

Received: 11 June 2026

Revised version accepted: 10 July 2026

Doi number: 10.5937/35ah-68166

Raman microspectroscopy as a tool for analysis of essential oil constituents in glandular trichomes of *Thymus* species

Ivan Šoštarić¹ , Jelena Arsenijević² , Stefan M. Kolašinac¹ 

¹ University of Belgrade, Faculty of Agriculture, Department of Agrobotany, Nemanjina 6, 11080 Zemun, Serbia

² University of Belgrade, Faculty of Pharmacy, Department of Pharmacognosy, Vojvode Stepe 450, 11221 Belgrade, Serbia
e-mail: sostaric@agrif.bg.ac.rs

SUMMARY

Thymus is one of the largest genera in the Lamiaceae family, and all its species are used as medicinal and aromatic plants. In this research, Raman spectroscopy is used as a fast and nondestructive tool for chemotype assessment by measuring spectra directly from glandular trichomes. Samples of two *Thymus pulegioides* subsp. *pannonicus* populations were collected from Banat (Serbia), analyzed using a 785 nm laser, and compared with reference spectra of citral and thymol standards. Spectra of samples from the Vršacke planine locality showed the presence of dominant bands related to citral (around 1671 and 1633 cm⁻¹). Spectra of samples from the Devojački bunar locality displayed dominant bands at around 1640 and 1668 cm⁻¹, corresponding to $\nu(\text{C}=\text{C})$ and $\nu(\text{C}=\text{O})$ vibrations related to conjugated and oxygenated monoterpene stretching, respectively. The dominant bands of thymol were at 740, 1062, and 1262 cm⁻¹, confirming that samples from Devojački bunar are not of the thymol chemotype. GC-FID/MS analysis revealed that germacrene D is a major compound in the essential oil from this population. Additionally, PCA revealed a clear separation of the two investigated populations, demonstrating that Raman spectroscopy can be used for chemotype differentiation.

Keywords: chemotypes, citral, thymol, Raman spectroscopy.

INTRODUCTION

The genus *Thymus* L. (Lamiaceae) is among the largest genera within the family, encompassing more than 200 species (Morales, 2002). Its representatives are native to arid, temperate, and cold regions of the Old World north of the equator, with their distribution extending westward to the coasts of Greenland (Morales, 1989). Following the classification by Jalas (1971), the genus is divided into eight sections: *Micantes*, *Mastichina*, *Piperella*, *Teucrioides*, *Pseudothymbra*, *Thymus*, *Hyphodromi*, and *Serpyllum*. Section *Serpyllum* is considered the oldest and most species-rich section, comprising approximately 150 taxa distributed throughout the entire range of the genus.

In the Flora of Serbia, 31 *Thymus* species with more than 60 varieties are recorded (Diklić, 1974). They predominantly colonize diverse meadow and pasture communities, as well as dry, sun-exposed rocky habitats on both limestone and serpentine substrates. With the exception of *Th. comptus* and *Th. striatus* (section *Hyphodromi*) and the allochthonous *Th. vulgaris* (section *Thymus*), all Serbian species belong to section *Serpyllum* (Miller) Benth. *Thymus pulegioides* subsp. *pannonicus* (All.) Kerguelen, treated in the Flora of Serbia as *Th. pannonicus* All. (Diklić, 1974), is a perennial herbaceous plant widely distributed across central and eastern Europe, occurring predominantly on open dry meadows, grasslands, and rocky terrain (Jalas, 1972). In Serbia, the taxon has been recorded in Vojvodina province as well as in the eastern, southeastern, and central parts of the country (Šoštarić et al., 2012).

All *Thymus* species are considered medicinal and aromatic plants, producing essential oils (EOs) in specialized secretory structures. More than 17,500 plant species synthesize EOs, with members of the Lamiaceae being among the most prominent producers (Regnault-Roger et al., 2012). Chemical polymorphism within the genus *Thymus* is extensively documented: individual populations may comprise plants belonging to distinct chemotypes that differ markedly in their EO composition (Slavkovska et al., 2006; Etri and Pluhár, 2024). For *Th. pulegioides* subsp. *pannonicus*, the occurrence of at least three chemotypes has been reported, including thymol/p-cymene, geranial/neral, and α -pinene-dominated types (Pluhár et al., 2007; Maksimović et al., 2008a; Šoštarić et al., 2012).

Knowledge of chemotype diversity in wild populations is essential for the initial assessment of herbal material quality. The standard analytical approach relies on gas chromatography coupled with a mass detector (GC-MS) and/or flame ionization detectors (GC-FID). However, Raman spectroscopy has increasingly demonstrated its utility in EO characterization owing to its speed, non-destructive nature, and minimal sample preparation requirements. It therefore represents a promising complementary tool for rapid chemotype screening (Baranska et al., 2005; Schulz et al., 2005). In previous studies conducted in the Banat region, the citral and thymol chemotypes were recorded (Maksimović et al., 2008b; Šoštarić et al., 2012). The aim of this study was to identify Raman spectral bands capable of discriminating between the chemotypes of *Th. pulegioides* subsp. *pannonicus* collected from two localities in the Banat region of Serbia.

MATERIAL AND METHODS

Plant material. Plant material from two populations of *Thymus pulegioides* subsp. *pannonicus* (All.) Kerguélen was collected from natural habitats in June 2026, when plants were in full bloom (Table 1). Information on altitude, latitude, and longitude is presented in Table 1. Plant taxa were identified according to Flora Europaea (Jalas, 1972) and Flora of Serbia (Diklić, 1974). Voucher specimens have been deposited at the Herbarium of the Department of Agrobotany, Faculty of Agriculture, University of Belgrade.

Table 1. *Thymus pulegioides* subsp. *pannonicus* — locality data.

Species	Locality	Altitude	Latitude (N)	Longitude (E)
<i>Thymus pulegioides</i> subsp. <i>pannonicus</i>	DB - Devojački bunar	150 m	45.0011	20.9386
<i>Thymus pulegioides</i> subsp. <i>pannonicus</i>	VP - Vršačke planine	450 m	45.1236	21.3447

Raman spectroscopy. Raman spectroscopy analysis was performed on the confocal Raman micro-spectroscopy Witec Alpha 300R (Dreieich, Germany) using a 785 nm laser, operated at 40 mW with an integration time of 60 s and a 63× water immersion objective, yielding a resolution of 1.24 cm⁻¹. Spectra were measured on fresh leaves from 14 different plants (7 per locality). Chemometric analysis was performed following a standard preprocessing workflow that included baseline correction, normalization, and data reduction. Data reduction was achieved using Principal Component Analysis (PCA), enabling the assessment of sample clustering patterns. All statistical analyses were conducted using Unscrambler X software (version 10.4).

Isolation of essential oil and gas chromatography–flame ionization detection/mass spectrometry (GC-FID/MS) analysis. Essential oil was isolated from 20 g of cut plant material by hydrodistillation using a Clevenger-type apparatus for 2 h. Gas chromatographic analysis (GC-FID/MS) was performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) and coupled to an Agilent 5975C mass spectrometric detector (MSD). Essential oil sample was prepared as a 1.5% (v/v) solution in hexane and separated on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was set at 200°C, and 1 µL of sample was injected in split mode (10:1). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed from 60 to 280°C at a rate of 3°C/min and held isothermally at 280°C for 5 min. The FID and MSD transfer line temperatures were maintained at 300 and 250°C, respectively. Electron ionization (EI) mass spectra were recorded at 70 eV over the m/z range of 35–550.

Compound identification was based on comparison of Kovats retention indices (KI), retention times (tR), and mass spectra with those reported in the NIST/NBS and Wiley mass spectral libraries, as well as with published literature (Adams, 2007). Linear retention indices were calculated relative to a homologous series of n-alkanes (C8–C20) analyzed under the same chromatographic conditions (van den Dool and Kratz, 1963). The relative contents of

individual constituents were determined from the FID peak area percentages without applying response factor corrections.

RESULTS AND DISCUSSION

Raman spectra of thymol and citral with main bands assignments. Raman spectra of citral (Carl Roth, Germany) and thymol (Sigma Aldrich, USA) were acquired using a 785 nm excitation laser and exhibited well-defined vibrational features characteristic of their molecular structures. The use of near-infrared excitation minimized fluorescence interference and enabled clear observation of the fingerprint region, facilitating reliable molecular identification.

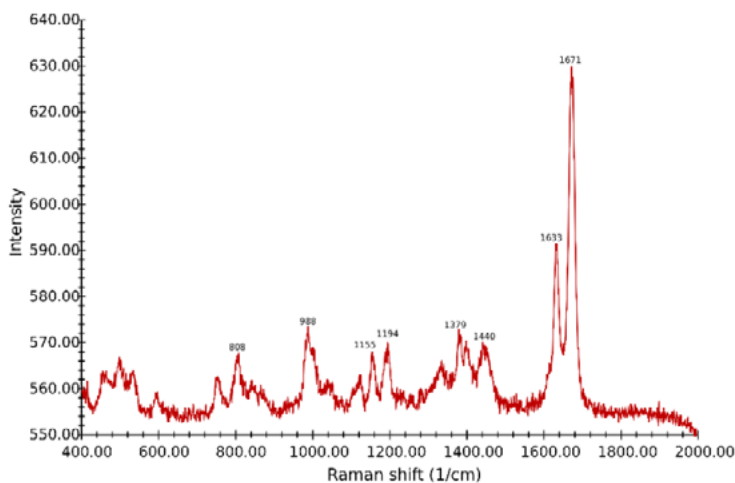


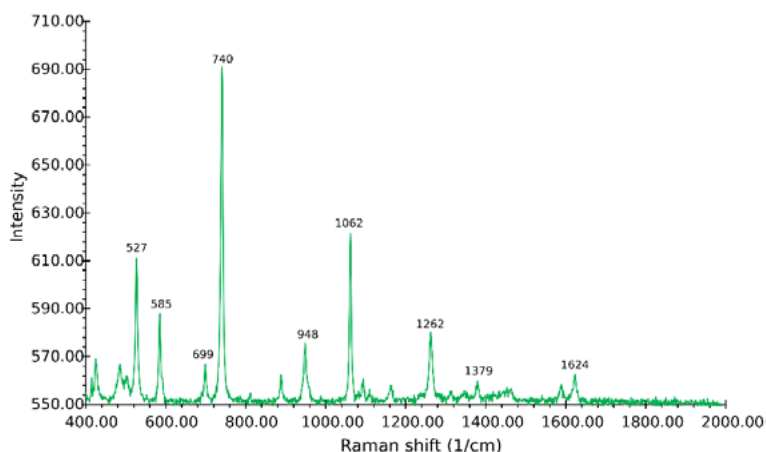
Figure 1. Raman spectrum of citral with main band assignments.

The Raman spectrum of citral (Figure 1) is dominated by bands associated with its conjugated aldehyde structure. The most characteristic feature is the intense band at 1671 cm^{-1} , assigned to the stretching vibration of the aldehydic carbonyl group, $\nu(\text{C}=\text{O})$. This band is typical of α,β -unsaturated aldehydes and serves as a distinctive spectroscopic marker for citral. A second prominent band at 1633 cm^{-1} corresponds to $\nu(\text{C}=\text{C})$ stretching of the conjugated double bond system. The presence of these two bands confirms the unsaturated aldehydic nature of the molecule. Vibrations observed at 1379 and 1440 cm^{-1} originate from CH_3 and CH_2 deformation modes, respectively, while bands at 1155 and 1194 cm^{-1} are attributed to skeletal C–C stretching vibrations and coupled methyl group motions within the aliphatic chain. The band at approximately 808 cm^{-1} is characteristic of out-of-plane deformation of olefinic C–H groups associated with substituted alkene moieties (Table 2; Figure 1).

Table 2. Raman bands of citral and their assignments.

Observed band (cm ⁻¹)	Assignment	Literature range (cm ⁻¹)	References
808	=C-H out-of-plane deformation of substituted alkene	800–840	Socrates (2004)
988	Skeletal C–C/C=C coupled vibration of monoterpenoid constituents	960–1000	Schulz and Baranska (2007)
1155	C–C skeletal stretching of aliphatic chain	1140–1180	Schulz and Baranska (2007)
1194	CH ₃ /CH ₂ wagging and deformation modes	1180–1210	Schulz and Baranska (2007)
1379	CH ₃ bending/deformation	~1380	Siatis et al., 2005
1440	CH ₂ /CH ₃ bending deformation	1442–1457	Vargas Jentsch et al., 2015
1633	ν (C=C) stretching of conjugated alkene	1630–1680	Schulz et al. (2005); Schulz and Baranska (2007)
1671	ν (C=C) stretching of acyclic monoterpenes	1660–1720	Schulz and Baranska (2007); Socrates (2004)

In contrast, the Raman spectrum of thymol (Figure 2) is dominated by vibrations arising from its substituted aromatic ring. The most characteristic band appears at approximately 740 cm⁻¹ and is widely recognized as a diagnostic Raman marker of thymol. This band originates from aromatic ring deformation and has been reported as one of the strongest and most distinctive Raman features of thyme-derived compounds. Additional ring deformation modes are observed at 527 and 585 cm⁻¹, reflecting skeletal vibrations of the aromatic framework (Table 3; Figure 2).

**Figure 2.** Raman spectrum of thymol with main band assignments.

The band at 1062 cm⁻¹ is attributed to aromatic ring breathing and in-plane C–H bending vibrations, whereas the band at 1262 cm⁻¹ is assigned to stretching of the phenolic C–O bond. The latter is particularly important because it reflects the presence of the hydroxyl substituent attached to the aromatic ring. The Raman band at 1379 cm⁻¹ corresponds to symmetric deformation of methyl groups directly attached to the aromatic system, while the strong band

at 1624 cm^{-1} originates from aromatic C=C stretching vibrations. Similar bands around 1620, 1460, 1380, and 1260 cm^{-1} have been reported previously for thymol and related phenolic monoterpenes (Table 3; Figure 2).

Table 3. Raman bands of thymol and their assignments.

Observed band (cm^{-1})	Assignment	Literature range (cm^{-1})	References
527	Aromatic ring skeletal deformation	500–550	Edwards (2006)
585	Aromatic ring deformation	560–600	Edwards (2006)
699	Aromatic C–H out-of-plane deformation	680–720	Smith and Dent (2019)
740	Characteristic thymol aromatic ring vibration	730–750	Siatis et al. (2005); Schulz and Baranska (2007); Vargas Jentzsch et al. (2015)
948	Aromatic C–H deformation	930–970	Schulz and Baranska (2007)
1062	Ring breathing/C–H in-plane vibration	1050–1070	Siatis et al. (2004); Vargas Jentzsch et al. (2015)
1262	Phenolic C–O stretching vibration	1240–1280	Siatis et al. (2004); Smith and Dent (2005)
1379	CH ₃ symmetric deformation	1370–1390	Schulz and Baranska (2007); Vargas Jentzsch et al. (2015)
1624	Aromatic C=C stretching vibration	1600–1630	Siatis et al. (2004); Schulz and Baranska (2007)

A direct comparison of the two spectra reveals substantial structural differences between the compounds. Citral exhibits intense bands associated with conjugated carbonyl and alkene functionalities, particularly at 1671 and 1633 cm^{-1} , which are absent in thymol. Conversely, thymol displays numerous strong bands within the 500–1300 cm^{-1} region corresponding to aromatic ring deformations and phenolic vibrations. The characteristic thymol marker at approximately 740 cm^{-1} and the citral carbonyl band at 1671 cm^{-1} provide unequivocal spectral signatures for differentiation between the two compounds. These observations demonstrate that Raman spectroscopy is a powerful and rapid technique for distinguishing aldehydic terpenes from phenolic monoterpenes and may be effectively applied for qualitative identification and authenticity assessment of essential-oil constituents.

Raman spectra of *Thymus peltate* glandular hair. Raman spectra were acquired directly from glandular trichomes located on the leaf surface of *Thymus* plants collected from two different localities (Figure 3). Since peltate glandular trichomes represent the primary sites of biosynthesis, accumulation and secretion of essential-oil constituents (Werker, 2000), the obtained spectra provide direct insight into the chemical composition of secretory structures without prior extraction or sample preparation. Consequently, the observed Raman features can be associated with the major metabolites accumulated within the trichome secretory reservoirs, predominantly monoterpenes and oxygenated monoterpenoids (Turner et al., 2000; Siatis et al., 2005).

The average spectrum of samples from the locality VP was dominated by an intense Raman band at 1672 cm^{-1} , assigned to the $\nu(\text{C}=\text{O})$ stretching vibration of an α,β -unsaturated aldehyde.

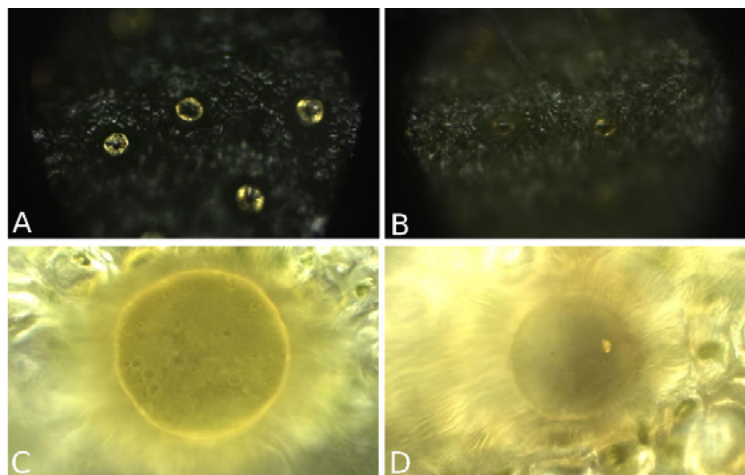


Figure 3. Micrographs of peltate glandular trichomes from the VP (A, C) and the DB locality (B, D) at 10 $\times$ and 63 $\times$ magnifications.

This band is characteristic of citral and represents the most distinctive spectral feature of the plants from the first locality. Additional strong bands at 1631 cm^{-1} and 980 cm^{-1} correspond to conjugated C=C stretching and terpene skeletal vibrations, respectively, further supporting the presence of citral-related constituents. The citral chemotype from this locality was previously described by Šoštarić et al. (2012). The combination of these bands indicates a significant contribution of oxygenated monoterpenes possessing conjugated aldehyde functionalities within the glandular trichome secretions (Schulz et al., 2005; Granda Santos et al., 2025) (Figure 4A; Table 4).

In contrast, samples collected from the locality DB (Figure 4B; Table 5) displayed a markedly reduced intensity of the carbonyl band in the 1665–1675 cm^{-1} region. Instead, relatively stronger signals were observed at 654, 670, 787, and 867 cm^{-1} , together with a pronounced band at approximately 1640 cm^{-1} (Figure 4B). These spectral features indicate a chemical composition distinct from that observed in samples from the locality VP and suggest a greater contribution of aromatic and/or phenolic constituents within the trichome secretions. However, comparison with the Raman spectrum of the thymol standard revealed substantial differences, indicating that the observed profile cannot be unequivocally attributed to a thymol chemotype, although previous paper indicated that thymol is the dominant compound in thyme essential oil at that locality (Maksimović et al., 2008b; Dajić Stevanović et al., 2008). Similar Raman bands have been reported for various aromatic monoterpenes and other phenolic compounds occurring in essential-oil-producing species of the Lamiaceae family, although definitive identification would require complementary chromatographic analyses such as GC–MS.

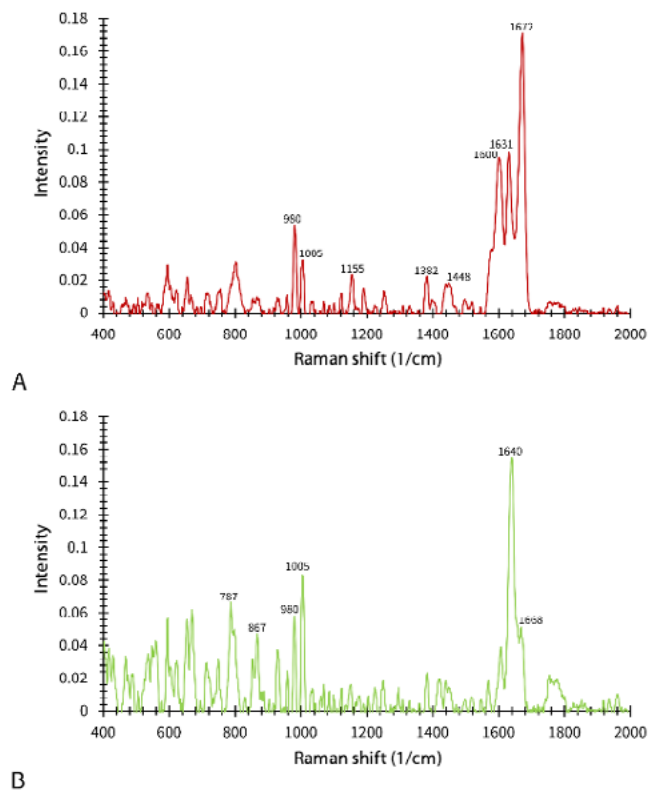


Figure 4. Baseline corrected, normalized, smoothed and averaged spectra of samples originated from VP - Vršačke planine (A) and DB - Devojački bunar (B).

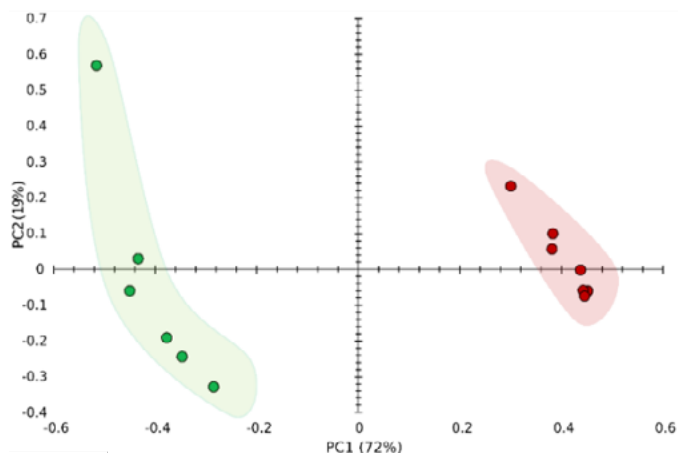
Table 4. Major Raman bands observed in glandular trichomes from the locality VP and comparison with literature data.

Experimental band (cm ⁻¹)	Literature band (cm ⁻¹)	Vibrational assignment	Reference
980	978-981	Terpene skeletal vibration (C–C/C=C coupled stretching)	Socrates (2004)
1005	1000-1006	Monoterpene skeletal vibration	Edwards (2006)
1155	1130-1160	C–C stretching vibration of terpene skeleton	Schulz and Barańska (2007); Smith and Dent (2019); Socrates (2004)
1382	1380	Symmetric CH ₃ deformation	Siatis et al. (2005)
1448	1453	CH ₂ /CH ₃ bending deformation	Siatis et al. (2005)
1600	1603	Aromatic/conjugated C=C stretching vibration	Edwards (2006)
1631	1633	ν(C=C) stretching of conjugated unsaturated system	Schulz and Barańska (2007)
1672	1670–1674	ν(C=O) stretching vibration of α,β-unsaturated aldehyde (citral-type constituent)	Socrates (2004); Edwards (2006)

Table 5. Major Raman bands observed in glandular trichomes from the locality DB and comparison with literature data.

Experimental band (cm ⁻¹)	Literature band (cm ⁻¹)	Vibrational assignment	Reference
787	783–787	Characteristic aromatic ring vibration of thymol-type compounds	Siatis et al. (2005); Daferera et al. (2002)
867	865–870	Aromatic C–H deformation vibration	Smith and Dent (2019); Socrates (2004);
980	981	Terpene skeletal vibration (C–C/C=C coupled stretching)	Edwards (2006); Socrates (2004)
1005	1003–1006	Monoterpene skeletal vibration	Schulz and Barańska (2007)
1640	1620–1642	Aromatic/conjugated $\nu(\text{C}=\text{C})$ stretching vibration	Vargas Jentzsch et al. (2015); Edwards (2006)
1668	1665–1675	$\nu(\text{C}=\text{O})$ stretching vibration of oxygenated monoterpenes	Schulz and Barańska (2007)

The results of PCA showed that the first two principal components explained 91% of the total data variability. Accordingly, the score plot demonstrated strong discrimination between samples from different localities, forming two separate clusters (Figure 5). The main band contributing to the discrimination of the DB samples was observed at approximately 1640 cm⁻¹, whereas samples from the VP locality are primarily clustered due to bands positioned at 1672 and 1631 cm⁻¹ (Figure 6). The most pronounced spectral differences between the two localities were observed in the carbonyl stretching region. The intensity of the 1672 cm⁻¹ band was considerably higher in the locality VP than in the locality DB, indicating differences in the relative abundance of aldehydic terpenoids accumulated within glandular trichomes. Since peltate glandular trichomes are highly specialized secretory structures whose metabolic profiles are influenced by genetic background and environmental conditions, these spectral differences may reflect chemotypic variability between populations originating from different habitats (Thompson et al., 2002).

**Figure 5.** PCA score plot. Spectra obtained from samples Devojački bunar (DB) are left on the plot (green), while spectra obtained from samples Vršacke planine (VP) are right on the plot (red).

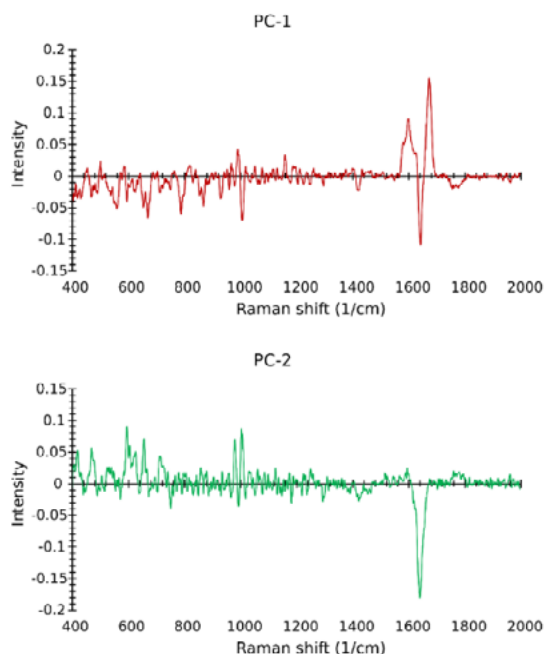


Figure 6. Loading plots.

Essential oil composition of *Thymus pulegioides* subsp. *pannonicus* (All.) Kerguélen population from Devojački bunar – DB. Although previous research identified the monoterpene phenol thymol as the major constituent of the DB population (Šoštarić et al., 2012), the Raman spectrum of thymol did not match the spectra obtained from the glandular hair residues. To elucidate the cause of this discrepancy, composition of the isolated essential oil was analyzed. In the essential oil from Devojački bunar, 47 compounds were identified, representing 97.69% of the total oil composition. Sesquiterpene hydrocarbons constituted the predominant class of constituents, followed by monoterpene hydrocarbons, while compounds belonging to other chemical classes were present only in minor amounts. Germacrene D was the dominant constituent, accounting for more than 50% of the total essential oil, explaining the differences in the obtained spectra. Similar to the present results, germacrene D was reported as the major compound in *Thymus pannonicus* populations from Bulgaria (Trendafilova et al., 2023) and eastern Serbia (Šoštarić et al., 2012).

Table 6. Essential oil composition from DB.

Constituent	Kiexp	Content (%m/m)
1 α -Thujene	923.6	0.28
2 α -Pinene	932.9	8.84
3 Camphene	949.0	0.75

4	Sabinene	970.7	0.38
5	1-Octen-3-ol	974.6	0.57
6	β -Pinene	977.7	1.37
7	3-Octanone	980.7	0.00
8	Myrcene	987.0	6.87
9	3-Octanole	993.3	0.20
10	α -Phellandrene	1006.1	t.
11	α -Terpinene	1015.8	t.
12	p-Cymene	1023.0	t.
13	Limonene	1027.9	1.88
14	β -Phellandrene	1029.9	0.39
15	1,8-Cineole	1031.5	1.19
16	E- β -Ocimene	1043.6	6.87
17	γ -Terpinene	1055.6	0.32
18	cis-Sabinene hydrate	1068.3	t.
19	Terpinolene	1083.4	t.
20	Linalol	1097.2	0.41
21	n-Nonanal	1102.2	0.33
22	Camphor	1147.1	0.22
23	α -Terpineol	1193.6	0.13
24	α -Copaene	1374.4	0.24
25	β -Bourbonene	1383.1	1.32
26	β -Elemene	1387.5	1.01
27	E-Caryophyllene	1420.6	5.98
28	β -Copaene	1429.0	0.30
29	Aromadendrene	1443.5	0.15
30	E- β -Farnesene	1450.5	0.46
31	α -Humulene	1455.4	0.37
32	allo-Aromadendrene	1459.7	0.20
33	cis-Muurolo-4(14),5-diene	1468.1	0.24
34	γ -Muurolene	1480.1	t.
35	Germacrene D	1487.3	50.29
36	γ -Amorphene	1490.9	0.13
37	Bicyclogermacrene	1496.3	1.47
38	E,E- α -Farnesene	1500.4	1.17
39	β -Bisabolene	1506.9	0.05
40	Germacrene A	1507.6	0.13
41	δ -Cadinene	1516.1	0.33

42	Caryophyllene oxide	1582.0	0.58
43	Salvial-4(14)-en-1-one	1592.2	0.14
44	α -Cadinol	1654.6	0.39
45	14-hydroxy-9-epi-(E)-Caryophyllene	1659.2	0.23
46	Germacre-4(15),5,10(14)-trien-1- α -ol	1684.9	1.25
47	Eudesma-4(15),7-dien-1-beta-ol	1690.5	0.25
Total identified			97.69
Monoterpene hydrocarbons			27.94
Oxygenated monoterpenes			1.95
Sesquiterpene hydrocarbons			64.44
Oxygenated sesquiterpenes			2.27
Other compounds			1.09

CONCLUSION

The ability to detect chemical differences directly from intact peltate glandular trichomes highlights a major advantage of Raman microspectroscopy. Unlike conventional chromatographic techniques, which require extraction and processing of plant material, Raman analysis enables rapid, non-destructive, and in situ characterization of secretory structures while preserving their spatial integrity.

The Raman spectra revealed clear chemical differentiation between glandular trichomes originating from the two studied localities. Samples from the VP locality were characterized by spectral features consistent with a higher abundance of citral-related constituents, particularly the prominent band assigned to the $\nu(\text{C}=\text{O})$ vibration of an α,β -unsaturated aldehyde. These results were in accordance with previous findings. In contrast, samples from the DB locality exhibited a distinct aromatic spectral profile and a markedly lower contribution of carbonyl-related bands. Comparison with the thymol standard indicated that the observed spectral pattern could not be unequivocally attributed to a thymol chemotype, suggesting the presence of an alternative chemical profile. GC-FID/MS analysis showed that germacrene D is a major constituent of the DB essential oil, indicating that further investigation of the Raman spectrum of a germacrene D standard should be conducted.

Overall, results demonstrate the potential of Raman microspectroscopy as a powerful tool for investigating the chemical composition of peltate glandular trichomes and assessing chemotypic variability in natural populations of essential-oil-producing plants. The observed differences between the studied populations highlight the value of Raman-based approaches for rapid screening of chemical diversity and provide a foundation for future studies combining Raman spectroscopy with complementary analytical techniques, such as GC-MS, for definitive metabolite identification.

ACKNOWLEDGMENTS

This research was supported by the European Union Horizon Europe program [HORIZON-MSCA-2023-SE-01; Grant number 101179991, VERDEDRIVE] and the Science Fund of the Republic of Serbia [Grant No. 7750160, R-SPECT].

REFERENCES

- Adams, R.P.:** Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th ed. Allured Publishing Corporation, Carol Stream, 2007.
- Baranska, M., Schulz, H., Reitzenstein, S., Uhlemann, U., Strehle, M.A., Krüger, H., Quilitzsch, R., Foley, W., Popp, J.:** Vibrational spectroscopic studies to acquire a quality control method of eucalyptus essential oils. *Biopolymers*, 78 (4), 237-248, 2005.
- Daferera, J.D., Tarantilis, P.A., Polissou, G.M.:** Characterization of Essential Oils from Lamiaceae Species by Fourier Transform Raman Spectroscopy. *Journal of Agricultural and Food Chemistry*, 50 (20), 5503-5057, 2002.
- Dajić Stevanović, Z., Šoštarić, I., Marin, P.D., Stojanović, D., Ristić, M.:** Population variability in *Thymus glabrescens* Willd. from Serbia: morphology, anatomy and essential oil composition. *Archives of Biological Sciences*, 60 (3), 475-483, 2008.
- Diklić, N.:** *Thymus* L. In: Josifović M (Ed.), *Flora SR Srbije*, Vol. VI. SANU, Beograd, Srbija, str. 475-509, 1974.
- Edwards, H.G.M.:** Spectra-structure correlations in Raman spectroscopy. In: Chalmers JM, Griffiths PR (Eds.), *Handbook of Vibrational Spectroscopy*. John Wiley and Sons, Chichester, 2006.
- Etri, K., Pluhár, Z.:** Exploring chemical variability in the essential oils of the genus *Thymus*. *Plants*, 13 (10), 1375, 2024.
- Granda-Santos, M., Reyna-Gonzales, K., Torrejón-Valqui, L., Valle-Epquín, M.G., Caetano, A.C., Díaz-Valderrama, J.R., Castro-Alayo, E.M., Cayo-Colca, I.S., Maicelo, J.L., Balcázar-Zumaeta, C.R.:** Characterization of terpenoids in aromatic plants using Raman spectroscopy and gas chromatography-mass spectrometry (GC-MS). *International Journal of Molecular Sciences*, 26 (23), 11254, 2025.
- Jalas, J.:** Notes on *Thymus* L. (Labiatae) in Europe. I. Supraspecific classification and nomenclature. *Botanical Journal of the Linnean Society*, 64 (3), 199-215, 1971.
- Jalas, J.:** *Thymus* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), *Flora Europaea*, Vol. 3. Cambridge University Press, Cambridge, 172-182, 1972.
- Maksimović, Z., Milenković, M., Vučićević, D., Ristić, M.:** Chemical composition and antimicrobial activity of *Thymus pannonicus* All. essential oil. *Central European Journal of Biology*, 3 (2), 149-154, 2008a.
- Maksimović, Z., Stojanović, D., Šoštarić, I., Dajić, Z., Ristić, M.:** Composition and radical-scavenging activity of *Thymus glabrescens* Willd. (Lamiaceae) essential oil. *Journal of the Science of Food and Agriculture*, 88 (11), 2036-2041, 2008b.
- Morales, R.:** El género *Thymus* L. en la región mediterránea occidental (Lamiaceae). *Biocosme Mésogéen*, 6 (4), 205-211, 1989.
- Morales, R.:** The history, botany and taxonomy of the genus *Thymus*. In: Stahl-Biskup E, Sáez F (Eds.), *Thyme: The Genus Thymus*. Taylor and Francis, London, pp. 1-43, 2002.
- Pluhár, Z., Héthelyi, É., Kutta, G., Kamondy, L.:** Evaluation of environmental factors influencing essential oil quality of *Thymus pannonicus* All. and *Thymus praecox* Opiz. *Journal of Herbs, Spices and Medicinal Plants*, 13 (3), 23-43, 2007.
- Regnault-Roger, C., Vincent, C., Arnason, J.T.:** Essential oils in insect control: low-risk products in a high-stakes world. *Annual Review of Entomology*, 57, 405-424, 2012.

- Schulz, H., Barańska, M.:** Identification and quantification of valuable plant substances by IR and Raman spectroscopy. *Vibrational Spectroscopy*, 43 (1), 13-25, 2007.
- Schulz, H., Özkan, G., Barańska, M., Krüger, H., Özcan, M.M.:** Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy. *Vibrational Spectroscopy*, 39 (2), 249-256, 2005.
- Siatis, N.G., Kimbaris, A.C., Pappas, C.S., Tarantilis, P.A., Daferera, D.J., Polissiou, M.G.:** Rapid method for simultaneous quantitative determination of four major essential oil components from oregano and thyme using FT-Raman spectroscopy. *Journal of Agricultural and Food Chemistry*, 53 (2), 202-206, 2005.
- Slavkovska, V., Lakušić, B., Jančić, R., Mimica-Dukić, N., Vujičić, D.:** Chemical composition of the essential oil of *Thymus bracteosus* Vis. ex Benth (Lamiaceae). *Journal of Essential Oil Research*, 18 (3), 310-311, 2006.
- Šoštarić, I., Arsenijević, J., Acić, S., Dajić Stevanović, Z.:** Essential oil polymorphism of *Thymus pannonicus* All. (Lamiaceae) in Serbia. *Journal of Essential Oil Bearing Plants*, 15 (2), 237-243, 2012.
- Smith, E., Dent, G.:** Modern Raman Spectroscopy: A Practical Approach. 2nd ed. John Wiley and Sons, Chichester, 2019.
- Socrates, G.:** Infrared and Raman Characteristic Group Frequencies: Tables and Charts. 3rd Ed. John Wiley and Sons, Chichester, 2004.
- Thompson, J.D.:** Population structure and the spatial dynamics of genetic polymorphism in thyme. In: Stahl-Biskup E, Sáez F (Eds.), *Thyme: The Genus Thymus*. Taylor and Francis, London, pp. 44-74, 2002.
- Trendafilova, A., Todorova, M., Ivanova, V., Zhelev, P., Aneva, I.:** Essential oil composition of ten species from sect. *Serpyllum* of genus *Thymus* growing in Bulgaria. *Diversity*, 15 (6), 759, 2023.
- Turner, G.W., Gershenzon, J., Croteau, R.:** Distribution of peltate glandular trichomes on developing leaves of peppermint. *Plant Physiology*, 124 (2), 655-664, 2000.
- van den Dool, H., Kratz, P.D.:** A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, 11, 463-471, 1963.
- Vargas Jentsch, P., Ramos, L.A., Ciobotă, V.:** Handheld Raman spectroscopy for the distinction of essential oils used in the cosmetics industry. *Cosmetics*, 2 (2), 162-176, 2015.
- Werker, E.:** Trichome diversity and development. *Advances in Botanical Research*, 31, 1-35, 2000.

Ramanska mikrospektroskopija kao metoda za analizu sastojaka etarskog ulja u žlezdanim dlakama vrsta roda *Thymus*

REZIME

Thymus je jedan od najvećih rodova u familiji Lamiaceae i sve njegove vrste koriste se kao lekovite i aromatične biljke. U ovom istraživanju, Ramanova spektroskopija primenjena je kao brza i nedestruktivna metoda za određivanje hemotipa analizom spektara dobijenih direktno iz žlezdanih dlaka. Uzorci dve populacije *Thymus pulegioides* subsp. *pannonicus* sakupljeni su sa područja Banata (Srbija), analizirani korišćenjem lasera talasne dužine 785 nm i upoređeni sa referentnim spektrima standarda citrala i timola. Spektri uzoraka sa Vršackih planina pokazali su prisustvo dominantnih traka vezanih za citral (oko 1671 i 1633 cm⁻¹). Spektri uzoraka sa Devojačkog bunara pokazali su dominantne trake na oko 1640 i 1668 cm⁻¹, koje odgovaraju ν(C=C) konjugovanim i ν(C=O) oksigenovanim istežućim vibracijama monoterpena. Dominantne trake timola nalazile su se na 740, 1062 i 1262 cm⁻¹, što potvrđuje da uzorci sa Devojačkog bunara ne pripadaju timol hemotipu. GC-FID/MS analiza je pokazala da je dominantno jedinjenje u ovom uzorku germakren D. Pored toga, analizom glavnih komponenti (PCA) utvrđeno je jasno razdvajanje dve ispitivane populacije, što pokazuje da se Ramanova spektroskopija može koristiti za diferencijaciju hemotipova.

Ključne reči: hemotipovi, citral, timol, Raman spektroskopija.