UDK 633.8
Naučni rad – Scientific paper

Datum prijema: 25.5.2023.
Datum odobrenja: 22.6.2023.
Doi broj: 10.5937/actaherb2301043A

Microscopic and spectroscopic characterization of nutlets and mucilage of *Ocimum basilicum* and *Thymus vulgaris*

Ana Alimpić Aradski[1], Ilinka Pećinar[2], Smiljana Janković[1], Petar D. Marin[1], Dejan Pljevljakušić[3], Katarina Šavikin[3], Sonja Duletić-Laušević[1]

[1]University of Belgrade - Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Studentski trg 16, 11000 Belgrade, Serbia
[2]University of Belgrade, Faculty of Agriculture, Department of Agrobotany, Nemanjina 6, 11000 Belgrade, Serbia
[3]Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia
e-mail: alimpic.ana@bio.bg.ac.rs

SUMMARY

Ocimum basilicum L. (basil) and *Thymus vulgaris* L. (thyme) are well-known medicinal plants from the *Lamiaceae* family. Although the micromorphological characteristics and mucilage production of basil and thyme fruits have already been partially studied, the aim of this work was to examine the samples collected in Serbia. The results obtained by stereomicroscope and scanning electron microscopy showed that the nutlets of these species differ in size, shape, abscission scar, and ornamentation pattern. Although the nutlets of both species produced a milky opaque mucilage with fibrils, the basil nutlets showed a faster and more abundant mucilage reaction. Also, this is the first report on the evaluation of the chemical composition of nutlets and mucilage of two *Lamiaceae* species grown in Serbia using Raman microspectroscopy. Some differences were found between the investigated species with regard to the chemical profile of both nutlets and mucilage. The differences between the nutlets are mainly determined by the content of phenolic compounds, fatty acids, and proteins. The examined nutlets are a good

sources of phenols, unsaturated fatty acids, and polysaccharides. Raman spectroscopy seems to be a suitable technique for the analysis of this type of samples, as it allows the identification and spatial distribution of the plant metabolites.
***Keywords*:** *Thymus, Ocimum*, *Lamiaceae*, nutlets, mucilage, micromorphology, spectroscopy.

INTRODUCTION

Lamiaceae (*Labiatae*), one of the largest families of flowering plants with a cosmopolitan distribution, includes over 7.000 species. The species belonging to the subfamily Nepetoideae are generally aromatic and are classified in the tribes *Menthae*, *Elsholtzieae*, and *Ocimeae* (Zhao et al., 2021). The tribe *Menthae* consists of 65 genera and more than 2.000 species, many of which are of great economic importance (Moon et al., 2009). One of them, *Thymus vulgaris* (thyme or garden thyme), is known for its characteristic aroma and great pharmacological potential (Jain and Choudhary, 2022). On the other hand, *Ocimum basilicum* (basil or sweet basil), classified in the tribe *Ocimeae*, is known in the traditional medicine of many people, especially in Ayurveda and Unani (Purushothaman et al., 2018).

Th. vulgaris L. is an aromatic perennial plant that reaches a height of 20-30 cm. This species is native to southern Europe, i.e. the Mediterranean region, and is cultivated worldwide as a culinary herb (Diklić, 1974). Thyme leaves are highly aromatic and are therefore often used fresh or dried for seasoning and for making herbal teas (Jain and Choudhary, 2022). *O. basilicum* L. is a herbaceous plant that reaches a height of 30-100 cm, depending on the variety and conditions. Basil is native to India and other tropical areas of Asia and Africa, as well as Africa, Central, and South America (Diklić, 1974). The plant is cultivated worldwide as a culinary herb and also has a number of pharmacological effects (Purushothaman et al., 2018).

The specific fruit of the *Lamiaceae* species, called mericarpium, consists of four one-seeded nutlets arising from two carpels. The species of the subfamily Nepetoideae are characterized by myxocarpy (the phenomenon of mucilage formation when the nutlets are wetted), which could be influenced by environmental conditions such as drought (Ryding, 2010; Moon et al., 2009). It plays an important role in the regulation of germination, adhesion to the ground, or in promoting dispersal (Kreitschitz and Gorb, 2018). The mucilage is composed of all of the typical cell wall polysaccharides (cellulose, pectins, hemicelluloses), but its structural organization remains unclear (Kreitschitz and Gorb, 2018). As pointed out by several authors (Marin, 1996; Duletić-Laušević and Marin, 1999; Moon et al., 2009), nutlet morphology (length, width, shape, color, surface ornamentation, the shape of nutlet base and apex, the shape and position of abscission scare, etc.) and mucilage features (amount, time of formation, transparency, color, fibrils presence, etc.) are a useful tool in taxonomic studies of the family *Lamiaceae*.

The nutlets of *Th. vulgaris* are small, 0.9 mm long and 0.8 mm wide, yellowish to brown and broadly elliptical in shape. Marin (1996) stated that *Thymus* species have very small nutlets (0.4-1.0 mm) and that the mass of the nutlet of *Th. vulgaris* is 0.3 mg. Moon et al. (2009) noted that the nutlets of *Thymus* species have an ornamental pattern characterized by the round

arrangement of the cells. Marin (1996) noted that oval/round, clearly differentiated cells were only found in *Thymus zygis* L. nutlets. Duletić-Laušević and Marin (1999) pointed out that *Th. vulgaris* has a strong mucilage reaction. The nutlets of *O. basilicum* are oval and smooth (Diklić, 1974). The pattern of ornamentation of the nutlets of *O. basilicum* is irregular and does not show any particular microstructures as observed in the other *Lamiaceae* taxa (Marin, 1996).

Basil mericarps have been reported to be a rich source of protein, fibers, and fatty acids, especially linoleic and linolenic acid, minerals, and phenolic compounds, making it a novel food and functional ingredient with beneficial properties (Bravo et al., 2021). The chemical composition of thyme nutlets showed the presence of proteins, lipids, and crude fibers, while the extracts contained tannins, alkaloids, phenols, saponins, etc., and showed antimicrobial effects (Abbas et al., 2011).

The aim of this study was to investigate the micromorphology of the nutlets of *Thymus vulgaris* and *Ocimum basilicum* grown in Serbia. Since the positive nutritional and health effects of the nutlets of both species are well known in the literature, this study was also aimed to investigate the chemical composition for the first time the chemical composition of the nutlets and mucilage of the samples from Serbia using Raman spectroscopy.

MATERIAL AND METHODS

Plant material. The plant material of *O. basilicum* and *Th. vulgaris* was obtained from the collection of the Institute for Medicinal Plant Research „Dr. Josif Pančić". The plants were collected at the fruiting stage, air-dried, and stored in paper bags until further processing. Immediately before analysis, the nutlets are separated from the flower parts.

Stereomicroscopic analyses. The nutlets of the studied species were examined and photographed with a Nikon SMZ18 stereomicroscope (Tokyo, Japan) without prior preparation. In addition, the length and width of the nutlets (N= 10) were measured using DIGIMIZER 4.3.4 and expressed as mean ± standard deviation. The intensity of myxocarpy was monitored for 8 hours after wetting and the nutlets were photographed with LEICA DMLS. In the first hour, myxocarpy was recorded at 15-minute intervals, while further changes were recorded at 60-minute intervals. After recording the intensity of mucilage formation, the nutlets of both species were moistened and the fully formed mucilage was individually stained by adding a drop of 0.05% ruthenium red and 0.05% methylene blue.

Scanning electron microscopy (SEM) analysis. For the SEM analysis, the nutlets of *O. basilicum* and *Th. vulgaris* were coated with a thin gold layerfor 100 seconds at 30 mA (BALTEC SCD 005 Sputter Coater), and then observed and photographed (JEOL JSM-6390W).

Raman measurements. Raman microspectroscopy of *O. basilicum* and *Th. vulgaris* nutlets and mucilage was done. The nutlets were longitudinally cut at room temperature and their mucilage was recorded using XploRA Raman spectrometer from Horiba Jobin Yvon. The mucilage samples were prepared according to the procedure described by Salgado-Cruz et al. (2013). Raman spectra for nutlets and mucilage were recorded using the laser at a wavelength of

532 nm equipped with a 1200 lines mm^{-1} grating, applying exposure time 20 s and scanning the sample 10 times, using a 100% filter. The spectral resolution was about 3 cm^{-1} and calibration was checked by a 520.47 cm^{-1} line of silicon. The spectral range in the interval from 200 and 1800 cm^{-1} was analyzed for nutlets from both species and from 250 to 3250 cm^{-1} for mucilage samples. Ten spectra were collected and averaged per each sample and species (Figures 4 and 5). Characteristic bands of specific functional groups were described from literature records. Raman spectra acquisitions were managed by the LabSpec software (Horiba Jobin Yvon). Raman data were analyzed by Origine Pro 8.6 software (OriginLab, Northampton, MA, USA) and were smoothed using the Savitzky–Golay method, based on 5 points.

RESULTS AND DISCUSSION

Morphological and micromorphological characteristics of the nutlets and mucilage of the studied species. The nutlets of *O. basilicum* and *Th. vulgaris* were examined with the stereomicroscope and the scanning electron microscope. All characteristics of the nutlets examined, including length, width, shape, color, abscission scar and ornamentation, are listed in table 1.

The nutlets of *O. basilicum* were larger than those of *Th. vulgaris* (almost three times as long and twice as wide). The nutlets of *Mentheae* are bilaterally symmetric, based on the position of the abscission scar, and their size varies from 0.6-4.3 mm in length (Moon et al., 2009). The nutlet dimensions of about 30 species of the Nepetoideae subfamily ranged from 0.6 mm for certain *Mentha* species to 2.7 mm for *Hyssopus officinalis* (Duletić-Laušević and Marin, 1999). Fully mature nutlets in *Mentheae* are usually brown, except for the genus *Salvia*, which has grey and yellow nutlets in several species (Moon et al., 2009). According to the classification of length/width ratio proposed by Clopton (2004), the nutlets of *O. basilicum* were ovoid, and those of *Th. vulgaris* were broadly ovoid (Table 1, Figures 1 and 2).

Most often the abscission scar is located either at the center of the basal end or slightly shifted towards the ventral side; without an extended area in Salvinae or with the U- or V-shaped extended area at the ventral side (Moon et al., 2009). According to figures 1 and 2, the species studied also differ in terms of the shape and position of the abscission scar. The abscission scar on the nutlets of *Th. vulgaris* (tribe *Mentheae*) was round and located on the ventral side, just below the nutlet base (Figure 1d and 1f, Figure 2d), which is in accordance with the findings of Moon et al. (2009) for other *Mentheae* taxa. On the contrary, the nutlets of *O. basilicum* had an irregularly shaped abscission scar, which was located exactly at the base of the nutlet (Figure 1b and 1c, Figure 2a).

Table 1. Morphological and micromorphological characteristics of the *Ocimum basilicum* and *Thymus vulgaris* nutlets

Tabela 1. Morfološke i mikromorfološke karakteristike orašica *Ocimum basilicum* i *Thymus vulgaris*

Species **Vrsta**	**Nutlets characteristics*** **Karakteristike orašica**						
	Length (mm)	Width (mm)	L/W ratio	Shape	Color	Abscission scar	Ornamentation**
O. basilicum	2.38±0.09	1.36±0.08	1.76	ovoid	brown	irregular	secondary - striae
Th. vulgaris	0.88±0.04	0.73±0.03	1.20	wide ovoid	dark brown	ovoid	primary-rounded cell arrangement

*N= 10; **Ornamentation is determined according to Moon et al. (2009)

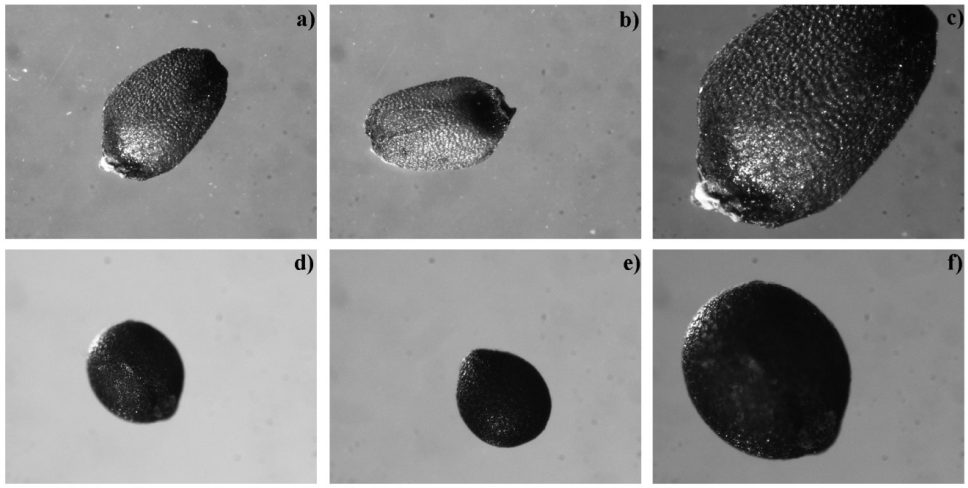

Figure 1. Nutlet appearance (stereomicroscope) of *O. basilicum* (a, b, c) and *Thymus vulgaris* (d, e, f) (magnification: a, b – 40x, c – 80x, d, e – 60x, f – 135x). The ventral side of *O. basilicum* nutlets with the irregularly shaped abscission scar is shown in the (b), while round abscission scar of *Th. vulgaris* is visible in the (d). The appearance of the dorsal side of *O. basilicum* (a, c) and *Th. vulgaris* (e) could be observed; (c) and (f) also showed the color and surface of the nutlets

Slika 1. Izgled orašica (binokularna lupa) *O. basilicum* (a, b, c) i *Thymus vulgaris* (d, e, f) (uvećanja: a, b – 40x, c – 80x, d, e – 60x, f – 135x). Ventralna strana orašica *O. basilicum* sa stopalnim ožiljkom nepravilnog oblika je prikazana na (b), dok je okrugao stopalni ožiljak orašica *Th. vulgaris* uočljiv na (d). Prikazan je i izgled dorzalne strane orašica *O. basilicum* (a, c) i *Th. vulgaris* (e); slike (c) i (f) takođe pokazuju boju i izgled površine orašica

The ornamentation of the nutlets of *O. basilicum* was characterized by the presence of secondary sculpturing elements, such as striae (Figures 2b and 2c). This ornamentation type was previously identified in some species of the genera *Salvia*, *Clinopodium*, *Cyclotrichium*, etc. Other species of the tribe *Mentheae* commonly possessed papillae or both striae and papillae as secondary surface elements (Moon et al., 2009). On the other hand, the nutlets of *Th. vulgaris* had a primary ornamentation pattern (Figures

2e and 2f) which is mostly recognized as a type E according to Moon et al. (2009). These authors defined that type E includes a rounded cell arrangement; each cell is rather flat and the cell boundary is slightly thick. Similarly, Marin (1996) indicated that certain *Thymus* species, such as *Th. zygis* had a round arrangement of exocarp cells. Both species lacked trichomes on the nutlets, which is consistent with the findings of Duletić-Laušević and Marin (1999) for the majority of the examined species of the *Nepetoideae* subfamily. These authors also pointed out that hairy or glandular nutlets do not show extensive mucilage production, so we expected the absence of trichomes in thyme and basil nutlets to correlate with an extensive mucilage reaction.

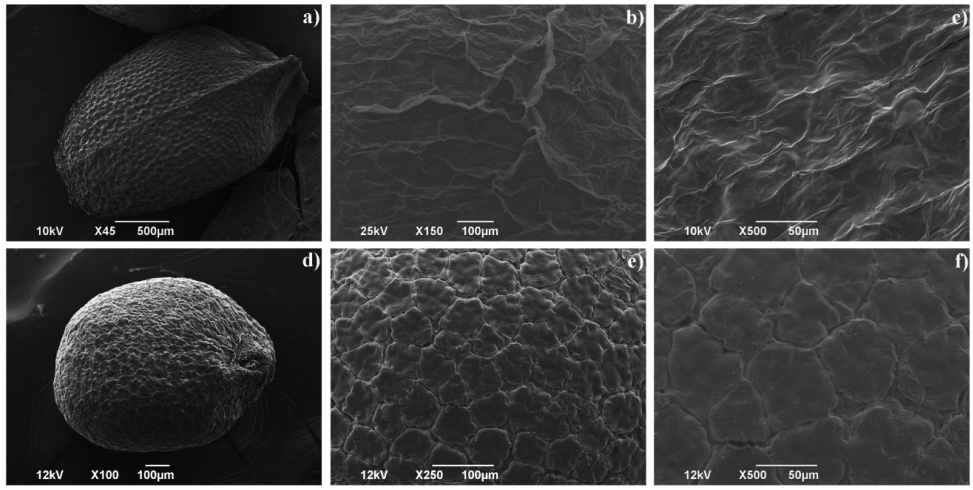

Figure 2. Nutlet appearance and ornamentation (SEM micrographs); *Ocimum basilicum* (a, b, c) and *Thymus vulgaris* (d, e, f). In the dorsal side of nutlets of both species, an abscission scar is distinguished (a, d). The surface sculpturing pattern is visible on the magnification of 150x and 250x (b, e), and 500x (c, f)
Slika 2. Izgled orašica i ornamentacija (SEM mikrografije); *Ocimum basilicum* (a, b, c) i *Thymus vulgaris* (d, e, f). Na dorzalnoj strani orašica obe vrste jasno je uočljiv stopalni ožiljak (a, d). Obrazac skulpturiranosti površine je vidljiv na uvećanjima od 150x i 250x (b, e), kao i 500x (c, f)

Duletić-Laušević and Marin (1999) found that the nutlets of most Nepetoideae species showed mucilage production upon wetting, although the reaction varies in intensity. The nutlets of both species showed a very fast reaction of myxocarpy, forming the maximum amount of mucilage 15 minutes after wetting. The nutlets of *O. basilicum* formed a mucilage layer four times thicker than *Th. vulgaris* (Table 2). According to Duletić-Laušević and Marin (1999), the mucilage layer >0.5 mm (*O. basilicum*) indicates a strong reaction, and 0.1-0.5 mm a moderately strong reaction (*Th. vulgaris*). Both species produced non-transparent, milky opaque mucilages with fibrils present (Table 2, Figure 3a and 3d). The mucilaginous cells are located in the exocarp of *Lamiaceae* species' fruit, and their cell walls contain spiral fibrils (Ryding, 1992; Duletić-Laušević and Marin, 1999; Ryding, 2010). The cellulose fibrils are attached to the surface of the nutlet, preventing the loss of the mucilage layer (Kreitschitz and Gorb, 2018).

Table 2. Morphological characteristics of *Ocimum basilicum* and *Thymus vulgaris* mucilage
Tabela 2. Morfološke karakteristike sluzi *Ocimum basilicum* i *Thymus vulgaris*

Species Vrsta	Mucilage characteristics* Karakteristike sluzi					
	Production time	**Mucilage width**	**Mucilage reaction intensity**	**Color**	**Transparency**	**Fibrils presence**
O. basilicum	15 min	1.04 mm	strong reaction	milky opaque	non-transparent	present
Th. vulgaris	15 min	0.25 mm	moderately strong reaction	milky opaque	non-transparent	present

*N= 10

The addition of ruthenium red (staining of pectins) and methylene blue (staining of cellulose) showed that the mucilage of *Th. vulgaris* consists of a pectin matrix and cellulose fibrils (Figures 3e and 3f). Ryding (2010) pointed out that mucilaginous cells present in the pericarp of tribe *Mentheae* species were colorless and contained globose bodies without starch as was confirmed in our study.

Myxocarpy was found in about 80% of the species of the tribe *Ocimeae*. The mucilage was often colorless, with spiral cellulosic fibrils (Ryding, 1992). The mucilage of *O. basilicum* contained pectins, fibrils, and distinguishable starch grains (Figures 3b and 3c). Our findings

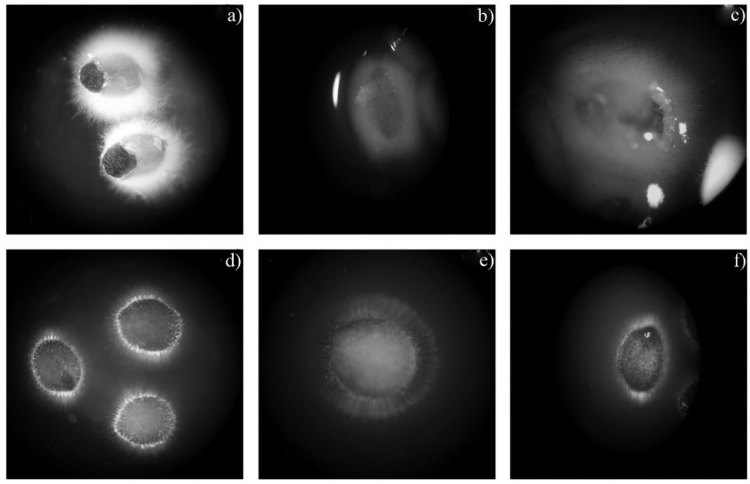

Figure 3. Mucilage of *O. basilicum* (a, b, c) and *Th. vulgaris* (d, e, f); *O. basilicum* (16x), intact mucilage after 15 minutes (a); mucilage of *O. basilicum* (32x) stained by ruthenium red (b); mucilage of *O. basilicum* (40x) stained by methylene blue (c); *Th. vulgaris* (40x), intact mucilage after 15 minutes (d); mucilage of *Th. vulgaris* (50x) stained by ruthenium red (e); mucilage of *Th. vulgaris* (40x) stained by methylene blue (f)
Slika 3. Sluz *O. basilicum* (a, b, c) i *Th. vulgaris* (d, e, f); *O. basilicum* (16x), intaktna sluz formirana nakon 15 minuta (a); sluz *O. basilicum* (32x) obojena pomoću rutenijum-crvenog (b); sluz *O. basilicum* (40x) obojena pomoću metilen plavog (c); *Th. vulgaris* (40x), intaktna sluz formirana nakon 15 minuta (d); sluz *Th. vulgaris* (50x) obojena pomoću rutenijum-crvenog (e); sluz *Th. vulgaris* (40x) obojena pomoću metilen plavog (f)

are supported by the study of Ryding (1992), who reported that spherical starch grains have been found in the mucilaginous cells of several probably unrelated genera of tribe Ocimeae, including *Ocimum* spp. Kreitschitz and Gorb (2018) reported that the mucilage of *O. basilicum* nutlets is covered by round starch grains spread as protrusions under the mucilaginous layer.

Raman signature of nutlets and mucilage. The nutlets from *O. basilicum* showed a high content of lipids (33%) and carbohydrates (43%) and a low protein content (10%) (Nazir et al., 2017). Previous results on the chromatographic profile of *O. basilicum* nutlet samples (Angers et al., 1996), indicated the predominance of unsaturated fatty acids and were verified with the major fatty acids being linolenic (up to 64.8%) and linoleic acid (up to 31.3%), with lesser amounts of oleic acid (up to 13.3%). On the higher content of linolenic fatty acids in seed samples indicated many reports (Alimpić Aradski et al., 2020; Špirović-Trifunović et al., 2021). To a lesser extent, saturated acids included palmitic (up to 11.0%) and stearic acid (up to 4.0%). The only information about the chemical composition of *Thymus* spp. nutlets coming from Marin et al. (1991), was subjected only to *Th. serpyllum* as well as *O. basilicum*. According to this study, nutlets of *Th. serpyllum* have a slightly higher percentage of linolenic (63.1%) than *O. basilicum* (59.1%) and have a similar percentage of linoleic acids 21.9 (*O. basilicum*) and 20.2% (*Th. serpyllum*). Marin et al. (1991) noted a higher index of unsaturated/saturated acids in *O. basilicum* (59.1) compared to *Th. serpyllum* (12.7) and a higher ratio between linolenic and linoleic acids in *Th. serpyllum*.

Using Raman spectroscopy, as a fast and nondestructive method, we demonstrate for the first time application of Raman microspectroscopy for the analysis of fresh nutlet samples of *O. basilicum* and *Th. vulgaris*. The Raman spectra exhibit several intensive bands, indicating specific nutlet constituents, with predominant bands of fatty acids arising from vibrations of the hydrocarbon chains (Figure 4). The assignments of the main spectral features are presented in supplementary table.

These results showed that there are two distinct spectral patterns ascribed to unsaturated (α linolenic, linoleic, and oleic) and saturated (palmitic and stearic) fatty acids. The medium intensity bands were responsible for the saturated and unsaturated fatty acids observed at 1439 and 1299 cm^{-1} (Figure 4), assigned to the CH_2 scissoring deformation vibration and C-H bending, respectively (Baranski et al., 2006; Fei et al., 2017). In the Raman spectra of α-linolenic and linoleic acid, as predominant acids in both species, (Baeten et al., 2001; Lv et al., 2016) bonds with higher wavenumbers are marker features for the presence of double bonds (Martini et al., 2018). These acids differ mainly in the position of the double bonds, consequently, their Raman spectra are highly similar (De Gelder et al., 2007). The bands at 1652 and 1261 cm^{-1} (Figure 4), could be assigned to the cis stretching vibration of C=C and the bending of C-H, respectively, all involving the unsaturation moieties of unsaturated fatty acids cis isomers, which relative intensity is in accordance with the level of unsaturation, especially in a case of the band at 1652 cm^{-1} (Martini et al., 2018).

The degree of fatty acid unsaturation can be also estimated from the peak area of the bands at 1261 and 1299 cm^{-1}, which are due to in-phase C=C symmetric rocking and methylene twisting vibration, respectively (Baranski et al., 2006). The previous Raman studies of fatty

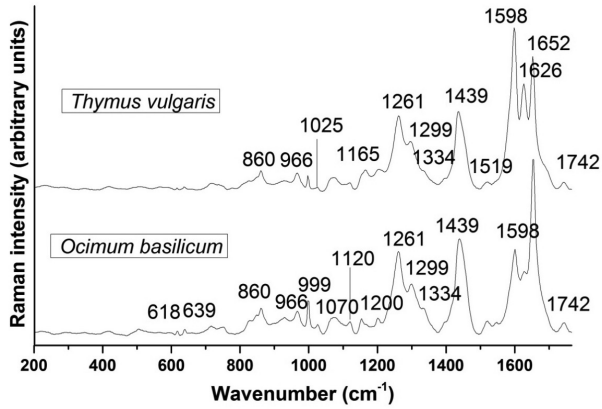

Figure 4. Raman spectra of *Ocimum basilicum* and *Thymus vulgaris* nutlets in the endosperm region, spectral range from 200 to 1750 cm^{-1}

Slika 4. Ramanovi spektri orašica *Ocimum basilicum* i *Thymus vulgaris* u regionu endosperma, spektralni opseg od 200 do 1750 cm^{-1}

acids found that the bands in the region from 800-900 and 1000-1150 cm^{-1} indicated on presence of C-C stretching bonds (Lv et al., 2016; Fei et al., 2017; Martini et al., 2018), such as the bands at 860 and 1025 cm^{-1} (Figure 4 and Supplementary Table). The band at 1742 cm^{-1} was assigned to the stretching of C=O from triacylglycerol structure that was present in all Raman spectra of different plant oil samples (Martini et al., 2018).

The highest intensity signals seen in the region from 1595-1630 cm^{-1} could be attributed to lignin from pericarp cell wall compounds. In this range, lignin gives a doublet band, with one maximum at 1598 cm^{-1} (aromatic vibrations) and a peak of lower intensity at 1626 cm^{-1} (Baranski et al., 2006). Higher intensity of the bands at 1598 and 1626 cm^{-1} observed for *Th. vulgaris* could suggest that they contain higher lignin content than for *O. basilicum*. The lower and sharp intensity band at 999 cm^{-1} could attributed to the presence of protein fraction in nutlet samples, probably to phenylalanine (Li-Chan, 1996) while the intensity of the band in *O. basilicum* could be infuenced by the slight increase in proteins.

Raman spectroscopy of mucilage. The chemical composition of *O. basilicum* mucilage arises from a total carbohydrate (up to 80%), fats (4-11.55%), starch (1.5%), proteins (1.3-2%) and soluble sugars (0.6%) (Razavi et al., 2009; Hosseini-Parvar et al., 2010). Previously, several studies of the mucilage polysaccharides extracted from *O. basilicum* nutlets (Anjaneyalu and Dowda, 1979; Hosseini-Parvar et al., 2010) have reported the highest presence of glucomannan, (1,4)-linked xylan and a minor present of glucan. Azuma and Sakamoto (2003) also reported the presence of highly branched arabinogalactan in addition to glucomannan and (1,4)-linked xylan. According to staining reactions of the mucilage envelope in *O. basilicum*, it was shown that mucilage was comprised of starch grains, cellulose, and pectin (Kreitschitz and Gorb, 2018). There is no published results about chemical composition based on the carbohydrate

nature of *Th. vulgaris* mucilage, as we know, only about protein content and lipid (lecitin) activity of mucilage (Fernández-Alonso et al., 2003). Raman spectra of *O. basilicum* and *Th. vulgaris* nutlet mucilage are shown in figure 5. The bands in the region 1100–1460 cm^{-1} could be primarily due to the glucose as well as hemicellulosic polysaccharides, *e.g.* glucomannan and xylan (Himmelsbach and Akin, 1998; Salgado-Cruz et al., 2013). The bands at 1457 and 1333 cm^{-1} are due to coupled vibrations of hydrogen atoms corresponding to CH, CH_2, and COH from the glucose as well as in the xyloglucan spectrum with characteristic bands at 1376 cm^{-1} (Himmelsbach and Akin, 1998; Malekfar et al., 2010; Synytsya et al., 2003). The spectral region between 1200 and 400 cm^{-1} is very often assigned in literature as the fingerprint of the glycosidic bond (Thygesen et al., 2003; Baranska et al., 2005). The bands at 478 and 1122 cm^{-1} were observed as a marker for starch and cellulose (Himmelsbach and Akin, 1998; Chylinska et al., 2014). The vibration originating from α-1,4 glycosidic linkages can be observed at 936 cm^{-1} (Almeida et al., 2010), while the characteristic band at about 859 cm^{-1} can be considered to be a marker band for α-glycosidic bonds in pectin (Synytsya et al., 2003; Chylinska at al., 2014). Vibrational C-H stretching was observed at 2908 cm^{-1} which is highly correlated with glucose as reported by Malekfar et al. (2010).

These results support the advantages of the Raman spectroscopy in methodology and provide, for the first time, characterization of dominant polysaccharides inside mucilage from *O. basilicum* and *Th. vulgaris*, without any specific and time-consuming procedures.

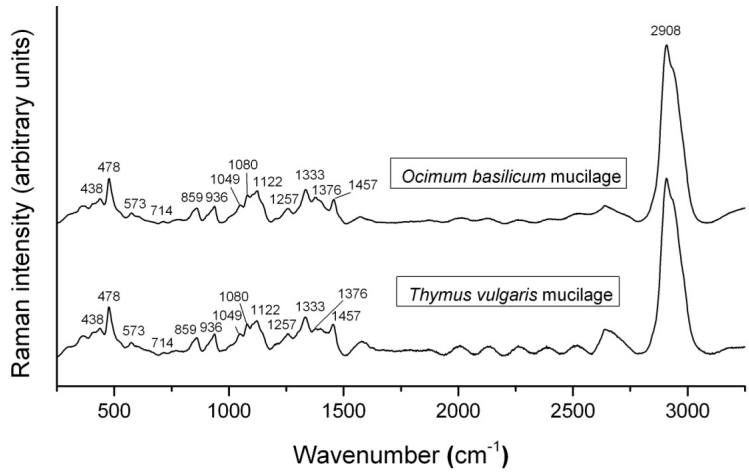

Figure 5. Raman spectra of *Ocimum basilicum* and *Thymus vulgaris* mucilage in the spectral range from 250 to 3250 cm^{-1}

Slika 5. Ramanovi spektri sluzi *Ocimum basilicum* i *Thymus vulgaris* u spektralnom opsegu od 250 do 3250 cm^{-1}

CONCLUSION

Thyme and basil are well-known medicinal plants from the *Lamiaceae* family of great economic importance. In contrast to the aerial parts, their nutlets were scarcely studied, so this work aimed to provide data on the micromorphology and chemical composition of the nutlets and their mucilages. Differences were found in the morphological characteristics as well as in the surface ornamentation of the nutlets of the species studied. Mucilage production was faster and more extensive in basil, but the properties of the mucilages were quite similar in both species. The mucilage was composed of a pectine matrix and cellulose fibrils, with additional starch grains visible in basil. As far as we know, there is no published report on the chemical composition of nutlets of both species and the mucilages contained in them, as determined by the Raman spectroscopy. Raman spectroscope, in combination with a microscope, is a suitable technique for the analysis of this type of samples, as it allows the visual identification of the tissues before spectroscopic analysis and then the spatial distribution of the plant metabolites. It was shown that the examined nutlets are good sources of phenols, unsaturated fatty acids, and polysaccharides and that the region below 1800 cm^{-1} in the Raman spectra is most important for their characterization.

ACKNOWLEDGMENT

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia [Grant No. 451-03-47/2023-01/200178, 451-03-47/2023-01/200003, 451-03-47/2023-01/200216].

REFERENCES

Abbas, A. J., Mousa, M. A., Al-aubadi, I. K.: Chemical composition of thyme seeds *Thymus vulgaris* and its antimicrobial activity. Anbar Journal of Agricultural Sciences, 9 (2), 2011.

Angers, P., Morales, M. R., Simon, J. E.: Fatty acid variation in seed oil among *Ocimum* Species. American Oil Chemists' Society, 73 (3), 393-395, 1996.

Anjaneyalu, Y. V., Gowda, D. C.: Structural studies of an acidic polysaccharide from *Ocimum basilicum* seeds. Carbohydrate Research, 75, 251-256, 1979.

Azuma, J. I., Sakamoto, M.: Cellulosic hydrocolloid system present in seed of plants. Trends in Glycoscience and Glycotechnology, 15 (81), 1-14, 2003.

Baeten, V., Dardenne, P., Aparicio, R.: Interpretation of fourier transform raman spectra of the unsaponifiable matter in a selection of edible oils. Journal of Agricultural and Food Chemistry, 49, 5098-5107, 2001.

Baranska, M., Schulz, H., Baranski, R., Nothnagel, T., Christensen, L. P.: In situ simultaneous analysis of polyacetylenes, carotenoids and polysaccharides in carrot roots. Journal of Agricultural and Food Chemistry, 53 (17), 6565-6571, 2005.

Baranski, R., Baranska, M., Schulz, H., Simon, P. W., Nothnagel, T.: Single seed raman measurements allow taxonomical discrimination of *Apiaceae* accessions collected in gene banks. Biopolymers, 81 (6), 497-505, 2006.

Calderón Bravo, H., Vera Céspedes, N., Zura-Bravo, L., Muñoz, L. A.: Basil seeds as a novel food, source of nutrients and functional ingredients with beneficial properties: a review. Foods, 10 (7), 1467, 2021.

Chylińska, M., Szymańska-Chargot, M., Zdunek, A.: Imaging of polysaccharides in the tomato cell wall with Raman microspectroscopy. Plant Methods, 10 (1), 1-9, 2014.

Clopton, R. E.: Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. Comparative Parasitology, 71 (2), 130-140, 2004.

Czamara, K., Majzner, K., Pacia, M. Z., Kochan, K., Kaczor, A., Baranska, M.: Raman spectroscopy of lipids: a review. Journal of Raman Spectroscopy, 46 (1), 4-20, 2014.

de Gelder, J., de Gussem, K., Vandenabeele, P., Moens, L.: Reference database of Raman spectra of biological molecules. Journal of Raman Spectroscopy, 38, 1133-1147, 2007.

Diklić, N.: Flora SR Srbije, Vol VI: SANU, Beograd, 1974.

Duletic-Lausevic, S., Marin P. D.: Pericarp structure and myxocarpy in selected genera of *Nepetoideae* (*Lamiaceae*). Nordic Journal of Botany, 19 (4), 435-446, 1999.

Fei, G., Lujia, H., Xian, L.: Vibration spectroscopic technique for species identification based on lipid characteristics. International Journal of Agricultural and Biological Engineering, 10 (3), 255-268, 2017.

Fernández-Alonso, J. L., Vega, N., Filgueira, J. J., Pérez, G.: Lectin prospecting in Colombian Labiatae. A systematic-ecological approach. Biochemical Systematic and Ecology, 31 (6), 617-633, 2003.

Himmelsbach, D. S., Akin, D. E.: Near-infrared–Fourier-transform–Raman spectroscopy of flax (*Linum usitatissimum* L.) stems. Journal of Agricultural and Food Chemistry, 46, 991-998, 1998.

Hosseini-Parvar, S. H., Matia-Merino, L., Goh, K. K. T., Razavi, S. M. A., Mortazavi, S. A.: Steady shear flow behavior of gum extracted from *Ocimum basilicum* L. seed: Effect of concentration and temperature. Journal of Food Engineering, 101, 236-243, 2010.

Jain, N., Choudhary, P.: Phytochemistry, traditional uses and pharmacological aspect of *Thymus vulgaris*: a review. Indian Journal of Pharmaceutical Sciences, 84 (6), 1369-1379, 2022.

Kreitschitz, A., Gorb, S. N.: The micro-and nanoscale spatial architecture of the seed mucilage - Comparative study of selected plant species. PloS one, 13 (7), e0200522, 2018.

Li-Chan, E. C. Y.: The applications of Raman spectroscopy in food science. Trends in Food Science and Technology, 7, 361-370, 1996a.

Lv, M. Y., Zhang, X., Ren, H. R., Liu, L., Zhao, Y. M., Wang, Z. et al.: A rapid method to authenticate vegetable oils through surface-enhanced Raman scattering. Scientific Reports, 6, 23405, 2016.

Malekfar, R., Nikbakht, A. M., Abbasian, S., Sadeghiand, F., Mozaffari, M.: Evaluation of tomato juice quality using surface enhanced Raman spectroscopy. Acta Physica Polonica A, 117 (6), 971-973, 2010.

Marin, P. D., Sajdl, V., Kapor, S., Tatic, B., Petkovic, B.: Fatty acids of the *Saturejoideae*, *Ajugoideae* and *Scutellarioideae* (*Lamiaceae*). Phytochemistry, 30 (9), 2979-2982, 1991.

Marin, P. D.: Nutlets and trichomes in the *Lamiaceae* family.University of Belgrade, Faculty of Belgrade, Belgrade, 1996.

Martini, W. S., Porto, B. L. S., de Oliveira M. A. L., Sant'Ana, A. C.: Comparative study of the lipid profiles of oils from kernels of peanut, babassu, coconut, castor and grape by GC-FID and Raman spectroscopy. Journal of Brazilian Chemical Society, 29 (2), 390-397, 2018.

Moon, H. K., Hong, S. P., Smets, E., Huysmans, S.: Micromorphology and character evolution of nutlets in tribe *Mentheae* (*Nepetoideae*, *Lamiaceae*). Systematic botany, 34 (4), 760-776, 2009.

Muik, B., Lendl, B., Molina-Díaz, A., Ayora-Cañada M. J.: Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy. Chemistry and Physics of Lipids, 134 (2), 173-82, 2005.

Nazir, S., Wani, I. A., Masoodi, F. A.: Extraction optimization of mucilage from Basil (*Ocimum basilicum* L.) seeds using response surface methodology. Journal of Advanced Research, 8 (3), 235-244, 2017.

Purushothaman, B., Prasanna Srinivasan, R., Suganthi, P., Ranganathan, B., Gimbun, J., Shanmugam, K.: A comprehensive review on *Ocimum basilicum*. Journal of Natural Remedies, 71-85, 2018.

Razavi, S. M., Mortazavi, S. A., Matia-Merino, L. Hosseini-Parvar, S. H., Motamedzadegan, A., Khanipour, E.: Optimisation study of gum extraction from Basil seeds (*Ocimum basilicum* L.). International Journal of Food Science and Technology, 44, 1755-1762, 2009.

Ryding, O.: Pericarp structure and phylogeny of tribe *Mentheae* (*Lamiaceae*). Plant Systematics and Evolution, 285, 165-175, 2010.

Ryding, O.: Pericarp structure and phylogeny within *Lamiaceae* subfamily *Nepetoidea*e tribe *Ocimeae*. Nordic Journal of Botany, 12 (3), 273-298, 1992.

Salgado-Cruz, M. D. L. P., Calderón-Domínguez, G., Chanona-Pérez, J., Farrera-Rebollo, R. R., Méndez-Méndez, J. V., Díaz-Ramírez, M.: Chia (*Salvia hispanica* L.) seed mucilage release characterisation. A microstructural and image analysis study. Industrial Crops and Products, 51, 453-462, 2013.

Schulz, H., Baranska, M.: Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spectroscopy, 43 (1), 13-25, 2007.

Strehle, M. A., Rösch, P., Baranska, M., Schulz, H., Popp J.: On the way to a quality control of the essential oil of fennel by means of Raman spectroscopy. Biopolymers, 77 (1), 44-52, 2005.

Synytsya, A., Copikova, J., Matejka, P., Machovic, V.: Fourier transform Raman and infrared spectroscopy of pectins. Carbohydrate Polymers, 54, 97-106, 2003.

Špirović-Trifunović, B., Nedeljković, D., Stojićević, D., Božić, D.: Masne kiseline u semenu divlje konoplje (*Cannabis sativa* L. ssp. *sativa* var. *spontanea* Vavilov). Acta herbologica, 30 (1), 65-73, 2021. doi: 10.5937/actaherb2101065Q

Thygesen, L. G., Løkke, M. M., Micklander, E., Engelsen, S. B.: Vibrational microspectroscopy of food. Raman vs. FT-IR. Trends in Food Science and Technology, 14 (1-2), 50-57, 2003.

Tobolova, M., Kresalek, V., Vaškova, H., Bučkova, M.: Investigation of the thermal degradation of vegetable oils by spectroscopic methods and its mathematical analysis. International Journal of Mathematics and Computers in Simulations, 8 (1), 213-219, 2014.

Vaškova, H., Bučkova, M.: Measuring and identification of oils. In Proceedings of the 18th International Conference on Systems (part of CSCC´14), Santorini Island, 211-215, 2014.

Vaškova, H., Bučkova, M.: Thermal degradation of vegetable oils: spectroscopic measurement and analysis. Procedia Engineering, 100, 630-635, 2015.

Weng, Y. M., Weng, R. H., Tzeng, C. Y., Chen, W.: Structural analysis of triacylglycerols and edible oils by near-infrared Fourier transform Raman spectroscopy. Applied Spectroscopy, 57 (4), 413-418, 2003.

Zhao, F., Chen, Y. P., Salmaki, Y., Drew, B. T., Wilson, T. C., Scheen, A. C. et al.: An updated tribal classification of *Lamiaceae* based on plastome phylogenomics. BMC Biology, 19, 1-27, 2021.

Mikroskopska i spektroskopska karakterizacija orašica i sluzi *Ocimum basilicum* i *Thymus vulgaris*

REZIME

Ocimum basilicum L. (bosiljak) i *Thymus vulgaris* L. (timijan) su dobro poznate lekovite biljke familije *Lamiaceae*. Iako su mikromorfološke karakteristike orašica i produkcija njihove sluzi do sada delimično proučeni, cilj ovog rada je bio analiza uzoraka iz Srbije. Rezultati dobijeni steremikroskopom i skenirajućom elektronskom mikroskopijom ukazuju da se orašice proučavanih vrsta razlikuju po obliku, veličini, stopalnom ožiljku i obrascu ornamentacije.

Iako su obe vrste produkovale mlečno belu sluz sa fibrilima, orašice bosiljka su se izdvojile po brzoj reakciji osluznjavanja, kao i produkciji veće količine sluzi. Takođe, ovo je prvi izveštaj o hemijskom sastavu orašica i sluzi dve vrste familije *Lamiaceae* koje su gajene u Srbiji i analizirane Ramanovom spektroskopijom. Evidentirane su pojedine razlike u hemijskom profilu orašica i sluzi među analiziranim vrstama. Razlike u sastavu orašica su uglavnom pripisane sadržaju fenolnih komponenti, masnih kiselina i proteina. Proučavane orašice predstavljaju bogat izvor fenola, nezasićenih masnih kiselina i polisaharida. Raman spektroskopija se pokazala kao veoma pogodna tehnika za analizu ovog tipa uzoraka, s obzirom da omogućava identifikaciju i ukazuje na prostorni raspored biljnih metabolita u uzorku.
Ključne reči: *Thymus, Ocimum*, *Lamiaceae*, orašice, sluz, mikromorfologija, spektroskopija.

Supplementary Table. The major assignment of Raman bands recorded from *O. basilicum* and *Th. vulgaris* nutlets
Dodatna tabela. Glavni pokazatelji Ramanovih veza snimljenih na orašicama *O. basilicum* i *Th. vulgaris*

Wavenumber (cm^{-1}) **Talasni broj**	**Vibrational modes** **Vibracije**	**Literature records** **Literaturni izvor**
860	C-C stretching vibrations	Schulz and Baranska, 2007; Lv et al., 2016; Fei et al., 2017
966	C=C waging vibration of cis double bonds	Muik et al., 2005; Fei et al., 2017
999	phenylalanine	Li-Chan, 1996
1025	C-C stretching	Czamara et al., 2014; Martini et al., 2018
1120, 1150	C-C stretching	Fei et al., 2017
1261	C=C symmetric rocking	Weng et al., 2003; Strehle et al., 2004; Schulz and Baranska, 2007; Czamara et al., 2014; Vaškova and Bučkova, 2015; Lv et al., 2016
1299	CH2 in-plane twist	Weng et al., 2003; Strehle et al., 2004; Muik et al., 2005; Schulz and Baranska, 2007; Vaškova and Bučkova, 2014, 2015; Lv et al., 2016
1439	CH2 scissoring mode of the saturated fatty acid part, methylene	Strehle et al., 2004; Muik et al., 2005; Schulz and Baranska, 2007; Czamara et al., 2014; Vaškova and Bučkova, 2014, 2015; Lv et al., 2016; Fei et al., 2017
1598	aromatic vibrations	Baranski et al., 2006
1626,1652	aromatic vibrations	Baranski et al., 2006
1742	C=O stretching	Martini et al., 2018