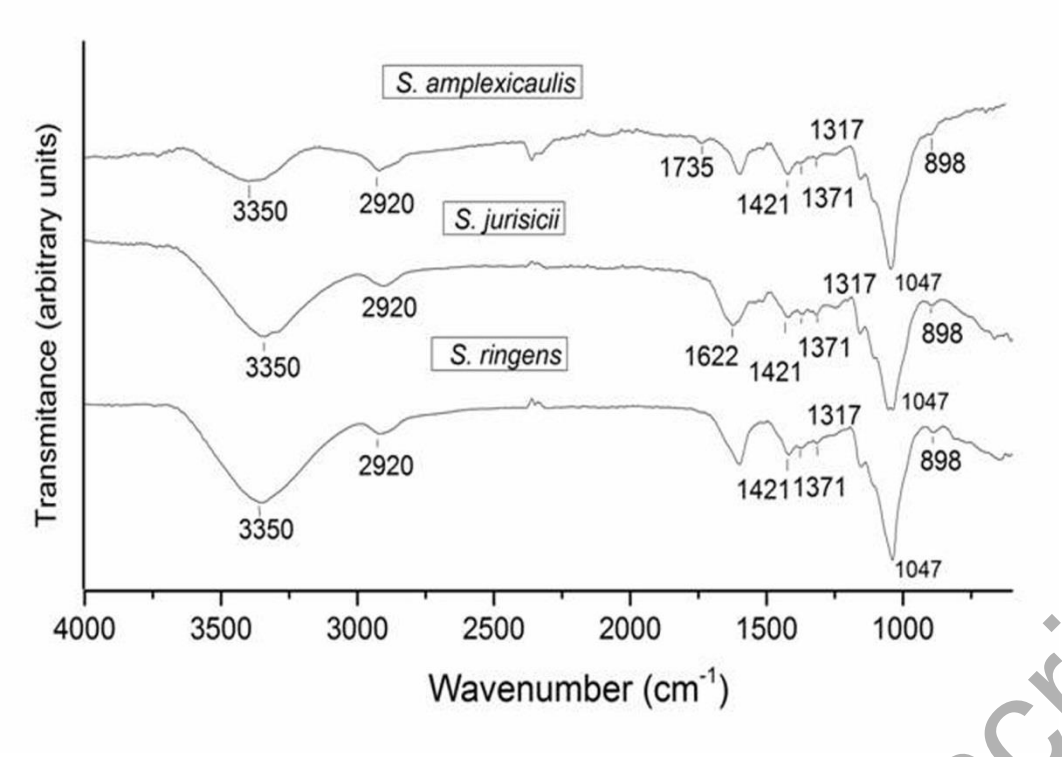


Figure 87. Fourier transform infrared (FT-IR) spectra of *S. amplexicaulis* Lam., *S. jurisicii* Košanin and *S. ringens* Sibth. & Sm. mucilage, collected at mid-infrared region (4000–600 cm^{-1}).

Table I. Nutlet characteristics of examined *Salvia* species.

		Species		
		S. amplexicaulis	***S. jurisicii***	***S. ringens***
Nutlet characteristics*	**Length (mm)**	1.97±0.07	2.34±0.15	3.08±0.16
	Width (mm)	1.05±0.09	1.61±0.15	2.27±0.16
	Length/width ratio	1.88	1.45	1.34
	Shape	prolate-spheroidal	spheroidal	spheroidal
	Colour	pale brown	pale brown	dark brown
	Abscission scar shape	triangular	round	round
	Trichomes	-	-	-
	Ornamentation	reticulate	reticulate	reticulate
Mucilage	**Layer width**	≥0.5 mm	≥0.5 mm	≥0.5 mm
	Period of production	15 min	15 min	45 min
	Colour	yellowish	yellowish	yellowish
	Transparency	transparent	transparent	transparent
	Fibrils	+	+	-

*N=10