

FLAVONOID COMPONENTS, CHEMOTYPES, AND CANDIDATE CHEMICAL MARKERS OF *TEUCRIUM* (LAMIACEAE) SPECIES USING HPLC-MQ-API-MS/MS

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Belonging to Lamiaceae family, the genus *Teucrium* L. is a rich source of secondary metabolites used in pharmacology and industrial medicine. This study aimed to investigate the flavonoid contents of methanol extract and the chemical markers of *Teucrium* at specific and infraspecific levels and identify the chemotypes of its accessions. Nineteen accessions, eight species, and five subspecies were collected from three sections (*Teucriis*, *Polium*, and *Scordium*) belonging to the natural habitats in the west, southwest, centre, and south of Iran. Total flavonoid extraction was attained by the air-dried leaf of *Teucrium* species and 90% methanol. HPLC coupled with triple quadrupole mass analyser was applied to screen and identify the chemical constituents. Multivariate analyses were implemented by cluster and principal component analysis. Chemical investigation specified 148 chemical compounds, of which 135 flavonoids were tentatively identified. The most abundant derivatives belonged to flavones (41 derivatives), flavonols (37 derivatives), and isoflavonoids (22 derivatives). Based on the cluster analysis, 13 groups of chemical markers were formed in sections *Teucriis* and *Scordium* and five groups in section *Polium* as chemotaxonomic indicators. The specified chemotypes were represented by ten in the sections *Teucriis* and *Scordium* and by six in the section *Polium*. A relationship was suggested between type and flavonoid quantity (number), altitude, and geographical location. The present study provided valuable information for pharmacological research, bioactivity assessment, and chemotaxonomic purposes. This is the first report on the presence of chemotypes, chemical markers, and quality assessments for the genus *Teucrium*.

Key words: chemotype, flavonoid, Lamiaceae, liquid chromatography, *Teucrium*

INTRODUCTION

The genus *Teucrium* L. belongs to Lamiaceae family and is a well-known medicinal plant with different biological activities (Elmasri *et al.* 2015, Reching 1982). This genus is represented by 400 species all over the world and 19 species in Iran (Jamzad 2012, Ozcan 2020). *Teucrium* species are generally distributed in the west of the Mediterranean region, northwest and south of America, north and south of Africa, Australia, New Zealand, and the temperate regions of Asia (Bukhari *et al.* 2015, Navarro 2020). Its vegetative forms

are shrub, subshrub, biennial, perennial, and annual in mountains, hills, wetlands, and deserts (Navarro 2020, Ozcan 2020, Reching 1982).

Teucrium species have long been utilised in ethnopharmacology and traditional and industrial medicine. It has different biological properties and a great potential for treating certain diseases. Based on recent research, antiseptic, anti-bacterial, anti-cancer, hepato-protective, anti-oxidant, anti-toxic, anti-spasmodic, anti-rheumatic, anti-nociceptive, anti-diabetic, and anti-inflammatory activities are associated with *Teucrium* species (Elmasri *et al.* 2015, Stefkov *et al.* 2012). It has also been well documented that the genus *Teucrium* is a rich source of secondary metabolites, particularly flavonoid, phenolic acids, essential oils, anthocyanins, iridoids, and phenylethanoid glycosides. As demonstrated, flavonoid compounds are the main metabolites of plants with antioxidant capacity (Alwahsh *et al.* 2015, Mitreski *et al.* 2014, Venditti *et al.* 2017).

Recently, more scientific attention has been paid to different metabolites in plants. From a phytochemical point of view, different studies have been published regarding the genus *Teucrium* over the recent years. Using Ultra Performance Liquid Chromatography (UPLC), HPLC, Proton Magnetic Resonance (PMR), Nuclear Magnetic Resonance (NMR), UV spectroscopy, Electron Ionisation/Electrospray Ionisation (EI/ESI), and Liquid Chromatography/diode array detector/Electrospray Ionisation (LC/DAD/ESI), phenolic or flavonoid derivatives were observed to consist of 5-demethylsinensetin, eupatorin, cirsimaritin, cirsiolol, apigenin, luteolin, diosmetin, hyperoside, salvigenin, chrysosplentin, methoxyflavone, and hydroxyflavone isolated from *T. orientale* L., *T. polium* L., and *T. barbeyanum* Aschers. (Alwahsh *et al.* 2015, Elmasri *et al.* 2015, Mitreski *et al.* 2014, Stefkov *et al.* 2012). Phenolic acids such as, rosmarinic, *p*-coumaric, hydrocinnamic, and hydrobenzoic acid were identified in *T. barbeyanum*, *T. scordium* L., and *T. polium* by HPLC, NMR, and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) (Alwahsh *et al.* 2015, Milošević-Djordjević *et al.* 2018, Özer *et al.* 2018). β -sitostrol (phytostrol) and steroids were further described for different *Teucrium* species (Grubešić *et al.* 2012). Different essential oils, including monoterpenes, neo-clerodane diterpenes, triterpenes, and sesquiterpenoids were reported in *T. mascatense* Boiss. and *T. polium* using Infrared (IR), MS, and NMR (Ur Rehman *et al.* 2014, Venditti *et al.* 2017).

Some of the previous studies revealed the presence of chemotypes and the infraspecific variability of metabolites. The populations growing in different natural habitats display various qualities and quantities of chemical ingredients for adapting to new conditions (Pourhosseini *et al.* 2018). Chemotypes are essentially required for providing chemical diversity in different habitats. Different metabolites were reported in the *T. polium* accessions using NMR (Venditti *et al.* 2017). The consequences associated with chemical diversity (chemotypes) in the phenolic compounds of *Teucrium* species have not

been fully considered despite the significance of those compounds as chemical protection.

Fingerprinting analysis of chemical compounds has improved the separation and identification of natural products. The combination of HPLC-photodiode array detector (PDA) technique and mass spectrometer shows the level of sensitivity, robustness, accuracy, potency, and the precise technological advances in characterising the chemical components of natural products and specifying unknown compounds. Moreover, liquid chromatography coupled with atmospheric pressure ionisation (API) technique is an ionisation process for analysing large, small, polar, and nonpolar compounds. This method can be applied to confirm the identity of an extensive array of compounds by presenting mass to charge (m/z) and fragmentation patterns (Pratima and Gadikar 2018).

There has been no report about the chemical constituents in *Teucrium* species using HPLC-MS/MS method; therefore, it is necessary to develop a metabolomics approach to assessing chemical composition and obtaining information regarding its secondary metabolites. Such chemical profiles can be useful for pharmaceutical, chemotaxonomic, and biological purposes. To this aim, the current work attempted to develop an HPLC-Micromass Quattro micro Atmospheric Pressure Ionisation Mass Spectrometer (HPLC-MQ-API-MS/MS) technique for Iranian *Teucrium* species so as to 1) identify the flavonoid constituents of derived fractions at specific and infraspecific levels, 2) evaluate the flavonoid diversity at infraspecific levels, 3) propose appropriate chemical markers with chemotaxonomic significance, and 4) clarify different chemotypes in its species. To our knowledge, this is the first attempt at identifying such a high number of spectral data and qualifying and characterising flavonoids for *Teucrium* species.

MATERIALS AND METHODS

Plant samples

During April–July 2018 and 2019, we collected 19 accessions, eight species, and five subspecies from three sections (section *Polium*: *T. polium* L., *T. capitatum* L., *T. gnaphalodes* L' Her. – section *Teucris*: *T. orientale* subdivided into four subspecies *T. orientale* subsp. *orientale*, *T. orientale* subsp. *taylori* (Boiss.) Rech. f., *T. orientale* subsp. *glabrescens* (Hauskn. ex Bornm.) Rech. f., *T. orientale* subsp. *gloeotrichum* Rech. f.), *T. oliverianum* Ging. ex Benth. as an endemic species, *T. parviflorum* Schreb. – section *Scordium*: *T. melissoides* Boiss. ex Hauskn. as an endemic species, *T. scordium* subsp. *scordioides* (Schreb.) Arcang) (Table 1). These species grow in the west, centre, southwest and south of Iran. All specimens were identified by Flora Iranica and Flora of Iran (Jamzad 2012, Rechinger

Table 1

List of collected *Teucrium* species and their populations from their natural habitats with specimen numbers, location, altitude, and geographical position

| Species/accession/herbarium number | Locality | Alt. (m) | Geographical position |
|---|--|----------|-----------------------|
| <i>T. polium</i> 46 | Kurdestan, between Sanandaj and Kamyaran, Sou village | 1,800 | 47.12° E, 35.9° N |
| <i>T. polium</i> 60 | Fars, Ghaemieh, Shiraz road | 1,121 | 51.34° E, 29.50° N |
| <i>T. capitatum</i> 5 | Kohgiluyeh va Boyer Ahmad, Boyer Ahmad, Ludab, Kouh-e Nour | 1,620 | 50.48° E, 30.53° N |
| <i>T. capitatum</i> 7 | Chaharmahal va Bakhtiari, Ardal, Emamzadeh Abdollah | 1,960 | 50.39° E, 32.0° N |
| <i>T. orientale</i> subsp. <i>glabrescens</i> 17 | Lurestan, Aligoudarz, after Azad University | 2,048 | 49.7° E, 33.37° N |
| <i>T. orientale</i> subsp. <i>glabrescens</i> 39 | Isfahan, Chadegan, Zayanderod | 2,279 | 50.69° E, 32.72° N |
| <i>T. orientale</i> subsp. <i>gloeotrichum</i> 26 | Lurestan, Khorramabad toward Poldokhtar, Shourab | 1,245 | 48.14° E, 33.46° N |
| <i>T. orientale</i> subsp. <i>gloeotrichum</i> 38 | Isfahan, Chadegan, Kalb Ali village | 2,396 | 50.69° E, 32.83° N |
| <i>T. orientale</i> subsp. <i>orientale</i> 29 | Chaharmahal va Bakhtiari, Boroujen, Naghaneh | 2,338 | 51.42° E, 31.91° N |
| <i>T. orientale</i> subsp. <i>orientale</i> 37 | Isfahan, road of Daran toward Taraz | 2,406 | 50.45° E, 32.94° N |
| <i>T. orientale</i> subsp. <i>taylori</i> 2 | Kohgiluyeh va Boyer Ahmad, Kohgiluyeh, Tang-e Higoun | 1,048 | 54.38° E, 36.30° N |
| <i>T. orientale</i> subsp. <i>taylori</i> 58 | Fars, Komarj village | 857 | 51.47° E, 29.61° N |
| <i>T. scordium</i> subsp. <i>scordioides</i> 40 | Isfahan, Semirom, water fall | 1,909 | 51.36° E, 31.25° N |
| <i>T. scordium</i> subsp. <i>scordioides</i> 45 | Kurdestan, Marivan, Zarivar | 1,250 | 46.13° E, 35.55° N |
| <i>T. parviflorum</i> 41 | Kurdestan, Sarvabad, Houraman, Jivar village | 1,900 | 46.20° E, 35.21° N |
| <i>T. melissoides</i> 49 | Kermanshah, Dar-e Gamaleh, Nosoud | 1,900 | 46.13° E, 35.11° N |
| <i>T. gnaphalodes</i> 54 | Boushehr, road of Ahrom toward Borazjan, Chahkoutah | 45 | 51.14° E, 29.08° N |
| <i>T. gnaphalodes</i> 64 | Kohgiluyeh va Boyer Ahmad, Gachsaran, Emamzadeh Ghotbeddin | 456 | 50.47° E, 30.49° N |
| <i>T. oliverianum</i> 50 | Boushehr, 12 km from Dalaki toward Borazjan | 108 | 51.26° E, 29.34° N |

1982). The authenticity of studied species was verified by Dr N. Kharazian, Department of Botany, Faculty of Sciences, Shahrekord University. All voucher specimens were deposited in the herbarium of Shahrekord University (HSU).

Extraction method

The air-dried leaves (10 g) were powdered in a grinder. The total flavonoid was obtained from dried ground materials using 90% methanol and maceration method. The extracted solutions were filtered through Whatman's filter paper and concentrated using a rotary evaporator (Eyela Co., Tokyo, Japan) at 40 °C. The dry methanol extracts were dissolved in distilled water and then filtered. Flavonoid isolation was performed successively by

1-Butanol through silica gel 60F 254 (16 mg, 70 ml water) thin layer chromatography (TLC; 5 μm , 20 \times 20 cm). The chromatogram was transferred in solvent systems such as chloroform-methanol (60:40; 70:30; 80:20) (Venditti *et al.* 2017). Spot detection with natural product reagents (diphenylboric acid 2-aminoethyl ester; Sigma Chemical; in methanol, followed by 5% solution of polyethylene glycol 400 in ethanol) was accomplished by ultraviolet-366 nm. The flavonoids were fractionated by column chromatography (50 \times 4 cm), followed by Sephadex LH₂₀ Sigma-Aldrich and methanol 20% mixture eluted with 100 ml chloroform-methanol 60:40 to afford several fractions. The flavonoid fractions for liquid chromatography process were prepared on an ultraviolet spectrum (200–400 nm) (Jafari Dehkordi *et al.* 2020).

HPLC-MS/MS method

The chemical fractions of each species were checked by UV-absorption. Subsequently, a total of 19 methanol fractions were exposed to HPLC-MS/MS. The liquid chromatography conditions were used as follows:

The technique was carried out on Waters Alliance 2695 (Waters, Corp., MA, USA) HPLC-Micromass Quattro micro API (Atmospheric Pressure Ionisation) Mass Spectrometer which is a combined HPLC detector and a triple quadrupole mass spectrometer planned for LC/MS/MS. Sample ionisation was conducted in the source at atmospheric pressure. An Atlantis T3-C18 column (3 μm , 2.1 \times 100 mm, the flow rate of 0.25 ml min⁻¹) was used at 30 °C in HPLC/MS. The mobile phase included acetonitrile, ultra-pure water, methanol, and formic acid (98%). It was comprised of two segments: A) 0.1% (v v⁻¹) formic acid in water and B) 0.1% formic acid in acetonitrile. The elution gradient was designed as follows: 0 min, 95% B; 25 min, 5% B; 35 min, 5% B, and injection volume 5 μl (Jafari Dehkordi *et al.* 2020).

The mass spectrometer (MS) conditions

The mass spectrometer (MS) was recorded on negative ionisation mode with full scanning arranged by a mass range m/z 260–800. Total ion chromatogram (TIC) and extracted electrospray (ES) were performed for mass spectra. Nitrogen (grade 5.0) was utilised as nebuliser with gas flow 200 l h⁻¹. The ion source temperatures and desolvation were considered at 300 °C and 120 °C, respectively. The ion spray voltage was organised as capillary voltage 3.5 kV, extractor 2 V, RF Lens 0.2 V, and sampling cone voltage 30 V. The collision energy was applied over the range of 30–40 eV, providing additional fragmentation dataset. The extractions were developed over a 0.2 μm filter. The molecular ions of MS/MS were applied using collision induced dissociation (CID).

Commercial standards were not applied for all flavonoid compounds. Some analytical-grade standards (such as kaempferol and tricetin with 98% purity), obtained from Sigma-Aldrich Chemical Co. (St Louis, MO), were further applied. Methanol was used to arrange the stock solution (1 mg ml⁻¹) and filtration (Jafari Dehkordi *et al.* 2020). For compounds with no available standards, full scanning with MS/MS spectra in negative ionisation mode resulted in a precise characterisation.

Statistical analysis

In order to show different chemotypes in *Teucrium* species in conjunction with chemical markers, a cluster analysis was done with Neighbour Joining method followed by Dice similarity index and principal component analysis using Paleontological Statistics Software Package for Education and Data Analysis (PAST 3.00) and chromatographic datasets (Hammer *et al.* 2001).

RESULTS

Based on HPLC separation and clarification of MS/MS spectra, a total of 148 chemical compounds were detected in the chromatogram of *Teucrium* species. Moreover, 135 flavonoid compounds with an excessive variety were tentatively identified in this genus. Flavones (41 derivatives), flavonols (37 derivatives), and isoflavonoids (22 derivatives) were the most abundant flavonoid derivatives. Less abundant derivatives were flavanones (12 derivatives), chalcones (9 derivatives), flavan-3-ols (9 derivatives), anthocyanins (5 derivatives), and flavonolignans (one derivative). Additional compounds, including hydroxycinnamic acids (6 derivatives) and quinic acids (4 derivatives), benzopyrans (2 derivatives), tannins (2 derivatives), and alkaloids (one derivative) were also identified. One compound remained unknown and the m/z of each molecular ion was in the range of 267–785 AMU.

Major chemical constituents of specific levels were detected in *T. orientale* subsp. *orientale* (45 compounds), *T. orientale* subsp. *glabrescens* (42 compounds), *T. scordium* subsp. *scordioides* (40 compounds), and *T. capitatum* (38 compounds). The lowest amounts were further detected in *T. polium* (19 compounds). The main chemical compounds from accessions were found in *T. orientale* subsp. *orientale* with accession no. 29 (32 compounds), *T. orientale* subsp. *glabrescens* with accession no. 39 (26 compounds), *T. scordium* subsp. *scordioides* with accession no. 40 (23 compounds), and *T. capitatum* with accession no. 5 (21 compounds). The least abundant compounds belonged to *T. gnaphalodes* with accession no. 64 (10 compounds) and *T. polium* with accession no. 60 (8 compounds).

The identification process of chemical compounds representing major detected peaks was designed based on molecular ion ($[M-H]^-$), MS/MS fragmentation database, retention times, ES in mass spectra, and reference standards

(<http://mona.fiehnlab.ucdavis.edu>; <http://massbank.eu>; <http://pubchem.ncbi.nlm.nih.gov>). Table 2 depicts MS/MS fragmentation, retention time, mass/charge, and compound identification.

A total of 108 flavonoid compounds were primarily reported for *Teucrium* species (Table 2). Additionally, flavonoid compounds, such as cirsiol, cirsilinoleol, and diosmin were commonly presented in eight *Teucrium* species. This is the first study to propose that several chemical markers of each species were potential candidate markers as identification indicators (Table 2, Figs 1–2). The candidate markers were suggested for the chemotaxonomy of *Teucrium* genus. In this connection, in section *Polium*, *T. polium* was specified by three markers, *T. gnaphalodes* was determined by five markers, and *T. capitatum* by 17 markers (Table 2). In section *Teucris*, *T. orientale* subsp. *gloeotrichum* and *T. orientale* subsp. *taylori* were each represented by five markers, *T. orientale* subsp. *orientale* by 14 markers, *T. orientale* subsp. *glabrescens* by nine markers, and *T. parviflorum* by two markers. This implies the important chemical markers for *T. orientale*

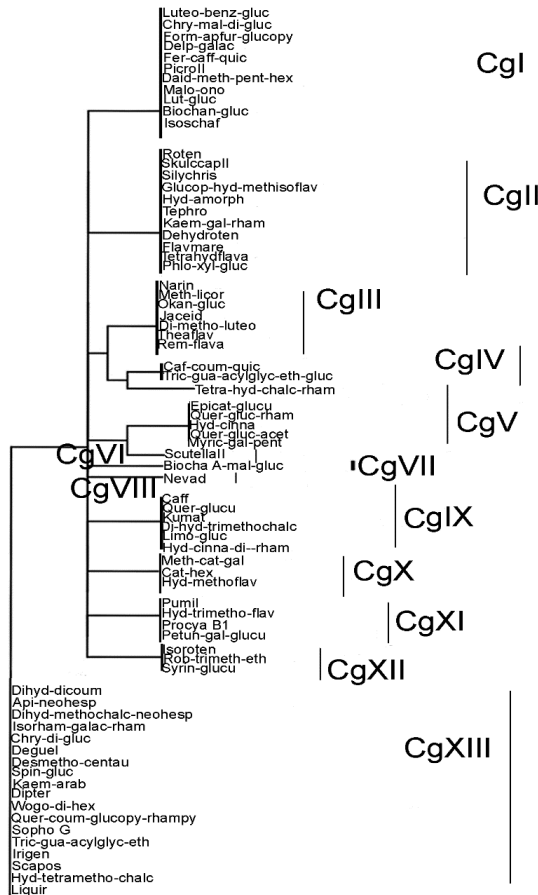


Fig. 1. Dendrogram related to the members of sections *Teucris* and *Scordium* using cluster analysis and chemical markers. Cg = chemical group. Cg I = *T. orientale* subsp. *orientale* 29, Cg II = *T. scordium* subsp. *scordioides* 45, Cg III = *T. orientale* subsp. *glabrescens* 39, Cg IV = *T. orientale* subsp. *glabrescens* 17, 39, Cg V = *T. orientale* subsp. *taylori* 2, 58, Cg VI = *T. orientale* subsp. *taylori* 58, Cg VII = *T. melissoides* 49, Cg VIII = *T. orientale* subsp. *gloeotrichum* 26, Cg IX = *T. oliverianum* 50, Cg X = *T. orientale* subsp. *orientale* 37, Cg XI = *T. orientale* subsp. *gloeotrichum* 38, Cg XII = *T. parviflorum* 41, Cg XIII = *T. scordium* subsp. *scordioides* 40. The chemical compounds are completely listed in Table 2

Table 2

The detailed identification of chemical constituents in *Teucrium* species and their accessions (N = compound number, tR = retention time, Mw = molecular weight, m/z = mass/charge, MS/MS = mass spectrum, Po = *T. polium*, gn = *T. ganphalodes*, c = *T. capitatum*, s = *T. scordium* subsp. *scordoides*, t = *T. orientale* subsp. *taylori*, glo = *T. orientale* subsp. *gleotrichum*, or = *T. orientale* subsp. *orientale*, gla = *T. orientale* subsp. *glabrescens*, ol = *T. oliverianum*, pa = *T. parviflorum*, m = *T. melissoides*)

| Species/accession | N | t _k (min) | Mw | m/z | MS/MS | Identification |
|--|----|----------------------|-----|-----|--|---|
| Po60, 46, gn64, 54, c5, s45, t2, 58, glo26 | 1 | 10.71, 11.41 | 624 | 623 | 623, 468, 315, 151 | Isorhamnetin-3-O- rutinoside |
| Po60, t2, 58, or29, glo38 | 2 | 10.92, 11.53 | 464 | 463 | 464, 463, 301, 271 | Isoquercitrin |
| Po60, gn64, pa41, gla39, or29, glo38, t2, 58 | 3 | 11.84 | 453 | 452 | 332, 317, 289, 161, | Caffeoyl-coumaroyl spermidine |
| Po60, m49, t12, 58, glo26 | 4 | 12.13, 12.66 | 638 | 637 | 619, 593, 443, 285, 175 | Luteolin-7-O-diglucuronide |
| Po60, 46, c5, pa41, m49, ol50, s45, gla39, 17, t2, 58, or29, 37, glo26, 38 | 5 | 16.00, 16.95 | 330 | 329 | 329, 314, 301, 299, 285, 272 | Cirsilol |
| Po60, c5, pa41, m49, ol50, gla39, 17, t2, 58, or29, 37, glo26, 38 | 6 | 17.95, 18.87 | 344 | 343 | 328, 315, 299, 298, 285, 270 | Cirsilineol |
| Po60, gla17, t2, 58, glo38, or29 | 7 | 19.77, 20.71 | 726 | 725 | 593, 563, 431 | Isovitexin-4'-O-glucoside-2''-O-arabinoside |
| Po60, t12, 58, or29, 37 | 8 | 22.86, 23.92 | 544 | 543 | 481, 463, 381, 301, 256 | Quercetin-O-hexoside-O-sulfate |
| Po46, c5, pa41, gn54 | 9 | 9.49 | 394 | 393 | 394, 365, 319, 293, 251, 248, 160 | Isorotenone |
| Po46 | 10 | 9.49, 9.73 | 640 | 639 | 640, 479, 315, 241 | Isorhamnetin-5-O-diglucoside |
| Po46, gn64, 54, c5 | 11 | 10.50, 11.56 | 772 | 771 | 771, 285 | Kaempferol-3-diglucoside-7-glucoside |
| Po46, pa41, m49, s45, gla39, 17, or29, 37, glo26, 38 | 12 | 11.13 | 594 | 593 | 593, 308, 285, 197 | Isosakuranetin-7-O-neohesperidoside |
| Po46, c5, pa41, m49, ol50, gn54, s45, gla39, or29, glo26, 38 | 13 | 11.54 | 608 | 607 | 675, 609, 301, 299, 285 | Diosmin |
| Po46, pa41, s40, 45 | 14 | 12.02, 13.64 | 424 | 423 | 422, 391, 322, 315, 254, 244, 231, 164 | Licoricidin |
| Po46, gn64, 54, pa41, gla39, 17, or37 | 15 | 13.20 | 608 | 607 | 299, 285, 256 | Diosmetin-coumaroylglucoside |
| Po46 | 16 | 13.10 | 493 | 492 | 347, 330, 299, 285, 243, 214, 187 | Malvidin-3-hexoside |
| S40, c7 | 17 | 11.58 | 379 | 378 | 379, 290, 254, 241 | Coumaroylquimic acid derivatives |
| Po46 | 18 | 15.12 | 379 | 378 | 314, 305, 287, 250, 240 | Coumaroylquimic acid derivatives |

Table 2 (continued)

| Species/accession | N | t _R (min) | Mw | m/z | MS/MS | Identification |
|---|----|----------------------|-----|-----|---|--|
| Po46, gn64, t2, 58 | 19 | 14.95, 15.54 | 466 | 465 | 465, 447, 434, 419, 330, 289, 193, 180 | Epicatechin glucuronide |
| Po46, c5, pa41, glal17, or29, 37, glo38 | 20 | 17.59, 18.50 | 314 | 313 | 325, 314, 298, 285, 283, 269, 255, 246, 175 | Cirsimaritin |
| Gn64 | 21 | 9.72, 9.84, 9.98 | 594 | 593 | 593, 534, 444, 353, 312, 269, 183 | Apigenin-6,8-digalactoside |
| Gn64 | 22 | 11.65 | 786 | 785 | 623, 477, 461, 315, 272 | Isorhamnetin-3-O-rutinoside-7-glucoside |
| Gn64, 54, c5 | 23 | 13.04 | 436 | 435 | 435, 337, 273, 123 | Phloretin-2'-O-glucoside |
| Gn64 | 24 | 13.05 | 494 | 493 | 475, 289, 231, 206 | Catechin-O-glucose (OAc) |
| Gn64, ol50, c7 | 25 | 13.25, 13.78 | 478 | 477 | 374, 373, 301, 274, 245, 200, 175, 125 | Quercetin 3-O-glucuronide |
| C5 | 26 | 10.23, 10.60 | 740 | 739 | 777, 760, 739, 593, 449, 431, 340, 328, 311, 282, 212 | Kaempferol-3-O-neohesperidoside-7-rhamnoside |
| C5, pa41, s45, gla39, 17, or29, 37, glo26, 38 | 27 | 10.97, 11.86 | 448 | 447 | 447, 448, 430, 411, 285, 271, 256, 178 | Isoorientin |
| C5 | 28 | 12.20, 12.31 | 756 | 755 | 755, 383, 431 | Alzelin-3-O-dihexoside |
| C5 | 29 | 12.50 | 594 | 593 | 593, 285, 228, 176, 158 | Datiscein-3-O-rutinoside |
| C5, gn54 | 30 | 12.96, 13.09 | 376 | 375 | 360, 344, 314, 301, 288 | Limocitrol |
| C5 | 31 | 13.26 | 652 | 651 | 651, 652, 351, 299, 175 | Chrysoeriol-7-O-di-glucuronopyranoside |
| C5, gla39 | 32 | 13.55, 14.08 | 316 | 315 | 652, 314, 285, 269, 202, 175 | 6-dimethoxyluteolin |
| C5 | 33 | 10.03 | 410 | 409 | 375, 363, 333, 319, 293, 261 | Amorphigenin |
| C5, gn54 | 34 | 14.44, 14.90 | 380 | 379 | 299, 285, 241 | Luteolin-3-methylether-7-sulphate |
| C5, s45 | 35 | 13.84, 14.77 | 426 | 425 | 425, 380, 370, 324, 311, 297, 218, 199, 177 | Hydroxyamorphigenin |
| C5 | 36 | 15.54 | 378 | 377 | 377, 362, 319, 270, 253, 218, 200, 175 | Methyl robustone |
| C5, glo38 | 37 | 15.45, 15.67 | 328 | 327 | 326, 299, 282, 247, 211, 209, 183, 171, 197, 166 | 3-hydroxy-3',4',5'-trimethoxyflavone |

Table 2 (continued)

| Species/accession | N | t _r (min) | Mw | m/z | MS/MS | Identification |
|--|----|----------------------|-----|-----|--|---|
| C5, s45 | 38 | 15.83, 16.13 | 410 | 409 | 392, 387, 365, 350, 320, 216, 200, 163 | Tephrosin |
| Pa41, t2, 58, or37, glo38 | 39 | 8.59, 9.53 | 408 | 407 | 410, 302, 286, 271, 269, 258, 243, 136 | 6,8-diprenylnarigenin |
| Pa41, gla39, 17, or29, 37, glo38 | 40 | 8.36 | 452 | 451 | 452, 433, 344, 329, 313, 301, 240, 179 | Aspalathin |
| Pa41 | 41 | 8.46, 8.85 | 344 | 343 | 342, 298, 299, 315, 268, 253, 239, 193 | Robinetin-trimethyl ether |
| Pa41, s40 | 42 | 9.44, 9.72 | 436 | 435 | 667, 492, 368, 337, 325, 283, 242, 175, 129, 110 | Artocaprin |
| Pa41, or29 | 43 | 11.80, 12.15 | 514 | 513 | 367, 498, 485, 469, 456, 444 | Icariside II |
| Pa41, or29 | 44 | 12.42, 12.96 | 560 | 559 | 439, 395, 321, 270, 269 | Apigenin-8-C-[6-deoxy-2-O-rhamnosyl]-xylohexos-uloside |
| Pa41, ol50, gla39, glo38 | 45 | 13.48 | 466 | 465 | 447, 373, 345, 328, 303, 284, 258, 178 | Taxifolin-hexoside |
| Pa41 | 46 | 14.00 | 508 | 507 | 506, 345, 328, 314, 288, 239, 229, 215, 195, 186, 174, 152 | Syringetin-3-O-galactoside |
| Pa41, ol50, gla39, or29, 37, glo26, 38 | 47 | 14.10 | 493 | 492 | 421, 400, 342, 316, 227, 214, 168 | Petunidin-glucuronide |
| Pa41, or37 | 48 | 15.14, 15.26 | 493 | 492 | 477, 301 | Quercetin-methoxyglucuronide |
| Pa41, gla39, 17, or29 | 49 | 15.20, 15.78 | 556 | 555 | 671, 592, 450, 431, 401, 357, 295 | Fukugetin |
| Pa41, ol50, gla39, 17, t2, 58, or29, 37, glo38 | 50 | 15.00 | 508 | 507 | 507, 345, 328, 315, 301, 260, 231, 215, 199, 175, 158 | Syringetin-3-O-glucoside |
| Pa41, m49, ol50, gla39, 17, or29, glo26, 38 | 51 | 17.96, 18.67 | 688 | 687 | 645, 525, 567, 328, 327 | Luteolin-5-O-hexosyl-8-C-(6''-acetyl)-hexoside derivative |
| Pa41, gla39, or29 | 52 | 20.17, 20.87 | 646 | 645 | 492, 482, 331, 267, 179, 151 | Laricitrin-(6''-O-galloyl)-3-O-hexose |
| S40, c7 | 53 | 4.63 | 594 | 593 | 578, 565, 549, 329, 285, 202, 220, 197, 176 | 2',6'-dihydroxy-4-methoxychalcone-4'-O-neohesperidoside |

Table 2 (continued)

| Species/accession | N | t _g (min) | Mw | m/z | MS/MS | Identification |
|-----------------------------|----|----------------------|-----|-----|--|--|
| S40, c7 | 54 | 6.09, 6.79 | 624 | 623 | 623, 591, 461, 477, 315, 299, 285, 271, 227, 192, 182, 158 | Isorhamnetin-3-galactoside-6'-rhamnoside |
| S40 | 55 | 8.20, 8.39 | 624 | 623 | 624, 533, 503, 412, 383, 299, 300, 284 | Chrysoeriol-6,8-di-C-glucoside |
| S40, c7 | 56 | 8.23 | 394 | 393 | 375, 363, 351, 349, 335, 323, 306, 175 | Deguelin |
| S40, c7 | 57 | 8.56, 9.2 | 608 | 607 | 607, 565, 443, 299, 283, 200, 161 | Wogonin-di-hexoside |
| S40 | 58 | 9.06, 9.14 | 756 | 755 | 754, 609, 593, 581, 300, 301, 298, 271, 254 | Quercetin-3-O-[O-coumaroyl]-glucopyranosyl]-rhamnopyranoside |
| S40, 45, m49, t2, 58, g1o26 | 59 | 10.33, 11.02 | 757 | 756 | 755, 593, 447, 394, 341, 284, 183 | Cyanidin-3-O-glucosylrutinoside |
| S40 | 60 | 10.04 | 424 | 423 | 405, 313, 300, 284, 273, 244 | Sophoraflavanone G |
| S40 | 61 | 10.40 | 379 | 378 | 299, 290, 273, 254, 218 | 4,4'-dihydroxy-dicoumarin derivative |
| S40 | 62 | 11.47, 11.77 | 526 | 525 | 506, 328 | Tricin-4'-O-(guaiaacylglyceryl) ether |
| S40 | 63 | 11.67, 12.22 | 360 | 359 | 359, 344, 331, 315, 288, 272, 241, 184 | Irigenin |
| S40 | 64 | 12.43 | 390 | 389 | 800, 719, 373, 345, 344, 245 | Scaposin |
| S40 | 65 | 12.36, 12.60 | 344 | 343 | 343, 328, 315, 283, 298, 271, 221, 211, 194, 185, 177, 155 | 4-hydroxy-2',3,4',6'-tetramethoxychalcone |
| S40 | 66 | 12.90 | 418 | 417 | 480, 416, 282, 255, 241, 202, 177 | Liquiritin |
| S40 | 67 | 13.09 | 330 | 329 | 314, 301, 299, 285, 272 | Desmethoxycentaureidin |
| S40 | 68 | 12.90 | 508 | 507 | 345, 343 | Spinacetin-7-glucoside |
| S40, t2, 58 | 69 | 13.43, 13.86 | 450 | 449 | 449, 317, 288, 271, 258, 243, 231, 191 | Myricetin-3-xyloside |
| S40 | 70 | 13.90 | 418 | 417 | 592, 416, 285, 210, 201, 185 | Kaempferol-3-O-arabinoside |
| S40 | 71 | 14.65, 14.91 | 314 | 313 | 327, 298, 283, 314, 283, 200, 163 | Dipteryxin |

Table 2 (continued)

| Species/accession | N | t _R (min) | Mw | m/z | MS/MS | Identification |
|-------------------------------------|----|----------------------|-----|-----|--|---|
| S40 | 72 | 21.18 | 578 | 577 | 645, 624, 578, 431, 269 | Apigenin-7-neohesperidoside |
| M49, or37, glo26 | 73 | 10.03, 10.86 | 416 | 415 | 415, 373, 326, 307, 295, 236, 204, 192, 186, 156 | Daidzein-8-C-glucoside |
| M49, glo26 | 74 | 10.45, 10.78 | 396 | 395 | 395, 372, 365, 347, 321, 318, 205, 192, 177, 165, 138 | Dihydrorotenone |
| M49, glo26 | 75 | 12.14 | 610 | 609 | 609, 582, 487, 469, 448, 389, 301, 295, 285, 228, 179 | Quercetin-3-O-neohesperidoside |
| M49, or37, glo26 | 76 | 12.41, 12.88 | 430 | 429 | 448, 430, 425, 385, 360, 328, 267, 210, 194, 183, 164 | Tectochrysin-7-O-glucoside |
| M49, or37, glo26 | 77 | 13.00, 13.76 | 472 | 471 | 442, 441, 406 | 3-methyl-epigallocatechin gallate |
| M49, glo26 | 78 | 13.53, 13.84 | 442 | 441 | 425, 409, 384, 379, 365, 309, 295, 285, 276, 267, 262, 214, 190, 108 | Villol |
| M49 | 79 | 16.04, 16.23 | 532 | 531 | 487, 355, 337, 326, 298, 283 | Biochanin A-8-C-malonylglucoside |
| M49, glo26 | 80 | 15.97 | 484 | 483 | 440, 331, 314, 271, 168, 126 | Digalloyl glucose |
| O150, gla39, or37 | 81 | 13.25 | 526 | 525 | 529, 509, 479, 449, 407, 397, 386, 361, 251, 178 | Bromadiolone |
| O150, gla17, 12.58, or29, 37, glo38 | 82 | 14.53, 15.25 | 495 | 494 | 493, 479, 332, 329, 317, 271, 243, 178 | Myricetin-1-methoxy-4-O-hexoside |
| O150 | 83 | 19.99 | 314 | 313 | 314, 298, 283, 211 | Kumatakenin |
| O150 | 84 | 21.32, 21.68 | 330 | 329 | 301, 299, 285, 284, 254, 177 | 2',4'-dihydroxy-3',4',6'-trimethoxychalcone |
| O150 | 85 | 22.16 | 296 | 295 | 202, 179 | Caffeic acid derivative |
| O150 | 86 | 23.40, 23.48 | 652 | 651 | 588, 547, 507, 489, 345, 330 | Limocitrin-O-glucoside-hydroxy-methylglutaryl |
| Gn54 | 87 | 10.19 | 394 | 393 | 432, 345, 319, 301, 239, 187, 111 | Unknown |
| Gn54, or37 | 88 | 12.05, 12.72 | 457 | 456 | 441, 407, 375 | Methyl-(epi) catechin gallate |
| Gn54 | 89 | 14.41, 14.47 | 374 | 373 | 526, 421, 403, 351, 317, 273, 189 | Myricetin-3,7,3',4'-tetramethylether |

Table 2 (continued)

| Species/accession | N | t _r (min) | Mw | m/z | MS/MS | Identification |
|-------------------|-----|----------------------|-----|-----|--|---|
| Gn54 | 90 | 14.12 | 436 | 435 | 435, 365, 350, 351, 295, 270 | Orotinichalcone |
| S45 | 91 | 9.54 | 456 | 455 | 521, 409 | 3-hydroxycinnamic dirhamnoside acid |
| S45 | 92 | 11.40 | 394 | 393 | 394, 363, 351, 323, 316, 291, 174 | Rotenone |
| S45 | 93 | 11.98 | 374 | 373 | 345, 329, 313 | Skullcapflavone II |
| S45 | 94 | 11.50 | 482 | 481 | 551, 481, 380, 351, 303, 282, 255, 243, 222, 212, 177, 151 | Silychristin |
| S45 | 95 | 12.00, 12.24 | 446 | 445 | 493, 430, 417, 403, 283, 269, 270, 175 | 7-O-glucopyranosyl-4'-hydroxy-5-methoxyisoflavone |
| S45 | 96 | 15.14, 15.26 | 392 | 391 | 391, 376, 361, 331, 302, 284, 228, 200 | Dehydrotrotenone |
| S45 | 97 | 16.21, 16.34 | 450 | 449 | 448, 329, 287, 243, 226, 185, 168, 170 | Flavanomarein |
| S45 | 98 | 16.64 | 288 | 287 | 287, 269, 259, 243, 230, 223, 215, 176 | 6,7,3',4'-tetrahydroxyflavanone |
| S45 | 99 | 16.77, 16.94 | 568 | 567 | 436, 435, 273, 274 | Phloretin-xyloglucoside |
| S45 | 100 | 17.14, 18.03 | 584 | 583 | 297, 285, 266, 258, 238, 199, 187, 175 | Kaempferol-3-O-galloylramnoside |
| Gla39, glo38 | 101 | 8.60, 9.26 | 346 | 345 | 343, 346, 289, 194, 170, 101 | Catechin-tetramethylether |
| Gla39, 17, or29 | 102 | 8.31 | 404 | 403 | 358, 271, 192, 182, 113, 101 | Naringenin-arabinoside |
| Gla39, 17 | 103 | 9.49 | 404 | 403 | 375, 359, 346, 331, 257, 182, 109, 102 | Butein-arabinoside |
| Gla39 | 104 | 9.60, 10.47 | 564 | 563 | 457, 397, 294, 199, 182, 176, 164 | Theaflavin |
| Gla39 | 105 | 10.79, 11.74 | 580 | 579 | 648, 581, 573, 451, 376, 311, 271, 119 | Naringin |
| Gla39 | 106 | 11.58, 11.71 | 438 | 437 | 438, 437, 423, 377, 347, 293, 164, 135, 108 | 5-O-methyllicoricidin |
| Gla39 | 107 | 12.21, 12.42 | 450 | 449 | 449, 431, 287, 269, 213, 169, 157, 137 | Okamin-4'-O-glucoside |

Table 2 (continued)

| Species/accession | N | t _R (min) | Mw | m/z | MS/MS | Identification |
|-------------------|-----|----------------------|-----|-----|---|---|
| Gla39 | 108 | 13.43, 13.51 | 360 | 359 | 360, 359, 346, 271, 243, 232, 201, 177, 166 | Jaceidin |
| Gla39 | 109 | 13.36, 13.48 | 408 | 407 | 241, 227, 219, 188, 179, 134 | Remangiflavanone |
| Gla39, or29 | 110 | 14.60, 14.70 | 286 | 285 | 767, 587, 399, 285, 226, 199, 187, 151 | Kaempferol |
| Gla17 | 111 | 14.13 | 500 | 499 | 481, 472, 354, 313, 191, 173 | 3-O-Caffeoyl-5-O-coumaroylquinic acid |
| Gla17 | 112 | 20.59 | 688 | 687 | 525, 329, 328 | Tricin-4'-(O-guaicylglyceryl) ether-7-O-glucoside |
| T2, 58 | 113 | 11.00 | 610 | 609 | 636, 610, 609, 463, 447, 301, 163 | Quercetin-3-glucoside-7-rhamnoside |
| T2, 58 | 114 | 14.10 | 453 | 452 | 317, 306, 288, 162 | Hydroxycinnamic acid derivative |
| T2, 58 | 115 | 18.93 | 506 | 505 | 463, 447, 343, 301, 256, 215, 153 | Quercetin-3-O-glucosyl-6'-acetate |
| T2, 58, glo38 | 116 | 19.62 | 550 | 549 | 549, 506, 301, 271, 255 | Quercetin-3-O-manoylglycoside |
| T2, 58 | 117 | 21.69, 21.87 | 602 | 601 | 449, 469 | Myricetin-3-O-(2'-O-galloyl)-pentoside |
| T58 | 118 | 16.50 | 508 | 507 | 507, 308, 266, 181, 162 | Scutellaroside II |
| Or29 | 119 | 10.58 | 568 | 567 | 541, 521, 485, 447, 342, 316, 285, 227, 177 | Luteolin-8-C-(6'-O-benzoyl)-glucoside |
| Or29 | 120 | 10.53, 10.62 | 710 | 709 | 667, 664, 623, 604, 385, 286 | Chrysoeriol-O-malonyl-di-glucoside |
| Or29 | 121 | 10.94 | 562 | 561 | 429, 399, 398, 267, 252 | Formononetin-7-O-apiofuranosyl-O-glucopyranoside |
| Or29 | 122 | 10.91 | 512 | 511 | 310, 285, 214, 191, 177 | Picroside II |
| Or29 | 123 | 12.99 | 530 | 529 | 366 | Feruloyl-caffeoyl quinic acid |
| Or29 | 124 | 13.50, 13.56 | 465 | 464 | 462, 302, 299, 284, 257, 227, 216, 179 | Delphinidin-3-galactoside |
| Or29 | 125 | 13.91 | 516 | 515 | 615, 429, 309, 295, 255, 245, 239, 196 | 6'-malonyl-ononin |
| Or29 | 126 | 14.91 | 516 | 515 | 447, 353, 309 | Luteone-O-glucoside |

Table 2 (continued)

| Species/accession | N | t _r (min) | Mw | m/z | MS/MS | Identification |
|-------------------|-----|----------------------|-----|-----|--|---|
| Or29 | 127 | 16.07 | 446 | 445 | 371, 325, 298, 283, 185, 176 | Biochanin-7-O-glucoside |
| Or29 | 128 | 20.55, 20.71 | 564 | 563 | 565, 545, 533, 504, 484, 475, 407, 394, 382, 193 | Isoschaftoside |
| Or29 | 129 | 22.36 | 563 | 562 | 560, 558, 547, 430, 400, 253, 224, 166 | Daidzein-methoxy-O-pentoside-hexoside |
| Or37 | 130 | 9.74 | 452 | 451 | 452, 289, 245, 205, 179, 167 | Catechin-hexoside |
| Or37, gl26 | 131 | 13.31 | 456 | 455 | 533, 522, 414, 409, 283, 219 | 3-hydroxycinnamic acid derivatives |
| Or37 | 132 | 29.75 | 268 | 267 | 266, 252, 239, 211, 169, 152, 133 | 7-hydroxy-2'-methoxyflavone |
| Gl26 | 133 | 23.39 | 344 | 343 | 710, 344, 315, 299, 298, 286, 297, 204, 196, 186, 179, 116 | Nevadensin |
| Gl26 | 134 | 11.51, 12.01 | 578 | 577 | 577, 573, 408, 381, 320, 225 | Procyanidin B1 |
| Gl26 | 135 | 12.30 | 512 | 511 | 511, 279, 280 | Pumiloside |
| Gl26 | 136 | 22.26 | 645 | 644 | 492, 468, 332, 316, 179, 151 | Petunidin-(6"-O-galloyl)β-O-glucuronide |
| Gl26 | 137 | 22.4 | 328 | 327 | 741, 456, 328, 299, 292, 282, 270 | Zapotinin |
| C7 | 138 | 3.50, 3.92 | 354 | 353 | 335, 309, 284, 269, 255, 227, 161 | Lico-isoflavone A |
| C7 | 139 | 5.04, 5.26 | 772 | 771 | 771, 770, 608, 447, 300 | Quercitrin-4-O-di-hexoside |
| C7 | 140 | 6.68 | 594 | 593 | 594, 431, 447, 285, 256, 212 | Kaempferol-3-O-glucoside-3"-rhamnoside |
| C7 | 141 | 7.68, 7.79 | 448 | 447 | 447, 327, 285, 256, 210 | Kaempferol-3-glucopyranoside |
| C7 | 142 | 10.75, 11.32 | 436 | 435 | 417, 375, 332, 289, 245, 206 | Catechin-3-O-rhamnoside |
| C7 | 143 | 12.02, 12.22 | 408 | 407 | 408, 313, 300, 287, 271, 261, 201 | 6-geranylharingenin |
| C7 | 144 | 14.88, 15.02 | 330 | 329 | 329, 314, 299, 271, 242, 227, 199, 151 | Tricin |
| C7 | 145 | 15.33 | 288 | 287 | 598, 287, 285, 269, 207, 180 | Dihydrokaempferol |

Table 2 (continued)

| Species/accession | N | t _R (min) | Mw | m/z | MS/MS | Identification |
|-------------------|-----|----------------------|-----|-----|-----------------------------------|-----------------------------------|
| C7 | 146 | 17.58, 17.69 | 450 | 449 | 450, 287, 269, 217, 187, 176, 151 | Eriodictyol-7-O-glucoside |
| C7 | 147 | 21.40 | 328 | 327 | 745, 678, 657, 285, 282 | Kaempferol-3,7,4'-trimethyl ether |
| C7 | 148 | 23.17 | 578 | 577 | 579, 577, 299, 285 | Kaempferol-di-O-rhamnoside |

at infraspecific levels. As an endemic species, the four markers obtained from *T. oliverianum* were first specified in detail (Table 2). In section *Scordium*, *T. melissoides* was characterised by one marker and *T. scordium* subsp. *scordioides* by 24 markers (Table 2). To verify the identified

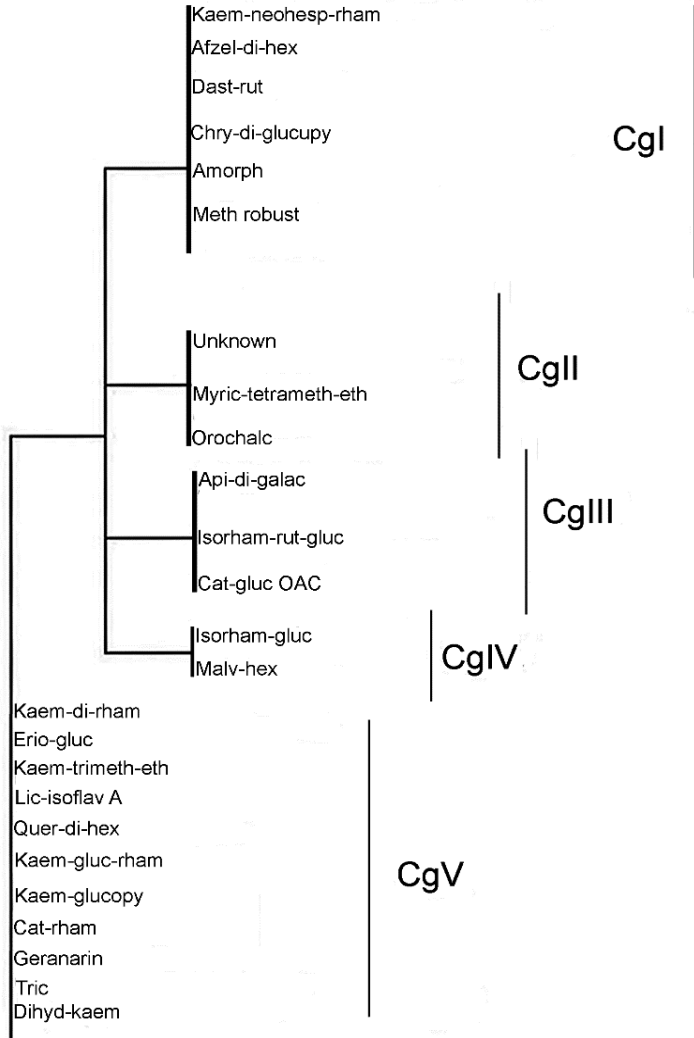


Fig. 2. Dendrogram related to the members of sections *Polium* using cluster analysis and chemical markers. Cg I = *T. capitatum* 5; Cg II = *T. gnaphalodes* 54; Cg III = *T. gnaphalodes* 64; Cg IV = *T. polium* 46, Cg V = *T. capitatum* 7. The chemical compounds are completely listed in Table 2

candidate markers in the *Teucrium* genus, more investigations need to be carried out by other specific analytical methods.

The cluster analysis combined with HPLC-MS dataset of markers clearly showed 13 groups in section *Teucriis* and *Scordium* and five groups in section *Polium* (Figs 1–2). Regarding cluster results, the members of three sections were completely separated. Obviously, specific and infraspecific levels verified the candidate markers.

As revealed by PCA analysis, all chemotypes with the exception of *T. melissoides*, *T. oliverianum*, and *T. parviflorum* were identified in *Teucrium* species. Ten chemotypes were identified in sections *Teucriis* and *Scordium*, and six chemotypes were observed in section *Polium*. Therefore, all chemotypes in each species were significantly discriminated by natural habitats. Total variability was considered for both principal components in sections *Teucriis*, *Scordium* (var. 33.86%), and section *Polium* (var. 57.18%). PC1 and PC2 components provided evidence that all chemotypes were divided based on geographical location (Fig. 3A, B). The natural accumulation of flavonoid types was further shown in each habitat. The identified chemotypes were completely different concerning the chemical constituents present in this study. As observed, each chemotype was assessed by a specific type of flavonoid.

Based on the PCA score plot in combination with HPLC-MS, there was a relationship between ecological conditions and certain predominant flavonoid types (Fig. 4). In sections *Teucriis* and *Scordium*, four groups of provinces were significantly rich in particular flavonoid compounds: 1) Kurdistan, Luristan, Chahrmahal va Bakhtiari, 2) Bushehr, Fars, Kohgilouyeh va Boyerahmad, 3)

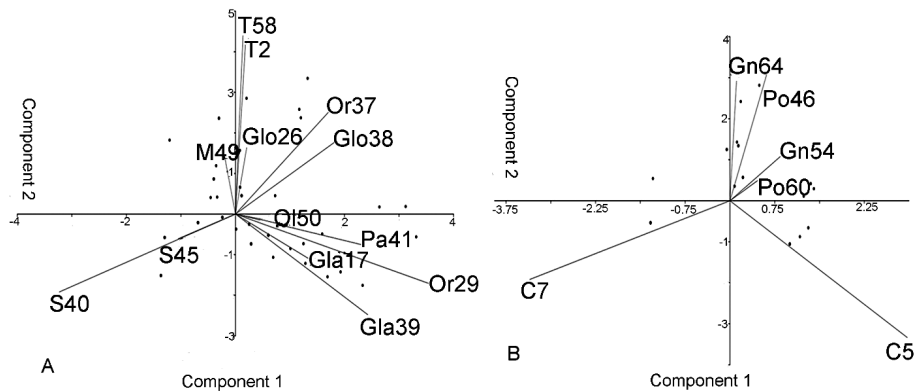


Fig. 3. Different chemotypes in *Teucrium* accessions belonging to A) sections *Teucriis* and *Scordium* and B) section *Polium* using Principal Component Analysis and chemical markers. T = *T. orientale* subsp. *taylori*, Or = *T. orientale* subsp. *orientale*, Glo = *T. orientale* subsp. *gloeotrichum*, Gla = *T. orientale* subsp. *glabrescens*, Ol = *T. oliverianum*, Pa = *T. parviflorum*, M = *T. melissoides*, S = *T. scordium* subsp. *scordioides*, Po = *T. polium*, Gn = *T. gnaphalodes*, C = *T. capitatum*

Isfahan, and 4) southern Isfahan. The accumulation of flavonol-type 1 was characterised in tropic and sub-tropic geographical regions. Flavone-type 1 and isoflavone-type 1 were considered in arctic regions, whereas flavonol-type 2 and flavone-type 2 were assigned to temperate regions. Semiarid regions were associated with flavan-type 1 (Fig. 4A).

Regarding section *Polium*, five groups were also detected: 1) Chahramahal va Bakhtiari, 2) northwest of Boyerahmad, 3) Kurdistan, 4) Bushehr, and 5) Fars and southwest of Boyerahmad. In terms of flavonoid compounds, flavonol-type 3 and flavone-type 3 frequently accumulated in the arctic geographical regions. In addition, flavonol-type 4 was found in sub-tropical regions and flavonol-type 5 belonged to semiarid regions. However, chalcone was concentrated in tropical regions (Fig. 4B). The present study showed that arctic regions were chiefly place to dominant ingredients, such as di-tri- and penta-hydroxyl, trihydroxy-methoxy, and methoxy flavone substitutions. Temperate regions were mostly rich in trihydroxy-methoxy flavone, hexoside, and xyloside flavonol substitutions. Moreover, tropic, sub-tropic, and arctic geographical regions principally showed hexoside and methylated flavonol substitutions. Sub-tropical regions were definitely discriminated in aglycones such as quercetin and isorhamnetin derivatives. Meanwhile, arctic and semi-arid geographical regions generally presented kaempferol derivatives along with hexoside and hydroxyl flavans. Based on our results, two geographical regions, namely Isfahan and Boyerahmad had a high chemical divergence.

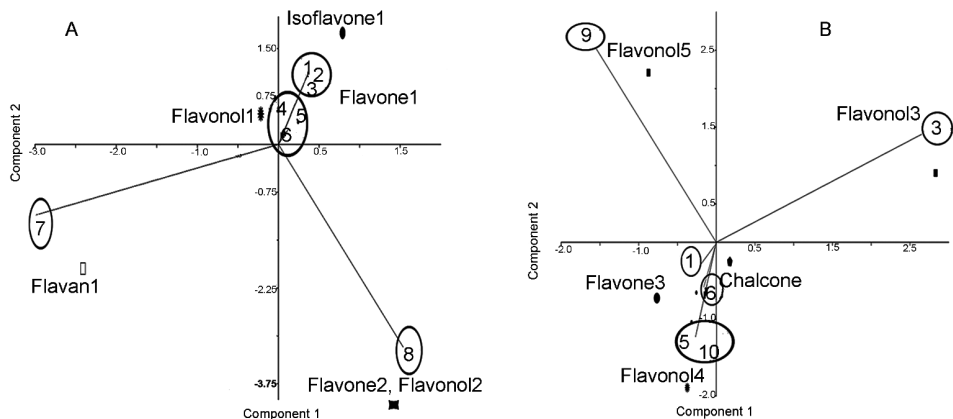


Fig. 4. A PCA score plot comparing the ecological habitats and the accumulation of flavonoid compounds belonging to A) sections *Teucrium* and *Scordium*. 1 = Kurdistan, 2 = Luristan, 3 = Chaharmahal va Bakhtiari, 4 = Kohgiluyeh va Boyer Ahmad, 5 = Fars, 6 = Bushehr, 7 = Isfahan, 8 = southern Isfahan, B) section *Polium*. 9 = northwest of Boyer Ahmad, 10 = southwest of Boyer Ahmad

It was revealed that environmental factors such as altitude could be responsible for the quantity (number) of chemical constituents. As seen in Tables 1 and 2, the quantity of compounds increased with the rise in altitude. Except for *T. melissoides* (h = 1,900 m), *T. orientale* subsp. *orientale* (2,406 m), and *T. polium* (h = 1,121 m), the quantity of compounds was reduced by the decrease in altitude, which might be attributed to the influence of temperature in southern geographical locations.

DISCUSSION

This section details the identification and verification of fingerprinting profiles in *Teucrium* species. The fragment ions in MS/MS spectra can further elucidate the subject (Fig. 5). The compounds were comprehensively assessed by the reference standards and literature data.

Flavone derivatives

One of the most known flavone compounds is luteolin as an aglycone. A detailed analysis of MS/MS spectra allowed for the characterisation of six luteolin derivatives in *Teucrium* species. In the fragmentation ions of some *Teucrium* species, a molecular ion at m/z 637 and its pseudo molecular ion (m/z 285) corresponded to luteolin-7-*O*-diglucuronide (Table 2). Moreover, the product ion at m/z 687 and its daughter ions (m/z 525, m/z 567, and m/z 645) could be tentatively derived from luteolin-5-*O*-hexosyl-8-*C*-(6''-acetyl)-hexoside derivative. Additional product ion at m/z 447 and released aglycone ion at m/z 285 are the characteristics of isoorientin. More aglycone derivatives, such as 6-di-methoxyluteolin (m/z 315; base peak aglycone at m/z 285) for *T. capitatum* 5 and *T. orientale*

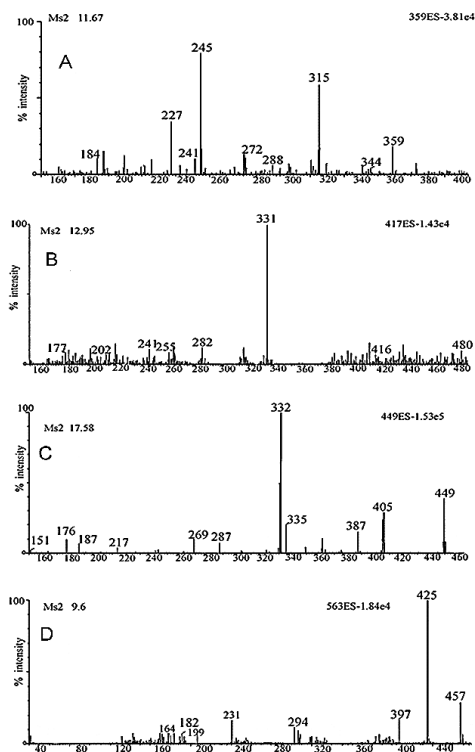


Fig. 5. MS/MS fragments of certain flavonoid compounds. A) irigenin (m/z 359) and B) liquiritin (m/z 417) in *T. scordium* subsp. *scordioides* (40), C) eriodictyol-7-glucoside (m/z 449) in *T. capitatum* (7), D) theaflavin (m/z 563) in *T. orientale* subsp. *glabrescens* (39)

subsp. *glabrescens* 39, were identified. Two tentative compounds by deprotonated parent peak at m/z 379 and m/z 567, respectively, detected luteolin-3-methylether-7-sulphate (fragment ion at m/z 285) and luteolin-8-C-(6''-O-benzoyl)-glucoside (released ions at m/z 521 and m/z 447) in certain *Teucrium* species (Table 2). All the evidence is in accordance with previous reports and reference standards (Bezerra *et al.* 2016, <http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, Noor Hashim *et al.* 2018, Sobeh *et al.* 2016). Compared with several previous studies, luteolin, luteolin glucoside or isoorientin, methoxyluteolin-7,3',4'-trimethylether, and luteolin-methylether derivatives were identified in *T. pilosum* (Pamp.) C. Y. Wu et Chow and *Ajuga laxmannii* (Murray) Benth. (Lamiaceae) (Atay *et al.* 2016, Hawas *et al.* 2008, Mihailović *et al.* 2020). Of note, luteolin derivatives showed remarkable hexoside substitution at C- and O-positions in *Teucrium* species.

Methoxy-luteolin or chrysoeriol is another flavone compound. Chrysoeriol derivatives, three in total, were also identified in the extract. In MS/MS spectrum, the identified compound was considered as chrysoeriol-7-O-diglucuronopyranoside (m/z 651; base peak at m/z 299) for *T. capitatum* 5. Two further derivatives followed by molecular ions at m/z 623 and m/z 709, respectively, led to the identification of chrysoeriol-6,8-di-C-glucoside (daughter ion at m/z 299) and chrysoeriol-O-malonyl-di-glucoside (additional fragment ions at m/z 623 and m/z 385) for *T. scordium* subsp. *scordoides* 40 and *T. orientale* subsp. *orientale* 29 (Table 2). All the evidence is in line with the reference standards and previously published research (Delazar *et al.* 2012, <http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>). Chrysoeriol-7-O-glucopyranoside and chrysoeriol-glucuronide were primarily identified in *Ajuga chamaepitys* (L.) Schreb., and *Phlomis fruticosa* L. (Lamiaceae) (Delazar *et al.* 2012, Marin *et al.* 2007).

Besides the above-mentioned flavones, tectochrysin and laricitrin as methoxy flavones, were detected in some *Teucrium* species. The fragmentation patterns exhibited mass molecular ions at m/z 645, generating daughter ions at m/z 331. Moreover, [M-H]⁻ ion at m/z 429 yielded a dissociated ion at m/z 267. Consistent with previous reference standards, those compounds were considered to be laricitrin-(6''-O-galloyl)-3-O-hexose and tectochrysin-7-O-glucoside (Atay *et al.* 2016) (Table 2). Chemical ingredients such as methoxy flavones were also present in the form of O-hexoside substitutions in *Teucrium* species.

More predominant hydroxy-methoxy flavone derivatives were mostly detected in *Teucrium* species. A derivative found in *T. scordium* subsp. *scordoides* 40 and *T. capitatum* 7 was wogonin-di-hexoside. Its mass molecular ion at m/z 607 produced a pseudo molecular ion at m/z 283 (<http://massbank.eu>). Moreover, typical derivatives such as cirsiol (m/z 329; released ions at m/z

301, m/z 285, m/z 272, and m/z 299), cirsilineol (m/z 343; daughter ions at m/z 315, m/z 299, and m/z 298), and cirsimaritin (m/z 313; fragment ions at m/z 285, m/z 269, and m/z 283) were identified in certain *Teucrium* species (Table 2). These compounds were previously found in *T. polium* and *T. barbeyanum* Aschers. (Alwahsh *et al.* 2015, Mihailović *et al.* 2020, Mitreski *et al.* 2014). Further dihydroxy-trimethoxy flavone derivatives with identical molecular ions at m/z 343, different retention times, and dissociated ions were tentatively identified in *T. parviflorum* 41 and *T. orientale* subsp. *gloeotrichum* 26. Given the retention time at 23.39, the successive losses of hydroxyl and methoxy groups by m/z 315, m/z 299 and m/z 298 were attributed to nevadensin. Robinetin trimethyl ether was identified at 8.46 and 8.85 retention times, supported by the reference standards (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, Mitreski *et al.* 2014). It has been demonstrated that nevadensin is first introduced for *Origanum vulgare* L. (Lamiaceae) (Napoli *et al.* 2020).

Additional types of hydroxy-methoxy flavones were identified in *T. capitatum* 5, *T. orientale* subsp. *orientale* 37, and *T. orientale* subsp. *gloeotrichum* 38. Deprotonated parent peaks at m/z 327, m/z 267, and m/z 327, respectively, represented 3-hydroxy-3',4',5'-trimethoxyflavone (characteristic ions at m/z 299, and m/z 282), 7-hydroxy-2'-methoxyflavone (daughter ions at m/z 252 and m/z 239), and zapotinin (m/z 327) (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>). It was concluded that 3-hydroxy-3',4',5'-trimethoxyflavone at 15.45 and 15.67 retention times was tentatively differentiated from zapotinin at 22.4 retention time (Table 2). The first published studies reported hydroxy-methoxyflavone derivatives for *Teucrium* species (Alwahsh *et al.* 2015, Mihailović *et al.* 2020). Zapotinin was first identified in *Casimiroa edulis* La Llave (Rutaceae) by Kotta *et al.* (2013). Regarding this information, hydroxy-methoxy flavone derivatives presented predominant variety in *Teucrium* species.

More derivatives of flavonoid as trihydroxy-dimethoxy groups were recognised in *T. capitatum* 7, *T. scordium* subsp. *scordioides* 40, and *T. orientale* subsp. *glabrescens* 17. Two tentatively proposed compounds, namely triclin-4'-O-(guaiacylglyceryl) ether (m/z 525) and triclin-4'-(O-guaiacylglyceryl)-ether-7-O-glucoside (m/z 687; base peak at m/z 525), were respectively identified. More compounds with the same mass molecular ion at m/z 329 were different at various retention times. The former was tentatively found desmethoxycentaureidin (m/z 329; additional fragment ions at m/z 301, m/z 285, m/z 272 and m/z 299, RT = 13.09) (Table 2). The latter was verified as triclin (m/z 329, RT = 14.88, 15.02) (Table 2), which is consistent with the reference standards and published reports (Ahmed *et al.* 2019, <http://mona.fiehnlab.ucdavis.edu>). Both triclin derivatives and desmethoxycentaureidin first corresponded to *Teucrium* species. Previous research revealed trihydroxy-dimethoxy flavones in *Teucrium* species (Hawas *et al.* 2008).

Scaposin and limocitrol, additional trihydroxy-tetramethoxy and tetrahydroxy-trimethoxy flavones, were primarily identified at m/z 389 and m/z 375 in *T. scordium* subsp. *scordioides* 40, *T. capitatum* 5, and *T. gnaphalodes* 54 (<http://mona.fiehnlab.ucdavis.edu>). Moreover, dihydroxy-tetramethoxy flavones were detected in *T. scordium* subsp. *scordioides* 45. In this context, a molecular ion at m/z 373 revealed pseudo molecular ions at m/z 345, m/z 329, and m/z 313 (Table 2). This flavone was concluded to be skullcapflavone II in structure (<http://mona.fiehnlab.ucdavis.edu>). Based on the literature, limocitrol has further been described for *Citrus × limon* L. Burm. f. (Rutaceae) (El Sayed *et al.* 2017).

Another trihydroxy-methoxy flavone derivative (Icariside II) first corresponded to *T. parviflorum* 41 and *T. orientale* subsp. *orientale* 29. The molecular ion at m/z 513 yielded characteristic ions at m/z 367, m/z 444, m/z 498, m/z 485, m/z 496, and m/z 456, indicating the successive loss of hydroxyl unit (<http://mona.fiehnlab.ucdavis.edu>).

As can be found in certain *Teucrium* species, two diosmetin derivatives were identified in the extract. Both derivatives were observed to have identical molecular ions at m/z 607, while it showed different retention times, 11.54 and 13.20 (Table 2). Most of the products detected in *Teucrium* species respectively led to the formation of diosmin with pseudo molecular ion at m/z 299 and diosmetin-coumaroylglucoside with contribution aglycone ion at m/z 299 (Table 2). All the evidence is in accordance with the reference standards (<http://massbank.eu>). Diosmetin derivative was further reported for *T. polium*, *T. gnaphalodes*, and *T. montanum* L. (Mihailović *et al.* 2020, Mitreski *et al.* 2014).

Apigenin, a characteristic aglycone, is considered as a significant flavone compound in medicinal plants. Its derivatives, five in total, were found in this research. A precursor ion at m/z 593 and its fragment ion at m/z 269 corresponded to apigenin-6,8-digalactoside for *T. gnaphalodes* 64. Apigenin-7-neohesperidoside (rhoifolin) (m/z 577; dissociated ion at m/z 269) was another derivative tentatively suggested for *T. scordium* subsp. *scordioides* 40. A mass molecular ion at m/z 559 and its pseudo molecular ions at m/z 395 and m/z 439 were tentatively attributed to apigenin-8-C-[6-deoxy-2-O-rhamnosyl]-xylo-hexos-uloside for *T. parviflorum* 41 and *T. orientale* subsp. *orientale* 29. Two parent ions at m/z 725 and m/z 563 resulted in the identification of isovitexin-4'-O-glucoside-2''-O-arabinoside (releasing daughter ions at m/z 563 and m/z 693) and isoschaftoside in some *Teucrium* species. This finding was confirmed by the reference standards and prior documents (Bezerra *et al.* 2016, <http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, Zhang *et al.* 2011). As previously published, apigenin, apigenin-glucoside or isovitexin derivative, and isoschaftoside were identified in *T. gnaphalodes*, *Vaccaria hispanica* (Mill.) Rauschert (Caryophyllaceae), and *Passiflora incarnate* L. (Passifloraceae) (Mihailović *et al.* 2020, Mitreski *et al.* 2014, Singh *et al.* 2012, Zhang *et al.* 2011). Apigenin derivatives were assigned to hexoside and xyloside substitutions.

Trihydroxy flavone derivative was also identified in *T. parviflorum* 41 and *T. scordium* subsp. *scordioides* 40. As compared with the existing reference standards, mass molecular ion at m/z 435 was indicative of artocaprin (<http://mona.fiehnlab.ucdavis.edu>). In addition, hexahydroxy flavones were found in some *Teucrium* species along with four proposed myricetin derivatives. A precursor ion at m/z 449 and aglycone ion at m/z 317 verified myricetin-3-xyloside. The molecular ion peak at m/z 494 followed by fragment ions at m/z 479 and m/z 332, indicated myricetin-1-methoxy-4-*O*-hexoside. A base peak at m/z 601 and its dissociated ions at m/z 449 and m/z 469 were attributed to myricetin-3-*O*-(2''-*O*-galloyl)-pentoside. The molecular peak at m/z 373 and its fragment ion at m/z 317 were suggested for myricetin-3,7,3',4'-tetramethylether (Table 2) (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, <http://pubchem.ncbi.nlm.nih.gov>, Sobeh *et al.* 2016). Myricetin and its derivatives, including myricetin-3-*O*-(2''-*O*-galloyl)-pentoside were first published for *T. scordium*, *T. polium*, and *Schotia brachypetala* Sond. (Fabaceae) (Mihailović *et al.* 2020, Milošević-Djordjević 2018, Sobeh *et al.* 2016).

Fukugetin, a morelloflavone or biflavonoid derivative, was observed in certain *Teucrium* species (Table 2). A mass molecular ion at m/z 555 identified this compound, as previously reported for *Garcinia xanthochymus* Hook. f. ex T. Anderson (Clusiaceae) (Rios *et al.* 2015).

Regarding the published evidence, flavonoid aglycones were mostly present in the form of *O*-glucosides or free aglycones in *Teucrium* species (Mitreski *et al.* 2014). Moreover, flavone derivatives exhibit the most abundant flavonoids in this genus (Mihailović *et al.* 2020).

Flavonol derivatives

Different isorhamnetin derivatives found in the extract were recognised in *Teucrium* species (Table 2). The aglycone derivatives with the same molecular ion at m/z 623 and different retention times were tentatively characterised by isorhamnetin-3-*O*-rutinoside (base peak at m/z 315, RT = 10.71, 11.41) and isorhamnetin-3-galactoside-6''-rhamnoside (m/z 461 and m/z 477, RT = 6.09, 6.79) for certain *Teucrium* species (Table 2). The presence of base peaks led to the identification of isorhamnetin-5-*O*-diglucoside (m/z 639; aglycone ion at m/z 315) and isorhamnetin-3-*O*-rutinoside-7-*O*-glucoside (a precursor ion at m/z 785; m/z 623 and m/z 477) for *T. polium* 46 and *T. gnaphalodes* 64. The peak obtained at m/z 315 also described the isorhamnetin in structure. The detected flavonol compounds were also identified by the reference standards (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, <http://pubchem.ncbi.nlm.nih.gov>, Jang *et al.* 2018, Lu *et al.* 2018, Reed 2009). In several studies, isorhamnetin and its derivatives such as isorhamnetin-rutinoside and isorhamnetin-

glucoside were identified in *T. polium* and *Rosmarinus officinalis* L. (Lamiaceae) (Mihailović *et al.* 2020, Mena *et al.* 2016, Özer *et al.* 2018).

In total, 11 quercetin derivatives, a distinctive common aglycone, were recognised at specific and infraspecific levels in our study. Different molecular ions were detected by MS/MS spectra. Accordingly, it was concluded that one of the quercetin derivatives was isoquercitrin (m/z 463; dissociated ions at m/z 301), corresponding to some *Teucrium* species (Table 2). Quercetin-O-hexoside-O-sulphate with a mass molecular ion at m/z 543 and its pseudo molecular ions at m/z 463 and 381, were attributed to certain *Teucrium* species. Quercetin-3-O-glucuronide (m/z 477; base peak at m/z 301) and quercetin-methoxyglucuronide (m/z 492; daughter ions at m/z 301, and m/z 477) were further determined in some *Teucrium* species (Table 2). Additional quercetin aglycone derivative was tentatively inferred. Quercetin-3-O-[O-coumaroyl]-glucopyranosyl]-rhamnopyranoside was recognised in *T. scordium* subsp. *scordioides* 40 through molecular ions at m/z 755 and its daughter ions at m/z 609, m/z 593, and m/z 581. In addition, quercetin derivatives, with an identical molecular ion at m/z 609, were tentatively identified by their different retention times as quercetin-3-O-neohesperidoside (m/z 609; base peak at m/z 301, RT = 12.14) and quercetin-3-glucoside-7-rhamnoside (m/z 609; base peaks at m/z 301 and m/z 447, RT = 11.00) for certain *Teucrium* species (Table 2). Quercetin-3-O-glucosyl-6''-acetate (m/z 505; fragment ions at m/z 447 and m/z 343) corresponded to *T. orientale* subsp. *taylori* 2, 58. The mass molecular ion at m/z 549 and its daughter ions at m/z 301 specified quercetin-3-O-manoyl-glucoside in *T. orientale* subsp. *taylori* 2, 58 and *T. orientale* subsp. *gloetrichum* 38. The other detected derivatives with a major peak at m/z 771 corresponded to quercitrin-4-O-dihexoside (m/z 771; base peak at m/z 447) in *T. capitatum* 7. The parent ions at m/z 465 indicated the presence of taxifolin hexoside (m/z 465; m/z 303). In terms of fragmentation patterns, the evidence was supported by the reference standards (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>). Compared with some previously published studies, aglycone quercetin and its derivatives with glucoside, glucuronide, hexoside-O-sulphate, and glucoside-4'-O-L-rhamnoside groups were found in *T. chamaedrys* L., *Origanum vulgare* L., *Ocimum basilicum* L. (Lamiaceae), and *Rubus idaeus* L. (Rosaceae) (Krauze-Baranowska *et al.* 2014, Mihailović *et al.* 2020, Marwat *et al.* 2011, Vlase *et al.* 2014).

Concerning the obtained MS/MS spectra, 11 kaempferol and its derivatives (found in extract) were evidenced in *Teucrium* species. Among highly common flavonols, kaempferol aglycone, with a generated mass molecular ion at m/z 285, was identified for *T. orientale* subsp. *glabrescens* 39 and *T. orientale* subsp. *orientale* 29. Other derivatives with major peaks at m/z 771 and m/z 417 were kaempferol-3-diglucoside-7-glucoside (a precursor ion at m/z 771; m/z 285) and kaempferol-3-O-arabinoside (m/z 417; fragment ion at m/z

285) in certain *Teucrium* species. In addition, galloylated derivatives such as kaempferol-3-*O*-galloylrhamnoside (m/z 583; m/z 285) were found in *T. scordium* subsp. *scordioides* 45. Seven derivatives existed in *T. capitatum*. Parent ions identified at m/z 447 and m/z 739 were kaempferol-3-glucopyranoside (m/z 447; base peak at m/z 285) and kaempferol-3-*O*-neohesperidoside-7-rhamnoside (m/z 739; daughter ions at m/z 593 and m/z 431), respectively. The molecular ions detected at m/z 593 and m/z 577 identified kaempferol-3-*O*-glucoside-3''-rhamnoside (m/z 593; m/z 431 and m/z 447) and kaempferol-di-*O*-rhamnoside (m/z 577; m/z 285). Full MS data and precursor ions at m/z 327 and m/z 287 were assigned to kaempferol-3,7,4'-trimethyl ether (m/z 327; m/z 285) and dihydrokaempferol (Table 2). The detected kaempferol derivatives supported previous reports and reference standards (Gonzales *et al.* 2015, <http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>). Kaempferol aglycone and its derivatives (glucoside, glucopyranoside, rhamnoside, and di-hydroxyl) were previously reported to occur in *T. polium*, *T. chamaedrys*, *Lamium album* L., and *Dracocephalum peregrinum* L. (Lamiaceae) (Calderon-Montano *et al.* 2011, Mihailović *et al.* 2020, Özer *et al.* 2018, Vlase *et al.* 2014).

Another identified flavonol compound was syringetin in structure. Two derivatives were observed with the same molecular ion at m/z 507 and different retention times (RT = 14.00, 15.00). The molecular ion produced characteristic ions at m/z 345 (Table 2). As confirmed by the reference standards, syringetin-3-*O*-galactoside (RT = 14.00) was tentatively suggested for *T. parviflorum* 41 followed by syringetin-3-*O*-glucoside (RT = 15.00) for some *Teucrium* species (<http://massbank.eu>). The results underscored the need for more comprehensive studies to provide chemical details. In a published study, syringetin was introduced for *Mentha pulegium* L. (Lamiaceae) (Taamalli *et al.* 2015).

In the current study, flavonol-hexoside derivatives were represented in *Teucrium* species. In MS/MS fragment patterns, afzelin-3-*O*-di-hexoside with a product ion at m/z 755 (daughter ion at m/z 431) and daticetin-3-*O*-rutinoside with a mass molecular ion at m/z 593 (daughter ion at m/z 285) were identified for *T. capitatum* 5. More derivatives such as spinacetin-7-glucoside with molecular ion at m/z 507 (released ion at m/z 346) and limocitrin-*O*-glucoside-hydroxy-methylglutaryl with product ion at m/z 651 (fragment ions at m/z 345 and m/z 330) were tentatively identified for *T. scordium* subsp. *scordioides* 40 and *T. oliverianum* 50. All the evidence is in agreement with published reports and reference standards (El Sayed *et al.* 2017, <http://mona.fiehnlab.ucdavis.edu>). Previously, chemical ingredients such as afzelin were described for *Mentha longifolia* (L.) L. (Patonay *et al.* 2017).

Two *O*-methylated flavonols were identified in *T. oliverianum* 50 and *T. orientale* subsp. *glabrescens* 39. Based on the fragmentation patterns, molecular ions at m/z 313 and 359 were considered to be kumatakenin and jaceidin confirmed by the reference standards (<http://mona.fiehnlab.ucdavis.edu>).

The latter compound was primarily identified in *Mentha pulegium* and certain *Teucrium* species by Mihailović *et al.* (2020) and Uritu *et al.* (2018).

It was evidenced that a majority of flavonol derivatives in *Teucrium* species were focused on particular substitutions as hexoside, di-hexoside, and dioxy-hexoside at *O*-position.

Isoflavonoid derivatives

Isoflavone is one of the most common flavonoid derivatives mentioned in the present research. A detailed analysis of MS/MS fragmentation led to the identification of 20 isoflavones in different *Teucrium* species.

In MS/MS spectra, rotenone and its derivatives were identified based on the presence of different molecular ions. Of note, the three compounds identified with identical molecular ions differed at different retention times. According to reference standards and published studies, identified compounds tentatively contained rotenone (m/z 393; RT = 11.40) in *T. scordium* subsp. *scordioides* 45, isorotenone (m/z 393, RT = 9.49), and deguelin (m/z 393; RT = 8.23) in some *Teucrium* species. Furthermore, the existence of $[M-H]^-$ ion at m/z 391 for dehydrorotenone and a major peak at m/z 395 for dihydrorotenone were respectively seen in *T. scordium* subsp. *scordioides* 45, *T. melissoides* 49, and *T. orientale* subsp. *gloeotrichum* 26. More compounds with the same molecular ions and different retention times were tentatively proposed as amorphenin (m/z 409, RT = 10.03) and tephrosin (m/z 409, RT = 15.83, 16.13) in *T. capitatum* 5 and *T. scordium* subsp. *scordioides* 45. The molecular ion at m/z 425 was indicative of the hydroxyamorphenin characterised for *T. scordium* subsp. *scordioides* 45 and *T. capitatum* 5 (Table 2). Some of the previous studies confirmed the detected compounds (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>). Tephrosin was presented in *Tephrosia purpurea* L. (Fabaceae) (Dixit *et al.* 2012).

Three *O*-methylated isoflavone derivatives were also found in *T. melissoides* 49, *T. scordium* subsp. *scordioides* 40, and *T. orientale* subsp. *orientale* 29. The first proposed compound was considered to be biochanin A-8-C-malonylglucoside (m/z 531; base peak at 283). Additionally, mass molecular ions at m/z 561 and m/z 359 corresponded to formononetin-7-*O*-apiofuranosyl-*O*-glucopyranoside (releasing characteristic ions at m/z 429 and m/z 399) and irigenin, respectively (Fig. 5A). Biochanin-7-*O*-glucoside was further identified by the presence of m/z 445 and the base peak at m/z 283 (Table 2). All findings were consistent with previous reports (<http://mona.fiehnlab.ucdavis.edu>). Irogenin and biochanin A were primarily identified in *Iris hookeriana* Foster (Iridaceae) and *Mentha australis* R. Br. (Dar *et al.* 2016, Tzima *et al.* 2018). We showed for the first time that biochanin A-8-C-malonylglucoside could be considered as a specific flavonoid for *T. melissoides* as an endemic species.

Hydroxy-methoxy isoflavones and their derivatives were evidenced in certain *Teucrium* species. According to MS/MS information and previously published reports, the proposed compound was villol with a precursor ion at m/z 441. A parent ion peak at m/z 415 (base peak at m/z 295) and deprotonated ion at m/z 562 (fragment ions at m/z 547, m/z 430, and m/z 400) represented daidzein-8-*C*-glucoside and daidzein-methoxy-*O*-pentoside-hexoside, respectively. The mass molecular ion detected at m/z 515 corresponded to luteone-*O*-glucoside (m/z 515; daughter ion at m/z 353). Additionally, deprotonated parent ions at m/z 353 and m/z 313 indicated lico-isoflavone A and dipteryxin, respectively. Isoflavone derivative was tentatively suggested as 7-*O*-glucopyranosyl-4'-hydroxy-5-methoxyisoflavone (m/z 445; dissociated ions at m/z 283, m/z 417, and m/z 430) (Table 2). All presented derivatives supported the previous reports (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, Reed 2009). The presence of daidzein, luteone, and lico-isoflavone A were previously shown in *Scutellaria laterifolia* L. (Lamiaceae), *Lupinus albus* L. (Fabaceae), and *Azorella madreporica* Clos (Apiaceae) (Hellal *et al.* 2020, Quesada *et al.* 2012, Uritu *et al.* 2018). It was also shown that *O*-methylated and hydroxy-methoxy isoflavones mostly exhibited *O*-hexoside substitutions.

Isoflavone glucoside with a molecular ion at m/z 515 was one of the derivatives detected in *T. orientale* subsp. *orientale* 29. Its fragment ion signal at m/z 429 revealed the proposed compound as 6'-malonyl-ononin (<http://massbank.eu>) (Table 2). It was formerly reported that ononin existed in *Scutellaria baicalensis* Georgi (Shin *et al.* 2013).

Two isoflavans were tentatively identified in certain *Teucrium* species. Molecular ions at m/z 423 and 437 were characteristic of licoricidin and 5-*O*-methyllicoricidin (<http://mona.fiehnlab.ucdavis.edu>). As a dominant ingredient, licoricidin was first identified in *Glycyrrhiza uralensis* Fisch. (Fabaceae) (Yang *et al.* 2017).

Also, as an isoflavanone derivative, methyl robustone was identified in *T. capitatum* 5 with a mass molecular ion at m/z 377, which is in line with the reference standards (<http://mona.fiehnlab.ucdavis.edu>).

Flavanone derivatives

Different flavanone derivatives were observed in *Teucrium* species as presented here.

A total of six naringenin derivatives were identified in the chromatogram of the species. In this process, three derivatives with the same molecular ions were specified through different retention times. Compared with reference standards and published reports, flavanone derivatives tentatively corresponded to 6,8-diprenylnaringenin (a precursor ion at m/z 407 with RT = 8.59, 9.53), 6-geranylnaringenin (m/z 407; base peak at m/z 271, RT = 12.02,

12.22), and remangiflavanone (m/z 407, RT = 13.36, 13.48). The presence of parent peak ions at m/z 423, m/z 403, and m/z 579 is respectively ascribed to sophoraflavanone G, naringenin-arabinoside (base peak at m/z 271), and naringin (daughter ion at m/z 271) (Table 2). The detected naringenin derivatives are consistent with the reference standards and previous reports (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, <http://pubchem.ncbi.nlm.nih.gov>, Nastić *et al.* 2018, Reed 2009). Prenylnaringenin and naringin were primarily extracted from *T. montanum* L. and *Humulus lupulus* L. (Cannabaceae) (Mihailović *et al.* 2020, Nastić *et al.* 2018, Štulíková *et al.* 2018).

Another flavanone derivative with a mass molecular ion at m/z 593 and its dissociated ion at m/z 285 tentatively corresponded to isosakuranetin-7-*O*-neohesperidoside in certain *Teucrium* species in agreement with those of the reference standards (<http://mona.fiehnlab.ucdavis.edu>).

Flavanone derivatives such as hydroxyflavanones were further yielded. As compared with reference standards, the proposed compound was considered to be liquiritin (m/z 417; base peak at m/z 255) found in *T. scordium* subsp. *scordioides* 40 (Fig. 5B). A precursor ion at m/z 287 and its fragments ion signals at m/z 259, m/z 243, m/z 230, and m/z 215, corresponded to 6,7,3',4'-tetrahydroxyflavanone for *T. scordium* subsp. *scordioides* 45. Two flavanone derivatives were tentatively identified by the identical molecular ions and different retention times. Accordingly, flavanomarein (m/z 449; base peak at m/z 287, RT = 16.21, 16.34) and eriodictyol-7-*O*-glucoside (m/z 449; base peak at m/z 287, RT = 17.58, 17.69) corresponded to *T. capitatum* 7 and *T. scordium* subsp. *scordioides* 45 (Table 2, Fig. 5C). Reference standards confirmed the identified compounds (<http://massbank.eu>). According to recent findings, eriodictyol-7-*O*-glucoside was first detected in *Thymus sibthorpii* Benth (Lamiaceae) (Kontogiorgis *et al.* 2016).

Chalcone derivatives

A total of nine chalcone derivatives were also seen in the chromatogram of *Teucrium* species. Different MS/MS spectra detected C-linked dihydrochalcone glucoside derivative or aspalathin (a precursor ion at m/z 451), and phloretin-2'-*O*-glucoside (m/z 435; base peak at m/z 273, RT = 13.04). A mass molecular ion at m/z 435 was further identified by RT = 14.12 in *T. gnaphalodes* 54. Compared with published reports, the detected compound was tentatively presumed to be orotinichalcone (Krauze-Baranowska *et al.* 2014) (Table 2). Additionally, the dihydrochalcone derivative of *T. scordium* subsp. *scordioides* 45 corresponded to phloretin-xyloglucoside (m/z 567; a pseudo molecular ion at m/z 273). A parent peak ion at m/z 449 and its daughter ion at m/z 287 resulted in the detection of okanin-4'-*O*-glucoside for *T. orientale* subsp. *glabrescens* 39. The presence of mass molecular ion at m/z 403 (base peak at m/z 271) provided butein-arabi-

noside for *T. orientale* subsp. *glabrescens* 39, 17. Approved fragmentation patterns were consistent with the reference standards and previous reports (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, Reed 2009, Sobeh *et al.* 2016). As formerly evidenced, aspalathin and butein-glucoside were primarily found in *Aspalathus linearis* (Burm. f.) R. Dahlgren (Fabaceae) and *Cosmos bipinnatus* Cav. (Asteraceae) (Iwashina 2015, Van Wyk and Gorelik 2017).

Chalcone derivatives such as hydroxy-methoxychalcone were also identified in *Teucrium* species. As a result, the identified derivative was 2',6'-dihydroxy-4-methoxychalcone-4'-*O*-neohesperidoside (m/z 593; fragment ions at m/z 285 [M-H-308]⁻, m/z 565 [M-H-CO]⁻, m/z 549 [M-H-CO₂]⁻ and m/z 578 [M-H-CH₃]⁻) found in *T. scordium* subsp. *scordioides* 40 and *T. capitatum* 7. The identity of the two derivatives was approved by the obtained peaks following 4-hydroxy-2',3,4',6'-tetramethoxychalcone (m/z 343; dissociated ions at m/z 315 and m/z 283) and 2',4-dihydroxy-3,4',6'-trimethoxychalcone (m/z 329; daughter ions at m/z 301, m/z 285, and m/z 284) for *T. scordium* subsp. *scordioides* 40 and *T. oliverianum* 50 (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>). It has recently been reported that chalcone is first extracted from *Salvia miltiorrhiza* Bunge (Lamiaceae) and certain *Teucrium* species (Deng *et al.* 2018, Mihailović *et al.* 2020).

Anthocyanin derivatives

In this process, anthocyanidin derivatives were found by different deprotonated molecular ions.

Four delphinidin derivatives were detected for *O*-methylated, hexoside, and glucuronide substitutions. Two derivatives with the same molecular ions at m/z 492 tentatively differed at different retention times. The evidence of MS/MS fragmentation patterns and reference standards represented malvidin-3-hexoside with RT = 13.10 (m/z 492; base peak at m/z 330) in *T. polium* 46. Additionally, petunidin-glucuronide with 14.10 retention time (m/z 492; daughter ion at m/z 316) was characterised for some *Teucrium* species (Table 2). Delphinidin-3-galactoside (m/z 464; fragment ion at m/z 302) and petunidin-(6''-*O*-galloyl)-3-*O*-glucuronide with molecular ion at m/z 644 and its cleavage to daughter ions at m/z 316, 492, and 468 were assigned to *T. orientale* subsp. *orientale* 29 and *T. orientale* subsp. *gloeotrichum* 38 (<http://mona.fiehnlab.ucdavis.edu>). Anthocyanin and delphinidin-3-galactoside were mainly considered as widely distributed in *Ajuga* L., *Salvia* L., and *Vaccinium* L. fruits (Ericaceae) (Iwashina 2015, Lee 2016).

As pentahydroxyflavylum derivatives, another anthocyanidin was found in *Teucrium* species. Cyanidin-3-*O*-glucosylrutinoside with a molecular ion at m/z 756 and characteristic ions at m/z 593 and 447 corresponded to certain *Teucrium* species (<http://mona.fiehnlab.ucdavis.edu>). Cyanidin-3-glucoside was previously described for *T. ramosissimum* Desf. (Ghazouani *et al.* 2016).

Flavan-3-ol derivatives

A total of three epicatechin and four catechin derivatives were found in the extract of *Teucrium* species.

Concerning the fragmentation patterns and reference standards, characteristic ions were assigned to epicatechin glucuronide (m/z 465; base peak at m/z 289) for *T. polium* 46, *T. orientale* subsp. *taylori* 2, 58, and *T. gnaphalodes* 64. Other tentatively characterised derivatives were 3-methyl-epigallocatechin gallate (m/z 471; characteristic ion at m/z 442) and methyl-(epi) catechin gallate (m/z 456; daughter ion at m/z 441) in some *Teucrium* species (Table 2) (<http://mona.fiehnlab.ucdavis.edu>, Pereira *et al.* 2015). Based on published research, epicatechin was detected in *Melissa officinalis* L. (Lamiaceae) and some *Teucrium* species (Mihailović *et al.* 2020, Ordaz *et al.* 2018).

Regarding the generated molecular and daughter ions, the proposed compounds were tentatively concluded to be catechin-O-glucose (OAc) (m/z 493; base peak at m/z 289) and catechin-hexoside (m/z 451; base peak at m/z 289) in *T. gnaphalodes* 64 and *T. orientale* subsp. *orientale* 37. A mass molecular ion at m/z 435 and its fragmentation at m/z 289 detected catechin-3-O-rhamnoside in *T. capitatum* 7. More proposed derivatives corresponded to catechin-tetramethyl ether (m/z 345; base peak at m/z 289) in certain *Teucrium* species (Table 2). All approved datasets were in agreement with former documents (<http://mona.fiehnlab.ucdavis.edu>, Reed 2009). Catechin was previously reported for *T. polium* and *T. scordium* (Mihailović *et al.* 2020, Milošević-Djordjević *et al.* 2018).

Two flavan derivatives were detected in *Teucrium* species. In fragmentation patterns, molecular ions at m/z 563 and 577, respectively, indicated theaflavin and procyanidin B1 for *T. orientale* subsp. *glabrescens* 39 and *T. orientale* subsp. *gloeotrichum* 38 (<http://mona.fiehnlab.ucdavis.edu>) (Fig. 5D). Procyanidin B1 was previously isolated from *T. polium* by Boumerfeg *et al.* (2012).

Flavonolignans

As a flavonolignan derivative, a molecular ion at m/z 481 allowed for the identification of silychristin in *T. scordium* subsp. *scordioides* 45, confirmed by the reference standards (<http://massbank.eu>). The identified compound was first extracted from *Silybum marimum* (L.) Gaertn. (Asteraceae) (El Sayed *et al.* 2019).

Additional compounds

Additional compounds were provisionally recognised in *Teucrium* species as presented here. Our research aimed to detect the flavonoid compounds, thus detailed information of additional compounds was not provided.

Hydroxycinnamic acid derivatives were tentatively inferred in *Teucrium* species. Caffeoyl-coumaroyl spermidine (m/z 452, RT = 11.84), hydroxycinnamic derivative (452, RT = 14.10), 3-hydroxycinnamic acid derivatives (m/z 455, RT = 9.54, RT = 13.31), scutellarioside II (m/z 507), and caffeic acid derivative (m/z 295) were recognised, which is consistent with the published reports (Hawas *et al.* 2008) (Table 2). Hydroxycinnamic acid derivatives and caffeic acid were described for the first time in *T. polium*, *T. scordium*, *T. chamaedrys* L., *T. montanum*, and *T. scordium* (Mihailović *et al.* 2020, Milošević-Djordjević *et al.* 2018, Mitreski *et al.* 2014, Özer *et al.* 2018).

Different quinic acid derivatives were proposed in some *Teucrium* species. Feruloyl-caffeoylquinic acid (m/z 529), 3-*O*-caffeoyl-5-*O*-coumaroylquinic acid (m/z 499), and coumaroylquinic acid derivative (m/z 378) were detected at different retention times (RT = 11.58 and 15.12) (Table 2) (<http://mona.fiehnlab.ucdavis.edu>, Reed 2009). Based on previous documents, 5-caffeoylquinic acid was primarily found in *T. polium*, *T. chamaedrys*, *T. montanum*, and *T. scordium* (Mitreski *et al.* 2014).

Moreover, two benzopyran derivatives, two tannins, and one alkaloid derivatives were proposed as 4,4'-dihydroxy-dicoumarin derivative (m/z 378, RT = 10.40), bromadiolone (m/z 525), picroside II (m/z 511, RT = 10.91), pumiloside (m/z 511, RT = 12.30), and digalloyl glucose or hexose (m/z 483) (Table 2) (<http://mona.fiehnlab.ucdavis.edu>, <http://massbank.eu>, <http://pubchem.ncbi.nlm.nih.gov>, Reed 2009, Santos *et al.* 2012). Different coumarin derivatives, picroside I, II, and galloyl-glucose derivatives were previously shown to exist in *Melittis melissophyllum* L., *Plectranthus barbatus* Andrews (Lamiaceae), and *Picrorhiza kurroa* Royle ex Benth. (Plantaginaceae) (Kumar *et al.* 2016, Santos *et al.* 2012, Szymborska-Sandhu *et al.* 2020).

Chemical markers and chemotypes of Teucrium species

As observed in Figures 1 and 2, specific chemical markers were primarily proposed in each *Teucrium* species. These chemical candidate markers can be employed for chemotaxonomic purposes. Previous studies proposed chemical compounds such as sesquiterpens in the *Vitex* genus as chemical markers for chemotaxonomic targets (Sena Filho *et al.* 2017). In particular, the presence of chemotypes determined the biomarkers.

On the contrary, similar cases were assigned to three sections. Sections *Teucriis* and *Scordium* were similar regarding the production of hydroxyflavone, trihydroxy-methoxyflavone, flavone-hexoside, flavonol-hexoside, flavanone-pentoside, isoflavan, and anthocyanins. In section *Polium*, the identified similarities were shown by the production of hydroxy-methoxyflavone, chalcone-hexoside, flavone-hexoside, flavan-hexoside, and flavanol-hexoside.

The present study is the first to detect 16 chemotypes for *Teucrium* species. Environmental conditions are the main factors influencing the chemical constituents. Extreme variations in chemical compositions and chemical polymorphism depend on the effect of environmental factors on gene expression (Pourhosseini *et al.* 2018). The obvious difference observed among chemotypes might be attributed to the presence of chemical polymorphism at infraspecific levels. Polymorphism of essential oils in *T. capitatum* contributes to genetic factors (Antunes *et al.* 2004). Flavonoid diversity was found in *T. polium*, and it exhibited infraspecific variations (Venditti *et al.* 2017). As presented in this paper, most chemical compounds in the *T. polium* accessions were completely different from the previously published studies. This difference is possibly ascribed to genetic variations, phenology, and plant parts. As previously evidenced, the presence of essential oils such as monoterpenes/sesquiterpenes derivatives in the *T. polium* accessions led to the identification of different chemotypes (Sadeghi *et al.* 2014). There was also an extensive chemical diversity in this taxon. Based on this finding, chemical compounds could be divided into three groups in section *Scordium*, ten groups in section *Teucris* and five groups in section *Polium*. Given the chemical diversity and morphological similarity in *Teucrium* species, their chemotypes were accurately identified using HPLC/MS/MS. The obtained data also showed that the altitude of the habitats determined the chemical differentiations and increased flavonoid quantity (number). Previous reports revealed the significant effect of altitude on the variations of essential oils in *T. polium* (Sadeghi *et al.* 2014).

Chemo-differentiation was found to be associated with the location of each species. Most of the *Teucrium* accessions were discriminated in terms of the ecological distribution. The western, southwestern, central and southern regions were definitely distinguished under altitudinal variations. Accordingly, ecological conditions probably initiated different chemical compounds in the *Teucrium* genus. It might be concluded that the adaptation response of the accessions generated different chemical compounds. Some chemical components showed the ecological adaptation of chemotypes, and motivations such as genetic differences, irrigations, seasonal variations, and geographical isolations likely generated chemotypes (Grignon-Dubois and Rezzonico 2012).

Nevertheless, no conclusive segregation was observed in *T. orientale* subsp. *taylori* accessions, which were caused by one tannin compound. It appears that ecological conditions slightly influenced the accessions. Despite the similar metabolites detected in both accessions, the proposed chemotypes were demonstrated by different quantities or intensities of molecular ions. However, future research is required to utilise other fingerprinting approaches. Concerning the significant discrimination of subspecies related to *T. orientale* and *T. scordium*, they can probably be considered as specific levels. Moreover, there was a significant difference among the chemotypes of *T. capitatum*. This might be attributed to the fact that geographical differences mostly af-

fect flavonoid types and determine climatic variability (Tykheev *et al.* 2018). Confirming our results, Grignon-Dubois and Rezzonico (2012) reported geographical variability in flavonoid compounds of *Zostera noltii* (Zosteraceae).

It was indicated that temperate geographical regions significantly facilitated the presence of xyloside and hexoside flavonol and hydroxy-methoxy flavone substitutions. Moreover, sub-tropical, tropical, and semiarid regions resulted in the accumulation of hexoside and methoxy substitutions in flavonol and flavan. Arctic regions tend to facilitate hydroxyl flavones and flavonols. As described for different locations, the significant difference existing between Isfahan and Boyerahmad regions was independently attributed to two different groups in each province. It is to be expected that in these areas, climatic conditions such as precipitation and aridity could be responsible for segregation. As previously reported, some predominant fatty acids and essential oils of *Bupleurum scorzonerifolium* Willd. (Apiaceae) were assumed to be impacted by moisture conditions (Tykheev *et al.* 2018). However, flavonoid accumulation might be influenced by several conditions requiring further investigations.

The chemotypes mainly show the significant differences of secondary metabolites. Therefore, they are essentially utilised in industrial productions and chemotaxonomic purposes (Tykheev *et al.* 2018). The presence of different chemotypes revealed the chemotaxonomic differentiation at specific and infraspecific levels. The existence of chemotypes in *Teucrium* species also significantly showed the spatial segregation in natural habitats. This is the first report on the presence of chemotypes at infraspecific levels.

Botanical perspectives

From a botanical perspective, the genus *Teucrium* has complexities at specific levels regarding wide distribution and morphological variabilities (Navarro 2020). The members of section *Teucris* exhibited morphological similarities at specific and infraspecific levels. Some of the previous studies reported that certain taxonomic relationships existed among the four *Teucrium* subspecies. Morphologically, in section *Teucris*, hirtellous indumentum on the surface of pedicel differentiated *T. orientale* subsp. *orientale*. *T. orientale* subsp. *glabrescens* differed by the glabrous and sub-glabrous indumentum at stem, inflorescence, bract, pedicel, and calyx. Villous indumentum at inflorescence and articulate trichome at pedicel and bract were observed in *T. orientale* subsp. *taylori*. The indumentum of glandular at stem, pedicel, and calyx was also ascribed to *T. orientale* subsp. *gloeotrichum* (Bagheri Moghadam *et al.* 2020). It was evidenced that HPLC-MS/MS also differentiated four subspecies of *T. orientale* by 32 identification markers, which is thoroughly supported by morphological results (Bagheri Moghadam *et al.* 2020). *T. oliverianum* and *T. parviflorum* were also distinguished by the indumentum of corolla lip (barbate, pannose) and the form

of leaf apex (Bagheri Moghadam *et al.* 2020). Based on fingerprinting analysis, both mentioned species differed by six candidate markers.

In section *Polium*, some complexities were observed in *T. polium*, *T. capitatum*, and *T. gnaphalodes*. Certain diagnostic morphological properties allowed for the separation of the mentioned species. The indumentum of stellate at leaf, bract, inflorescence, pilose at corolla tube, and emarginate apex of leaf were identified in *T. capitatum*. Moreover, the indumentum of hirsute and barbate at stem, floccose at inflorescence, stellate and glandular at bracteole, glandular at corolla lip, hirsute, velutinous, and lanate at calyx, hirsute at pedicel, and barbate and hirsute at filament was assigned to *T. polium*. The presence of pannose indumentum at bracteole was characterised for *T. gnaphalodes*. Consistent with previous reports, *T. polium* complex was resolved by 25 candidate markers using fingerprinting analysis (Bagheri Moghadam *et al.* 2020).

In section *Scordium*, *T. scordium* subsp. *scordioides* exhibited sub-cordate leaf base and obtuse bract apex, and lanuginose indumentum at stem, inflorescence, and bract. Moreover, ovate-elliptic bract form was described as a diagnostic feature in *T. melissoides* (Bagheri Moghadam *et al.* 2020). Among our HPLC-MS results, 25 chemical candidate markers obviously separated *T. scordium* subsp. *scordioides* from *T. melissoides*.

It has been proposed that the existence of chemotype possibly correlates with morphological variations. The provided morphological evidence revealed a significant diversity in the *Teucrium* genus (Bagheri Moghadam *et al.* 2020). The flavonoid diversity detected in the studied chemotypes confirmed the previously published documents. The most flavonoid diversity was recognised in this genus and revealed a chemotaxonomic status (Mihailović *et al.* 2020). The significant variations in morphological characteristics further corroborate our results (Bagheri Moghadam *et al.* 2020).

HPLC-MQ-API-MS/MS accurately assessed *Teucrium* species. This technique is the first to exhibit candidate markers at specific and infraspecific levels. Thus, it could be applied to differentiate chemotypes at molecular levels. All the proposed chemical makers were considered as the chemotaxonomic concepts of this genus. Furthermore, the flavonoid compounds of *Teucrium* species and its accessions showed a wide diversity in terms of habitats. The presence of chemotypes exposed the different characteristics of each accession and significantly improved the species and their biological properties and increased population variability. On the other hand, documentation of chemotypes revealed significant biomarkers. Environmental factors or genetic differences possibly affected the accumulation of flavonoid types. Consequently, HPLC-MS/MS was able to provide basic phytochemical information, quality control of medicinal plants, and valuable taxonomic information. This research provided the first evidence on the existence of chemotypes and chemo markers and fully characterised flavonoid profile in *Teucrium* species.

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