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RESEARCH PAPER

Morphologic and Chemotaxonomic Studies of Some *Teucrium* L. (Lamiaceae) in Zagros Region, Iran

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Abstract

The genus *Teucrium* L. is a well-known medicinal plant belonging to Lamiaceae family and Ajugoideae subfamily. The present study aims to evaluate the taxonomic status of the *Teucrium* species using both morphological and flavonoid characteristics and to determine the flavonoid classes. A total of 64 accessions belonged to eight species, and five subspecies were collected from different Zagros regions, Iran. Twenty-nine quantitative and qualitative morphological characters were studied. Extraction of leaf flavonoid was accomplished using a rotary evaporator and MeOH 90%. Identification of the chemotaxonomic position and flavonoid class of *Teucrium* species was investigated by thin layer chromatography, column chromatography and High Performance Liquid Chromatography-Micromass Quattro micro Atmospheric Pressure Ionization Mass Spectrometer. All information was analyzed using NTSYS pc. 2, PAST v.3.18 and Cluster Vis v. 1.8.2. According to the findings, *T. polium* accessions significantly revealed the highest value of morphological and flavonoid variations. All subspecies of *T. orientale* were definitely separated, but some relationships were observed between its subspecies, including *taylori* and *glabrescens*. All members of two sections of *Polium* and *Teucris* were definitely recognized. A total of seven flavonoid classes were determined from which the highest amounts of abundance were attributed to flavones (18) and isoflavones (17). In conclusion, morphological and flavonoid markers were introduced as appropriate characteristics in the taxonomic relationships of *Teucrium* species. All the evidence of the present research was first described for Iran.

Keywords *Teucrium* · Lamiaceae · Chemotaxonomy · Flavone · Morphology

1 Introduction

The genus *Teucrium* L. belongs to Lamiaceae family and Ajugoideae subfamily and is known as one of the large and complex genera (Rechinger 1982; Ecevit Gen et al. 2015). This genus is represented by 260 species throughout the world, and 19 species in Iran from which four endemic species were recognized for the country (Jamzad 2012; Salmaki et al. 2016). Its species are generally distributed in Europe, western Mediterranean regions, southeast Australia, northwest and south America, North Africa and temperate of Asia particularly in Irano-Turanian regions (Bukhari et al. 2015; Salmaki et al. 2016). Mediterranean regions are the main diversity centers for the genus *Teucrium* (Salmaki et al. 2016). The species grow as shrub, dwarf-shrub, biennial, perennial and annual forms in dry, open, sandy and rocky areas and hillsides (Rechinger 1982; Jaradat 2015; Ecevit Gen et al. 2015; Salmaki et al. 2016).

Teucrium species are traditionally used to treat different disorders. They have a rich source of chemical compounds with several and valuable biological activities and therapeutic effects, including antiseptic, antibacterial, anticancer, wound healing (Elmasri et al. 2015), hypoglycemic, hypolipidemic, hepatoprotective, antipyretic, anti-inflammatory, anti-ulcer, anti-tumor (El-Ashmawy 2018), anti-urogenital, anti-indigestion, antirheumatism (Sabet et al. 2013), antioxidant, anti-nociceptive and antitoxic (Stefkov et al. 2012). Almost its potent biological activities previously reported have been attributed to the presence of polyphenols involving radical-scavenging activities (Alwahsh et al. 2015).

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From a taxonomical point of view, several classifications for the genus *Teucrium* were presented. However, its proposed classifications remain controversial. Bentham (1835) introduced nine sections (*Teucrium* Ging. ex Benth., *Teucropsis* Ging. ex Benth., *Leucosceptrum* (Sm.) Benth., *Chamaedrys* Benth., *Polium* Benth., *Scordium* (Mill.) Rchb., *Pycnobotrys* Benth., *Scorodonia* Benth., *Stachyobotrys* Benth.). Briquet (1897) adopted the Bentham's classification, but considered two further sections (*Spinularia* Boiss., *Isotriodon* Boiss.). Rechinger (1982) classified *Teucrium* as seven sections (*Polium, Chamaedrys, Isotriodon, Scordium, Scorodonia, Stachyobotrys, Teucris* Benth.). Yuzepchuk (1954) considered five sections (*Teucris, Stachyobotrys, Scordium, Chamaedrys, Polium*) for this genus. Kastner (1989) also divided the genus *Teucrium* into five sections and 13 subsections. He also omitted the sections *Polium*, *Spinularia* and *Pycnobotrys*. Additionally, Navarro and El Oualidi (2000) proposed nine sections and four subsections (sub-sect. *Polium, Simplicipilosa* S. Puech., *Rotundifolia* Cohen ex Valdes Berm & Sanchez-Crespo, *Pumilum* Rivas- Mart.). However, Jamzad (2012) has not considered any classification for this genus. Ranjbar et al. (2017) taxonomically reviewed the section *Teucrium*. A few taxonomic changes were achieved in this section. According to different classifications, the taxonomy of *Teucrium* was disputed. Consequently, this genus has complexities mostly resulting from the presence of wide distribution, high morphological variability, different ploidy levels and natural hybridization (Salmaki et al. 2016).

Based on the morphological literature, different reports are currently available in *Teucrium* species. Bukhari et al. (2015) investigated some morphological characteristics of leaves and flowers in *T. polium* L. from Saudi Arabia. Pavlova and Vasileva (2010) studied different serpentine ecotypes of *T. polium* from Bulgaria. Navarro and El Oualidi (2000), Jurisic Grubesi et al. (2007), Eshratifar et al. (2011), Ecevit Genc et al. (2015) and Marzouk et al. (2017) provided further reports of micromorphological studies, including trichome, pollen and nutlet of *Teucrium* species in Mediterranean, Croatia, Iran, Turkey, and Lybia. The results provided by these data sources confirmed that these characters had taxonomical value at sectional and specific levels.

Morphological variations have been the subject of few researches. Some reports in *T. polium* characterized that seasonal periods and edaphic conditions affected the density, thickness and height of trichomes and the height of stem and leaf (Pavlova and Vasileva 2010; Bosabalidis 2013). On the contrary, Bukhari et al. (2015) indicated no correlations between environmental conditions and trichomes.

In the context of published works in chemical compounds, several researches into *Teucrium* species were presented. The major chemical constituents were ascribed to its species, including phenolic acids, polyphenols, flavonoids (Valant-Vetschera et al. 2003; Stefkov et al. 2012; Jurisic Grubesic et al. 2012; Mitreskia et al. 2014; Alwahsh et al. 2015; Boghrati et al. 2016; Venditti et al. 2017; Gecibesler et al. 2019), essential oils as monoterpenoids, diterpenoids, triterpenoids (Ahmad et al. 2014; Jaradat 2015; Elmasri et al. 2015; Semiz et al. 2016; Rahimi et al. 2019), iridoids, phenylethanol glycosides (Elmasri et al. 2015), alkaloids, anthraquinones, tannins, (Shah and Shah 2015), benzene ester (Ahmad et al. 2014), and β-sitosterol (Jurisic Grubesi et al. 2012).

From a chemotaxonomic point of view, there is limited evidence of flavonoid compounds used in the genus *Teucrium*. Some authentic reports have demonstrated the isolation of essential oils. Chemotaxonomic status of *T. arundini* L. (Kremer et al. 2012), *T. scordium* L. (Radulovic et al. 2010), *T. pumilum* Loefl. ex L. (Perez et al. 2000) and *T. stocksianum* Boiss. (Sonboli et al. 2013) were previously investigated using essential oils. Sonboli et al. (2013), Venditti et al. (2017) and Rahimi et al. (2019) specified the variation in chemical compounds in *T. polium*, *T. hircanum* L. and *T. stocksianum* as chemo type.

According to the literature survey, there are no reports of morphometric and chemotaxonomy of flavonoid compounds in *Teucrium* species in Iran. Moreover, owing to the presence of valuable genetic resources in the Zagros region, the present study focuses on the following aims: (1) comprehensive morphometric studies of quantitative and qualitative morphological characteristics, (2) investigation of the morphological variations at intraspecific levels, (3) chemotaxonomic study of flavonoid profiles at specific and intraspecific levels and 4) determination of the flavonoid classes of each species from the Zagros region. All data were first described for Iran.

2 Materials and Methods

2.1 Morphology Section

In this section, 63 accessions from eight species including five subspecies from section *Polium* (*T. polium* L., *T. capitatum* L., *T. gnaphalodes* L.' Her), section *Teucris* (*T. orientale* subsp. *orientale* (recently named under *T. orientale* L.), *T. orientale* subsp. *taylori* (Boiss.) Rech.f., *T. orientale* subsp. *glabrescens* (Hausskn. ex Bornm.) Rech. f., *T. orientale* subsp. *gloeotrichum* Rech. f., *T. oliverianum* Ging. ex Benth., *T. parviflorum* Schreb.) and section *Scordium* (*T. melissoides* Boiss. ex Hausskn., *T. scordium* subsp. *scordioides* (Schreb.) Arcang.) were collected and characterized from the north, west, southwest, center and south of Zagros regions (Table 3; "Appendix"). All specimens were collected from various natural habitats from March to September from 2018 to 2019. The voucher specimens were deposited in the Herbarium of Shahrekord University. The identification process of all collected specimens was accomplished by Flora Iranica and Flora of Iran (Rechinger 1982; Jamzad 2012).

To study the morphometric, morphological variations and taxonomic status in *Teucrium* species, 11 quantitative and 18 qualitative morphological variables were selected and measured in each species as provided in Table 1. All morphological characteristics were measured using stereomicroscope Olympus SZX-ZB12. Data matrix was subjected to a cluster analysis followed by simple matching coefficient and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method with NTSYS Pc. 2.0 (Rohlf 2000). An analysis of variance (ANOVA) and Kruskal–Wallis test also assessed the quantitative and qualitative morphological variables among species by the IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA) software.

2.2 Phytochemical and Chemotaxonomy Study

Extraction of flavonoids was accomplished using the procedure suggested by Rahman (2005). The total flavonoid of air-dried leaves (10 gr) from eleven taxa was isolated using 90% MeOH at 60 °C. The flavonoid extraction was accomplished using a rotary evaporator at 70 °C. Flavonoid purification was conducted by n-BuOH and consecutively screened through silica gel 60F 254 (16 mg, 70 ml H_2O) thin layer chromatography (TLC; 5 μM, 20 × 20 cm). The chromatogram was treated in different solvent systems such as $CHCl_3$–MeOH (60:40; 70:30; 80:20), CH_2O_2–CH_3COOH–H_2O (40:40:20; 30:30:40) and $C_4H_8O_2$–MeOH–H_2O (98:1.5:0.5) (Cakir et al. 2006; Venditti et al. 2017; Gecibesler et al. 2019). Spot detection with natural product reagents (H_2SO_4 5% in MeOH) was completed by ultraviolet-366 nm (Rahman 2005). The flavonoids were separated by column chromatography (50 × 4 cm), followed by Sephadex LH_{20} Sigma- Aldrich (Sephadex and MeOH 20% mixture) in 100 mL $CHCl_3$–MeOH 60:40 to yield several fractions. Identification of flavonoid classes was achieved on ultraviolet spectrum (200–400 nm), MeOH solution and shift reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/H_3BO_3 and MeOH. All fractions acquired were analyzed using HPLC-Micromass Quattro micro API (Atmospheric Pressure Ionization) Mass Spectrometer to provide the mass to charge (m/z) value in each species. Chromatography condition was prepared at Atlantis T3-C18 column (3 μ, 2.1 × 100 mm, flow rate of 0.25 ml/min) at 30 °C. The mobile phase includes acetonitrile, methanol and formic acid (98%). Moreover, flavonoid standard from SIGMA Aldrich Co. includes apigenin with 98% purity (Hossain et al. 2010; Aghakhani et al. 2018).

To explore chemotaxonomy and flavonoid variations concerning 63 accessions, a cluster analysis followed by the UPGMA method and Dice coefficient and principle component analysis (PCA) using the PAST v.3.18 (Hammer et al. 2001) and the Cluster Vis v. 1.8.2 (Raden 2012) software were assessed. The presence (1) and absence (0) of color spots were scored in this manner. In addition, the spots retention factor (Rf) in each sample is provided.

3 Results and Discussion

3.1 Morphology Results

The present work demonstrates the existence of some morphological variability at intraspecific levels. Most measurements of qualitative morphological variables were indicated for the indumentum of stem, leaf, inflorescence, bract, pedicel, calyx, corolla tube, corolla lips, filament and style (Table 1). Moreover, the highest value of quantitative morphological variations was mainly found in the length of leaf, inflorescence axis, bract, calyx and filament, and width of leaf, bract and calyx. Among quantitative morphological characters, some features including the length of stem (*T. melissoides*), length and width of leaf and length of inflorescence axis (*T. parviflorum*), length of bract (*T. polium*), width of bract (*T. oliverianum*), length of corolla lip (*T. orienale* subsp. *taylori*), length of calyx (*T. orienale* subsp. *orientale*), and length of filament and width of calyx (*T. orienale* subsp. *glabrescens*) were found as diagnostic characters in *Teucrium* species.

It is important to state that several unique qualitative morphological characteristics were presented as diagnostic characteristics in four subspecies of *T. orientale*. Identified characters included indumentum of stem surface (subsp. *taylori,* subsp. *gloeotrichum*, and subsp. *glabrescens*) followed by indumentum of leaf (subsp. *glabrescens*), indumentum of petiole (subsp. *glabrescens*), indumentum of inflorescence axis (in subsp. *taylori* and in subsp. *glabrescens*), indumentum of pedicel (subsp. *taylori*, subsp. *gloeotrichum*, and subsp. *glabrescens*), indumentum of corolla lips (subsp. *taylori*), indumentum of calyx (subsp. *taylori*, subsp. *gloeotrichum*, and subsp. *glabrescens*) and indumentum of filament (subsp. *glabrescens*) (Table 1). It is well known that *T. orientale* subsp. *orientale* is differed based on hirtellous indumentum on the surface of pedicel.

Some morphological characteristics of *T. polium* complex were also elucidated as taxonomic characters based on the following evidence: indumentum of leaf (*T. polium*),

Table 1 Quantitative and qualitative morphological characters in *Teucrium* species

Characters/species	Orien	Tayl	Gloeo	Glab	Parv	Oliv	Pol	Cap	Gna	Scor	Melis
Stem length (cm)	12–23	9–53	8–27	8–85	29–52	35–60	14–39	9–32	11–45.8	30–46	60–80
Leaf length (mm)	12–21	9.1–30	8.1–9	7.1–20	34–44	24–36	9.1–17.3	12–17.3	7.1–19.3	26–35	21–29
Leaf width (mm)	4–6.1	3.9–25	6.25–9.5	3.6–13	18–39	11.8–13.9	1–5.1	2–9.1	1.8–7.1	9.5–11.5	11.1–12.4
Length of inflorescence axis (cm)	7–35	9–29	11–40	6–40	13–55	17–47	3.1–23	2–19	2–29	5–26	33–53
Bract length (mm)	4–9	2–9	2.5–4	3–8	5.6–12.5	3–4.9	4.6–17.6	8.5–13	7.3–14	6–15	8.1–13
Bract width (mm)	0.5–7.2	0.3–7.5	1.2–4	0.4–6	3.5–6.2	0.5–1	0.9–2.8	1.8–3.5	1.7–3.7	4–5.4	4.6–5.9
Calyx length (mm)	6–8	4.2–7.5	4–5	4–7.5	4–6	5.5–6.3	2.6–5.5	2.5–6	4.7–6	5	5
Calyx width (mm)	5–7	2.8–6	4–5	3–8	2.1–3	3.8–6.5	1.4–3.4	0.2–3.1	2.5–4	3.5	2–2.5
Length of corolla tube (mm)	1.2–4	2–4	1.2–4	1.2–2.5	1.3–2	1.2–1.3	1–2	1.2–3	1.2–2	1.3	1.2–2
Length of corolla lip in upper surface (mm	7–9	6–12	9–10	5–12	1–2.1	8–11	1.1–3	1–2.1	1–2.1	1.2	2–3
Filament length (mm)	12–15	10–17	12–17	7–18	3–3.5	11–17	2.1–6.7	2–5	2–6	5.5–6	5–6
Stem indumentum	Pilose	Tomentose, Pilose, Articulate, Tuberculate, Pannose, Hirtellous, Villous, Branched	Glandular, Articulate, Tuberculate, Pilose, Villous, Branched	Glabrous	Pannose, Hirtellous, Tuberculate, Pilose	Pannose, Tuberculate	Lanate, Hirtellous, Barbate, Pilose, Tuberculate, Tomentose, Branched, Hirsute, Stellate, Pannose	Tomentose, Pilose, Stellate, Branched, Pannose, Tuberculate, Lanate, Articulate, Hirtellous	Pilose, Branched, Pannose, Hirtellous, Tomentose	Tuberculate, Lanate, Languinose, Pilose, Pannose	Villous, Pilose, Tuberculate
Leaf indumentum	Hirtellous, Pannose, Hirsute, Tuberculate	Hirtellous, Articulate, Villous, Tuberculate, Pannose, Pilose	Hirtellous, Articulate, Glandular, Tuberculate	Pilose, Articulate, Tuberculate, Villous, Hirtellous, Pannose, Glandular, Hirsute, Tomentose	Hirtellous, Pilose, Tuberculate	Hirtellous, Tuberculate, Pannose	Hirtellous, Pannose, Tuberculate, Lanate, Pilose, Branched, Tomentose, Villous	Hirtellous, Tuberculate, Tomentose, Pilose, Stellate, Branched, Pannose	Pannose, Tuberculate, Hirtellous	Hirtellous, Tuberculate, Tomentose, Pilose, Pannose, Lanate, Glandular	Hirtellous, Tuberculate, Pannose

Table 1 (continued)

Characters/species	Orien	Tayl	Gloeo	Glab	Parv	Oliv	Pol	Cap	Gna	Scor	Melis
Leaf form	Pinnatifid	Pinnatifid	Pinnatifid	Pinnatifid	Pinnatifid	Oblanceolate	Linear, Oblanceolate	Linear, Oblanceolate	Linear, Oblanceolate, Oblong	Oblong, Ovate-elliptic	Oblong, Ovate-elliptic
Leaf margin	Entire, Revolute	Entire, Revolute	Entire, Revolute	Entire, Revolute	Entire, Revolute	Entire	Sub-crenate, Revolute	Sub-crenate, Revolute	Sub-crenate, Revolute	Revolute, Crenate	Crenate
Leaf base	Obtuse, Acute	Obtuse, Acute, Cuneate	Acute	Obtuse, Acute	Cuneate	Cuneate, Acute	Obtuse, Acute, Cuneate	Obtuse, Acute, Cuneate	Obtuse, Acute	Sub-cordate	Acute
Leaf apex	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded, Lobed	Rounded	Rounded, Emarginate	Rounded	Rounded	Rounded
Petiole indumentum	Pannose	Hirtellous, Articulate, Pilose, Tuberculate, Pannose, Villous	Glandular, Hirtellous, Articulate	Pilose, Articulate, Hirtellous, Pannose, Glandular, Tomentose, Hirsute	Hirtellous, Tuberculate, Villous	Hirtellous, Tuberculate	–	–	–	–	–
Inflorescence indumentum	Pannose	Pilose, Hirtellous, Tuberculate, Pilose, Articulate, Glandular, Villous, Tomentose	Tuberculate, Glandular, Pilose, Articulate	Glabrous, sub-Glabrous	Hirtellous, Pannose, Tuberculate	Hirtellous, Pannose, Tuberculate	Lanate, Pilose, Tuberculate, Tomentose, Hirtellous, Pannose, Articulate, Floccose, Branched	Tomentose, Pilose, Stellate, Branched, Hirtellous, Tuberculate	Pilose, Branched, Pannose, Lanate, Tomentose	Lanate, Lanuginose, Pannose, Tuberculate	Tomentose, Pilose
Bract form	Pinnatifid	Pinnatifid	Pinnatifid	Pinnatifid	Pinnatifid	Pinnatifid	Linear, Oblong	Oblong	Linear, Oblong	Oblong	Oblong, Ovate-elliptic
Bract apex	Acute, Rounded	Acute, Rounded	Rounded	Acute, Rounded	Acute	Acute	Acute	Acute	Acute	Obtuse	Acute
Bract Indumentum	Pannose, Pilose, Tuberculate	Pannose, Pilose, Articulate, Hirtellous, Glandular, Villous	Tuberculate, Glandular	Glabrous, sub-Glabrous, Tuberculate	Hirtellous, Pannose, Tuberculate	Hirtellous, Pannose, Tuberculate, Pilose	Lanate, Tuberculate, Pilose, Tomentose, Hirtellous, Pannose, Branched, Villous	Branched, Hirtellous, Tuberculate, Pannose, Pilose, Stellate, Tomentose	Pannose, Pilose, Branched, Tuberculate, Hirtellous	Glandular, Tomentose, Lanate, Pannose, Tuberculate, Lanuginose	Pannose, Tuberculate, Hirtellous
Bracteole indumentum	–	–	–	–	–	–	Lanate, Pilose, Tuberculate, Tomentose, Glandular, Stellate, Hirtellous	Pilose, Tomentose, Tuberculate, Branched, Lanate, Hirtellous	Pilose, Lanate, Branched, Pannose, Tuberculate, Hirtellous	–	–

Table 1 (continued)

Characters/species	Orien	Tayl	Gloeo	Glab	Parv	Oliv	Pol	Cap	Gna	Scor	Melis
Pedicel indumentum	Hirtellous, Pilose	Pilose, Articulate, Tomentose, Tuberculate, Pannose	Tuberculate, Glandular	Glabrous	Pannose, Pilose, Barbate, Hirsute	Hirtellous, Pannose	Tuberculate, Pilose, Hirsute, Pannose, Tomentose, Branched, Lanate	Pilose, Tomentose, Lanate, Branched, Hirtellous	–	Pilose, Tuberculate, Tomentose, Barbate	Pilose, Tuberculate
Calyx indumentum	Pannose, Tuberculate	Pilose, Tuberculate, Articulate, Pannose, Hirtellous, Villous	Tuberculate, Glandular	Glabrous, Tuberculate, Villous	Tomentose, Pannose, Hirtellous, Tuberculate	Pannose	Pilose, Branched, Lanate, Velutinous, Tuberculate, Tomentose, Articulate	Tuberculate, Villous, Hirtellous	Villous, Pilose, Tuberculate, Branched	Tuberculate, Tomentose, Pilose	Tuberculate, Hirtellous, Tomentose, Pilose
Indumentum of corolla tube	Glabrous	Glabrous, Glandular, Tuberculate	Glabrous	Glabrous, Tuberculate, Glandular	Glabrous	Glabrous	Glabrous	Tuberculate, Pilose	Glabrous	Tuberculate	Pilose, Tuberculate
Indumentum of corolla lip	Pilose, Hirtellous, Tuberculate, Articulate	Tuberculate, Villous, Articulate, Hirtellous, Hirsute, Pilose	Tuberculate, Hirtellous, Pilose, Articulate, Hirsute	Tuberculate, Hirtellous	Hirtellous, Barbate, Pilose, Tuberculate, Pannose, Articulate	Tuberculate, Villous, Pilose	Tuberculate, Villous, Pilose, Hirtellous, Branched, Glandular	Tuberculate, Pilose, Villous, Hirtellous	Tuberculate, Pilose, Villous	Tuberculate, Pilose	Villous, Tuberculate, Hirtellous
Filament indumentum	Glandular, Hirtellous, Tuberculate	Tuberculate, Glandular, Hirtellous	Tuberculate, Glandular	Tuberculate, Hirtellous, Glandular, Glabrous	Hirtellous, Glandular, Tuberculate	Villous, Pilose, Articulate, Glandular	Villous, Pilose, Hirsute, Branched, Tuberculate, Glandular, Barbate	Glandular, Tuberculate, Villous, Hirtellous, Glandular	Pilose, Villous	Villous, Tuberculate	Villous, Tuberculate, Hirtellous
Style indumentum	Glabrous	Glabrous, Tuberculate	Glabrous	Tuberculate, Glabrous	Glabrous, Sub-glabrous	Glabrous, Tuberculate	Glabrous, Tuberculate, Sub-glabrous, Villous	Tuberculate, Glabrous, Villous	Glabrous	Sub-glabrous	Sub-glabrous

Orien, *T. orientale* subsp. *orientale*; tayl, *T. orientale* subsp. *taylori*; gloeo, *T. orientale* subsp. *gloeotrichum*; glab, *T. orientale* subsp. *glabrescens*; parv, *T. parviflorum*; oliv, *T. oliverianum*; pol, *T. polium*; cap, *T. capitatum*; gna, *T. gnaphalodes*; scor, *T.scordium* subsp. *scordioides*; melis, *T. melissoides* indumentum of stem (*T. polium*), indumentum of inflorescence axis (*T. polium* and *T. capitatum*), indumentum of bract (*T. polium*, and *T. capitatum*), indumentum of bracteole (*T. polium* and *T. gnaphalodes*), indumentum of pedicel (*T. polium*, and *T. capitatum*), indumentum of calyx (*T. polium* and *T. capitatum*), indumentum of corolla tube (*T. capitatum*), indumentum of corolla lips (*T. polium*), indumentum of style (*T. polium*) and indumentum of filament (*T. polium* and *T. capitatum*) (Table 1).

Among all taxa under investigation, some diagnostic morphological characters were identified (Figs. 1, 2, 3). *T. capitatum* revealed indumentum of stellate at leaf, bract and inflorescence, pilose at corolla tube and emarginate apex of leaf. Moreover, *T. polium* was differentiated by the presence of hirsute and barbate indumentum 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. The presence of pannose indumentum at bracteole was characterized in *T. gnaphalodes*.

In section *Scordium*, morphological characters including sub-cordate base of leaf and obtuse apex of bract, indumentum of lanuginose at stem, inflorescence and bract were observed in *T. scordium* subsp. *scordioides.* Moreover, ovate-elliptic form of bract was found as diagnostic character in *T. melissoides*.

In section *Teucris*, *T. orientale* subsp. *glabrescens* was discriminated by the presence of hirsute and tomentose indumentum at petiole. The indumentum of glabrous and sub-glabrous was characterized at stem, inflorescence, bract, pedicel, calyx and filament, whereas the existence of villous indumentum at inflorescence, and articulate at pedicel and bract corresponded to *T. orientale* subsp. subsp. *taylori*. The indumentum of glandular at stem, pedicel and calyx was also attributed to *T. orientale* subsp. *gloeotrichum*. Moreover, both species *T. oliverianum* and

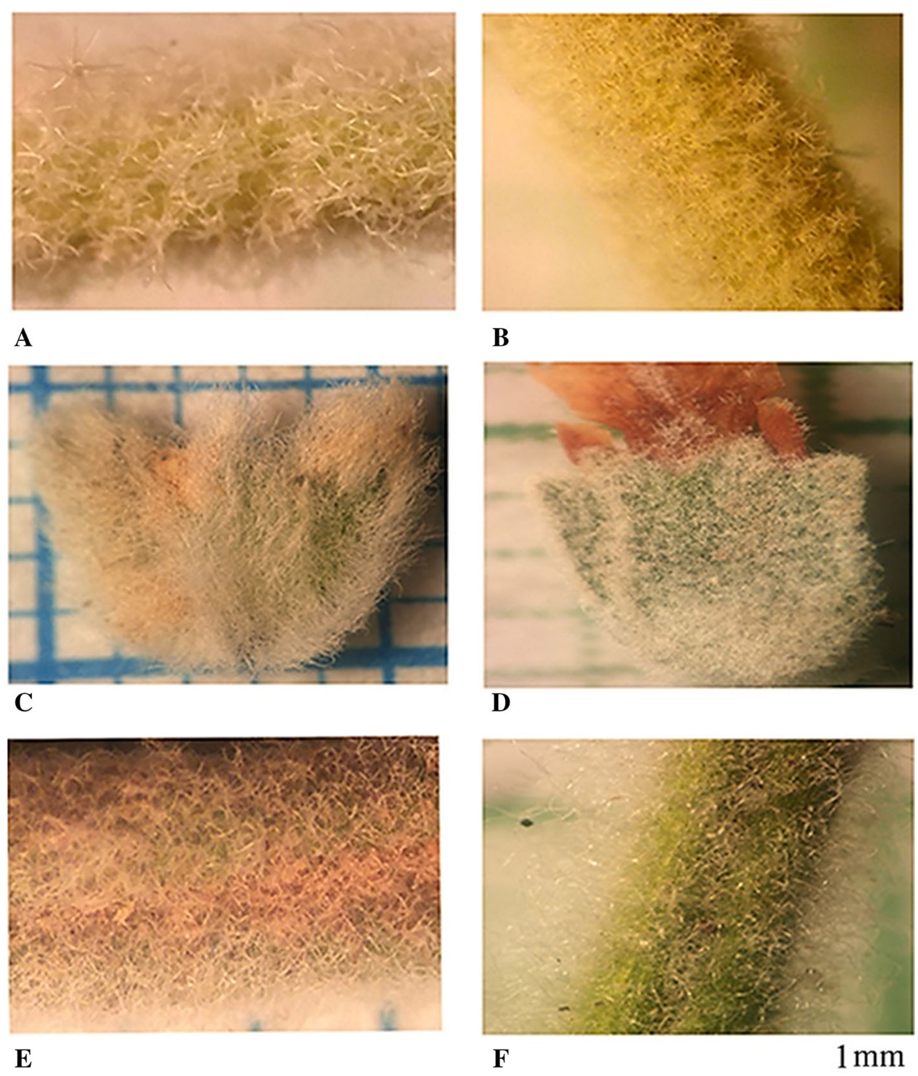

Fig. 1 Representative indumentum of *T. capitatum, T. polium, T. gnaphalodes* and *T. scordium* subsp. *scordioides*. **a**, **b** stellate at inflorescence and stem (*T. capitatum*), **c**, **d** lanate and velutinous at calyx (*T. polium*), **e** pannose at bracteole (*T. gnaphalodes*), **f** lanuginose at inflorescence (*T. scordium* subsp. *scordioides*)

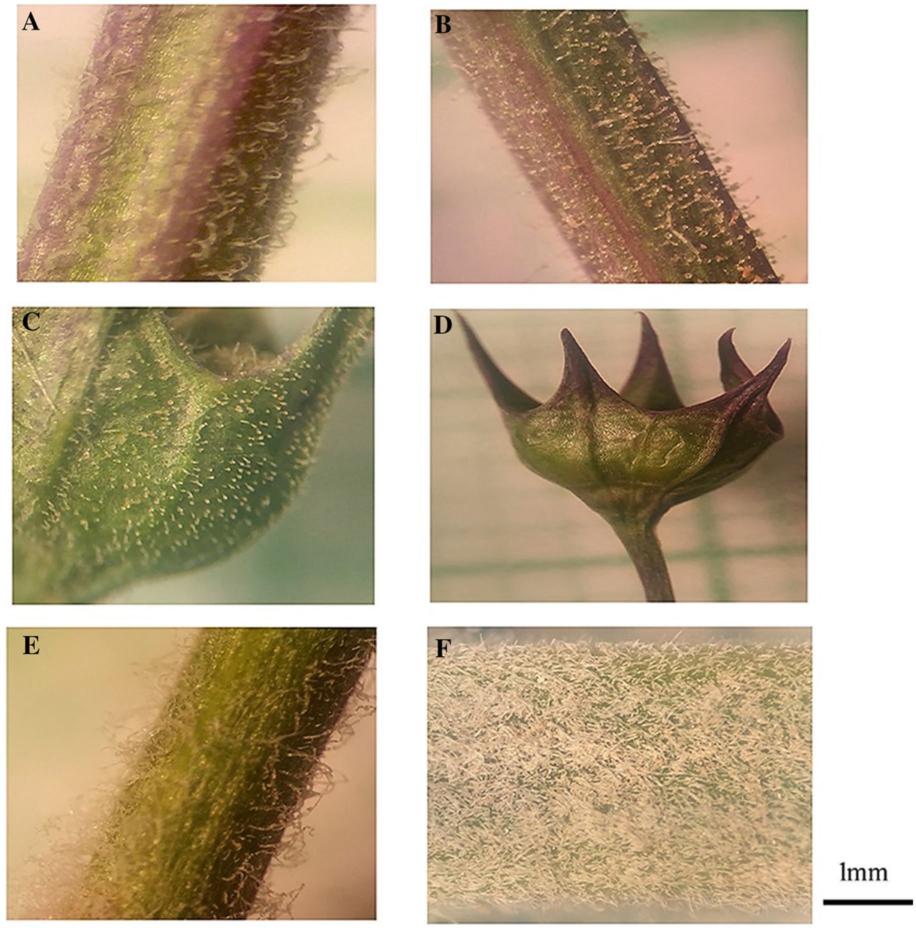

Fig. 2 Representative indumentum of *T. orientale* subspecies. **a** hirtellous at pedicel (*T. orientale* subsp. *orientale*), **b**, **c** glandular at stem and calyx (*T. orientale* subsp. *gloeotrichum*), **d** glabrous at pedicel and calyx (*T. orientale* subsp. *glabrescens*), **e**, **f** villous and tomentose at inflorescence and stem (*T. orientale* subsp. *taylori*)

T. parviflorum were differentiated by indumentum of corolla lip (barbate, pannose) and form of leaf apex.

The ANOVA test was assessed for quantitative morphological characteristics. Significant differences (*$p < 0.05$; *F-value* = 2.07–5.7) were provided in *Teucrium* species. They were assigned in length of leaf, stem, inflorescence, petiole, bract, bracteole, corolla tube and calyx. The Kruskal–Wallis test statistically approved the significant difference (*$p < 0.01$) for qualitative characteristics, including indumentum of inflorescence, bracteole, calyx, corolla lips, margin, base and apex of bracteole.

Based on the data presented in Fig. 4, the UPGMA dendrogram of morphological information showed two distinct groups. The first group is comprised of the members of section *Teucris*; *T. orientale* subsp. *taylori*, subsp. *gloeotrichum*, subsp. *glabrescens*, subsp. *orientale*, *T. oliverianum* and *T. parviflorum*. The second group was also formed by the members of the section *Polium*; *T. polium*, *T. capitatum, T. gnaphalodes* and the section *Scordium*; *T. scordium* subsp. *scordioides* and *T. melissoides*. According to the information, different groups were recognized, including *T. polium* with eleven groups, *T. orientale* subsp. *taylori* with seven groups, *T. capitatum* and *T. gnaphalodes* with four groups, *T. scordium* subsp. *scordioides*, *T. gloeotrichum*, *T. oliverianum*, *T. orientale* subsp. *orientale*, and *T. parviflorum* with two groups, and *T. melissoides* with one group. The main value of morphological variation was observed in the accessions of *T. polium* and *T. orientale* subsp. *taylori*. It has been found that in section *Teucris*; three subspecies of *T. orientale* were precisely separated. Nevertheless, it seems that the subsp. *taylori* with accession numbers 2, 6, 18 and 23 revealed few relations with subsp. *gloeotrichum*, subsp. *glabrescens* and subsp. *orientale*. It is important to point out that some accessions of these subspecies grow as sympatric distribution in Zagros regions. The presence of the specific indumentum of subsp. *taylori* led to display different groups such as accession no. 2 with hirtellous at stem and villous at inflorescence, accession no.18 with branched trichome at stem, tuberculate at petiole, style and leaf, accession no. 23 with pilose at bract and petiole and accession no. 6 with hirtellous at filament. In section *Scordium*, two accessions of *T. scordium* (nos. 40 and 45) were distinctly separated. It excludes variations caused by ecological and geographical conditions in Zagros regions as a possible reason. The morphological characteristics of

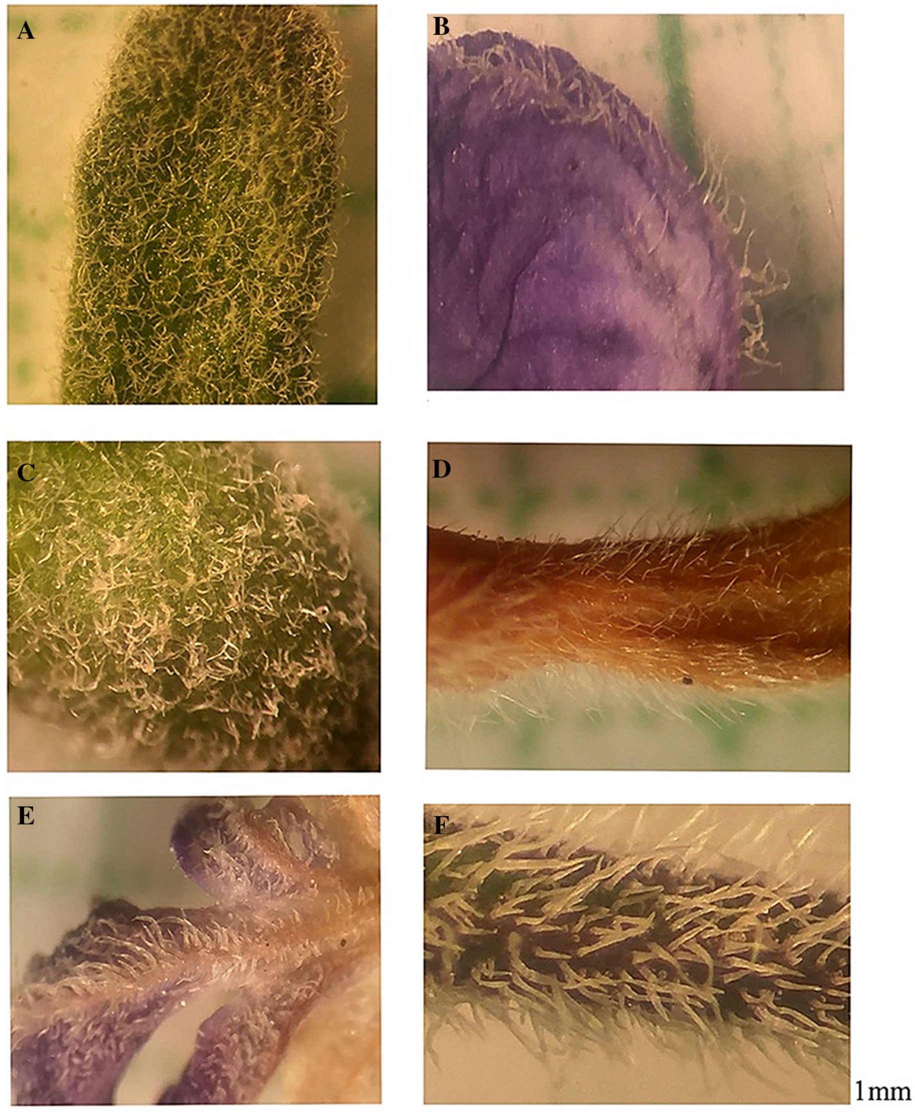

Fig. 3 Representative indumentum of *T. polium, T. oliverianum, T. melissoides,* and *T. parviflorum*. **a** villous at leaf (*T. polium*), **b** villous at corolla lip (*T. oliverianum*), **c** hirtellous at calyx (*T. melissoides*), **d** pilose at corolla tube (*T. melissoides*), **e** barbate at corolla lip (*T. parviflorum*), **f** hirsute at pedicel (*T. parviflorum*)

T. scordium with accession no. 45 were differed from its accession no. 40, namely as oblong leaf, indumentum of pilose, tuberculate and lanuginose at stem, indumentum of lanate, pannose, glandular, tomentose and lanuginose at bract, and indumentum of tomentose and barbate at pedicel. Moreover, in section *Polium*, *T. capitatum* accessions were distinctly grouped, but *T. gnaphalodes* with accession number 51 was grouped with two *T. capitatum* accessions studied. Different characteristics, including indumentum of pilose at style and bracteole, led to this differentiation. It is remarked that morphological characteristics strongly resolve the complexity of sections *Polium* and *Teucris*.

3.2 Chemotaxonomy Results

The flavonoid profiles of thin layer chromatography in *Teucrium* species showed an appropriate solvent system as $CHCl_3$–MeOH (60:40). The extracts analysis in the present study revealed the existence of 353 color spots in *Teucrium* accessions. The variation in color spots was frequently provided as blue, fluorescent blue, green, dark yellow, yellow and fluorescent yellow. The color spots were presented under natural product identifiers, including blue, fluorescent blue, violet, yellow and fluorescent yellow. The retention factor (Rf) and m/z (mass to charge) were also estimated for each spot ranging from 0.49 to 0.59 and from 221 to 801 (Table 2).

Based on the following evidence on UPGMA dendrogram of flavonoid data (Fig. 5a), two main clusters were revealed. According to these findings, different groups were recognized. Nevertheless, the highest value of variability was observed in section *Teucris*, including *T. orientale* subsp. *glabrescens* with ten groups, *T. orientale* subsp. *taylori* with seven groups, and in section *Polium,*

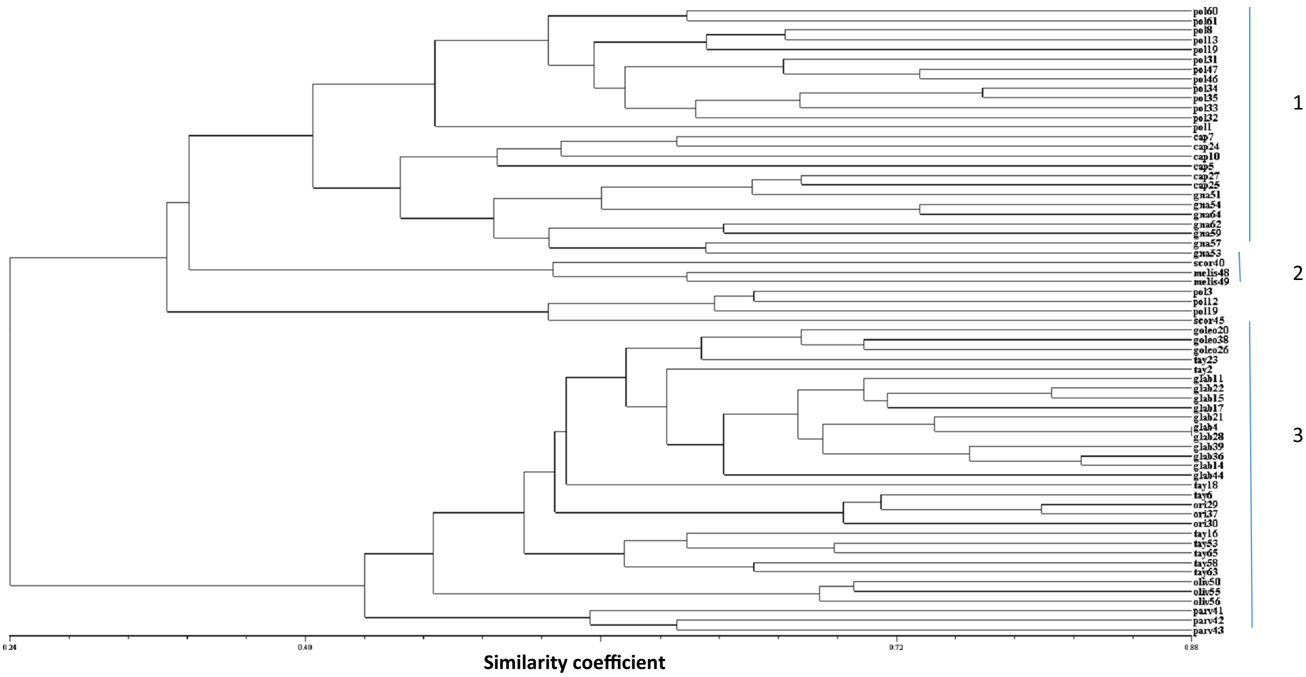

Fig. 4 Dendodgram of *Teucrium* species and their accessions using morphological characteristics. Pol: *T. polium,* cap: *T. capitatum*, gna: *T. gnaphalodes*, scor: *T. scordium* subsp. *scordioides*, melis: *T. melissoides*, gloeo: *T. orientale* subsp. *gloeotrichum*, tayl: *T. orientale* subsp. *taylori*, ori: *T. orientale* subsp. *orientale*, glab: *T. orientale* subsp. *glabrescens*, oliv: *T. oliverianum*, parv: *T. parviflorum*.1: section *Polium*, 2: section *Scordium,* 3: section *Teucris*

including *T. polium* with five groups. The lowest value of variability was provided with one group in section *Teucris,* including *T. orientale* subsp. *orientale*, *T. parviflorum* and *T. oliverianum* and in section *Scordium,* including *T. scordium* subsp. *scordioides* and *T. melissoides*. In section *Teucris*, further variations belonging to *T. orientale* subspecies *taylori*. *T. orientale* subsp. *taylori* with accession numbers 53 and 65 were significantly different from its accessions. It was identified that its accession no. 2 was clustered with *T. orientale* subsp. *glabrescens* with accession no. 11. This document was proved to be a factor showing the correlation and diversity of flavonoid at subspecific levels. Moreover, there was a relation between *T. orientale* subsp. *orientale* with accession number 37 and *T. orientale* subsp. *taylori* with accession number 23 (Fig. 5a). However, they comprised of independent clusters. It is recognized that *T. orientale* subsp. *orientale* was discriminated by color spot (green).

Despite morphological similarity between the members of section *Polium*, the two species *T. polium* and *T. gnaphalodes* exhibited a clear-cut separation. The present evidence was observed exactly in *T. polium* and *T. capitatum*. *T. capitatum* with accession number 7 was accurately different from its other accessions. Flavonoid variation of *T. polium* accessions was significant, and in some cases, they were grouped with some *T. capitatum* accessions (Fig. 5a).

Principle component analysis (PCA) was also consistent with cluster analysis (Fig. 5b). Following this approach, in section *Polium*, *T. polium* accessions were strongly categorized from the two species *T. capitatum* and *T. gnaphalodes*. Moreover, in section *Teucris*, *T. orientale* subsp. *glabrescens* and *T. orientale* subsp. *taylori* exhibited high variations, but two subspecies were exactly classified using dark yellow and fluorescent blue spots, and the type of flavonoid classes.

To better characterize the exact position of the section *Teucris* members (*T. orientale* and its subspecies) and section *Polium* (*T. polium*, *T. capitatum* and *T. gnaphalodes*), cluster analysis was separately performed. The subspecies of *T. orientale* were definitely clustered. However, some relations exist between *T. orientale* subsp. *taylori, T. orientale* subsp. *orientale* and *T. orientale* subsp. *glabrescens*. Similarly, *T. polium* accessions were exactly distinguished even though some affinities were provided with *T. capitatum* (Fig. 5c, d).

3.3 Flavonoid Classes

According to flavonoid classes, seven groups were mostly recognized in the *Teucrium* species with the presence of flavone (18), isoflavone (17), flavonol (13), flavanone (3), chalcone (3), dihydroflavonol (2) and aurone (1) (Table 2). It is specifically found diagnostic flavonoid classes in the

Table 2 Flavonoid class identified in each *Teucrium* species with the shift reagent $AlCl_3$/HCl, Rf, m/z (mass to charge) and λmax in Methanol, and color spots

Class/species	Orien	Tayl	Gloeo	Glab	Parv	Oliv	Pol	Cap	Gna	Scor	Melis	shifts	m/z [M-H]-	λmax
Flavone1				+				+	+			52/23	269	247,274,323
Flavone2		+						+				81/35	579	257,272,349
Flavone3	+	+	+	+	+	+	+	+	+	+	+	2/31	269	281, 364
Flavone4								+				43/36	223	244,269,299,350
Flavone5								+				46/39	299	240,252,267,291,344
Flavone6				+								46/44	431	271,336
Flavone7				+								45/42	593	271,336
Flavone8				+								67/68	253	247,268,313
Flavone9				+								47/41	313	240,248,269,293,340
Flavone10				+								61/60	237	268,296,333
Flavone11				+								29/76	373	250,279,336
Flavone12									+			53/49	431	268,333
Flavone13			+								+	1/2	221	250,294,307
Flavone14											+	2/2	561	255,311,325
Flavone15						+						57/56	609	270,326
Flavone16							+					39/–	577	247,255,305,341
Flavone17										+		80/35	447	242,255,271,349
Flavone18		+										54/45	446	247,274,315,342
Isoflavone1			+					+				49/50	281	241,250,252,295,345
Isoflavone2				+				+				–/–	237	242,299,305
Isoflavone3						+						57/56	579	270,326
Isoflavone4	+	+		+		+	+			+	+	69/68	419	274,353
Isoflavone5		+		+							+	1/1	589	249,261,292
Isoflavone6				+								44/44	269	261,328
Isoflavone7				+								29/33	447	262,290,343
Isoflavone8				+								35/38	359	268,336
Isoflavone9				+								1/1	281	251,306
Isoflavone10				+						+		52/52	253	259,303,315
Isoflavone11							+		+			40/13	484	238,254,325
Isoflavone12		+	+		+		+					–/1	283	256,283,317
Isoflavone13			+									1/6	265	257,298,323
Isoflavone14											+	1/2	299	258,320
Isoflavone15											+	1/–	429	251,258,301
Isoflavone16						+						9/51	415	256,313
Isoflavone17							+					6/21	285	245,270,350
Flavonol1				+								76/43	463	257,269,299,362

Table 2 (continued)

Class/species	Orien	Tayl	Gloeo	Glab	Parv	Oliv	Pol	Cap	Gna	Scor	Melis	shifts	m/z [M-H]-	λmax
Flavonol2				+								2/4	401	242,252,266,333
Flavonol3									+			88/56	331	258,272,293,371
Flavonol4									+			51/49	301	254,264,370
Flavonol5									+			83/38	447	255,267,293,346
Flavonol6									+			88/58	301	255,269,301,370
Flavonol7						+			+			80/51	447	256,265,301,350
Flavonol8							+					56/55	753	253,267,299,320,367
Flavonol9							+					43/80	447	254,310,340
Flavonol10										+		58/57	315	259,276,327,377
Flavonol11		+										51/50	801	254,269,349
Flavonol12		+										50/48	739	244,265,315,350
Flavonol13										+		59/62	343	271,340
Flavanone1									+			1/1	255	276,312
Flavanone2						+						57/53	609	283,326
Flavanone3				+	+		+		+			5/7	447	280,317
Chalcone1	+							+				4/3	223	243,284,344
Chalcone2			+				+					–/–	223	224,318
Chalcone3		+										48/–	239	265,316,365
Dihydroflavonol1			+				+		+			7/83	449	234,280,311
Dihydroflavonol2				+	+							37/1	303	275,308
Aurone1			+						+			47/6	253	283,312
Rf	0.57	0.54	0.56	0.57	0.51	0.56	0.49	0.59	0.53	0.51	0.53	–	–	–
Color spots	Green, yellow	Dark yellow, fluorescent blue	Yellow, blue	Yellow, blue	Fluorescent Yellow	Yellow	Yellow, blue	Yellow	Yellow, blue	Yellow	Yellow, blue			

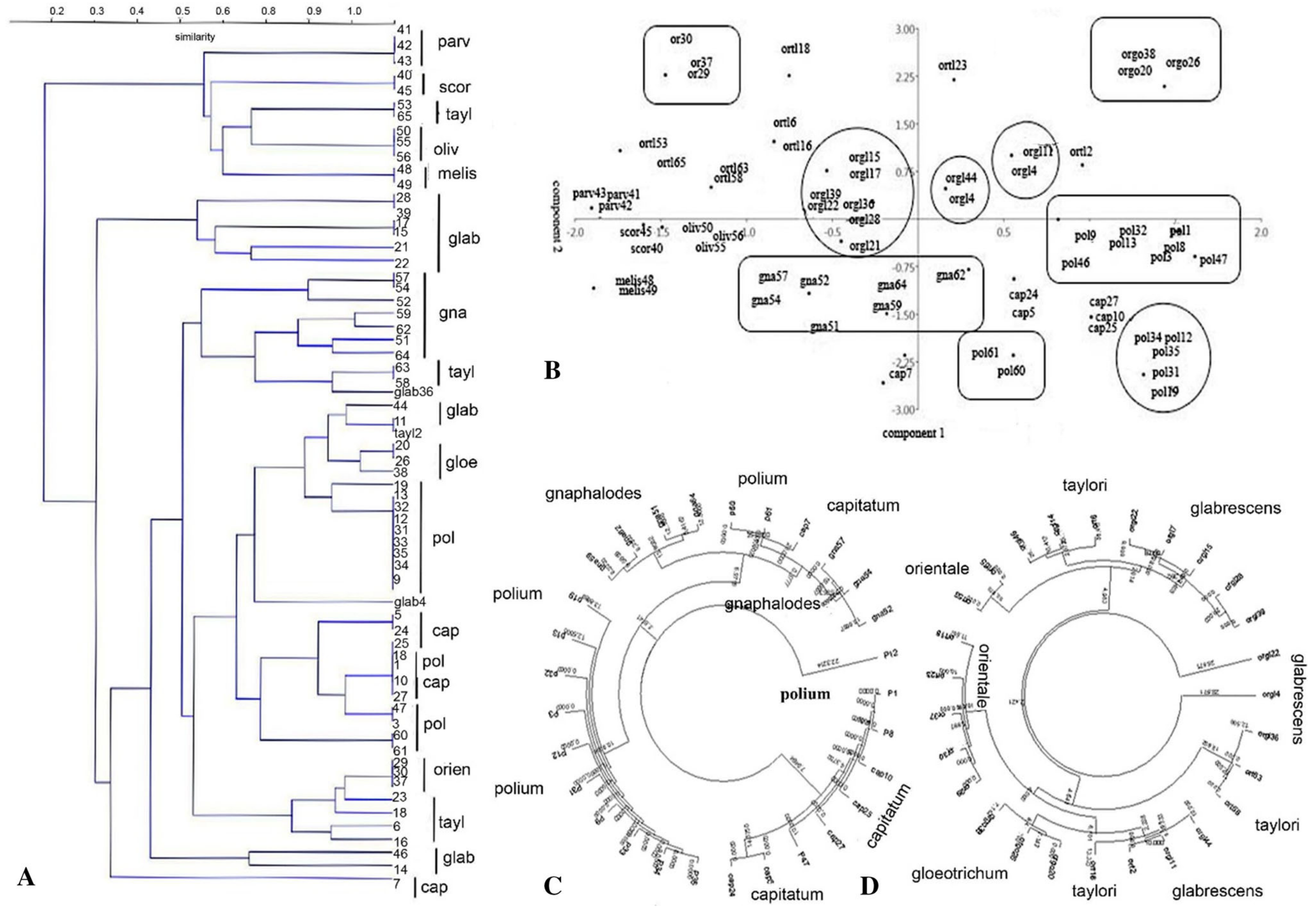

Fig. 5 **a** Dendrogram of *Teucrium* species and their accessions using flavonoid data. Pol: *T. polium,* cap: *T. capitatum*, gna: *T. gnaphalodes*, scor: *T. scordium* subsp. *scordioides*, melis: *T. melissoides*, gloeo: *T. orientale* subsp. *gloeotrichum*, tayl: *T. orientale* subsp. *taylori*, orien: *T. orientale* subsp. *orientale*, glab: *T. orientale* subsp. *glabrescens*, oliv: *T. oliverianum*, parv: *T. parviflorum*. **b** PCA analysis of *Teucrium* species and flavonoid data. orgo: *T. orientale* subsp. *gloeotrichum*, ortl: *T. orientale* subsp. *taylori*, or: *T. orientale* subsp. *orientale*, orgl: *T. orientale* subsp. *glabrescens*. **c** Circular dendrogram from the members of section *Polium* and **d** section *Teucris* using flavonoid data

members of three sections. All sub-specific levels of *T. orientale* were discriminated with different flavonoid classes, including flavone nos. 6-11, isoflavone nos. 6-9 and flavonol nos. 1, 2 in subsp. *glabrescens*, flavone no. 18, flavonol nos. 11, 12 and chalcone no. 3 in subsp. *taylori* and isoflavone no. 13 in subsp. *gloeotrichum*. In addition, flavone no. 15, isoflavone nos. 3, 16 and flavanone no. 2 were observed in *T. oliverianum*. However, *T. orientale* subsp. *orientale* and *T. parviflorum* were identified by green and fluorescent yellow spots, respectively (Table 2). In addition, further flavonoid classes were ascribed to flavone nos. 4, 5 in *T. capitatum*, flavone no. 12, flavonol nos. 3-6 and flavanone no. 1 in *T. gnaphalodes*, and isoflavone no. 17, flavone no. 16 and flavonol nos. 18, 19 in *T. polium* (Table 2). Flavone no. 17 and flavonol nos. 10, 13 were assigned to *T. scordium* subsp. *scordioides*, and flavone no. 14 and isoflavone nos. 14, 15 were recognized in *T. melissoides*.

It was recognized that flavone 3 was the most common flavonoid class in all *Teucrium* species. The UV-spectral shift reagent for flavonoid classes ranged from 1 to 88 nm, and the λ max absorbance ranged from 224 to 377 nm (Table 2).

Further cluster analysis was performed using both morphological characters and flavonoid data (Fig. 6). This analysis completely separated the members of three sections through two different groups. Consequently, this analysis more thoroughly supports the taxonomic status of *Teucrium* species. Moreover, high diversity was observed in *Teucrium* accessions including *T. orientale* subsp. *glabrescens* (seven groups), *T. polium* (seven groups), *T. orientale* subsp. *taylori* (six groups), *T. gnaphalodes* (four groups) and *T. capitatum* (three groups). Other species consist of one group. As mentioned above, some relationships exist in a few accessions, e.g., *T. orientale* subsp. *taylori*, *T. orientale* subsp. *orientale* and *T. orientale* subsp. *glabrescens*, and *T. polium* and *T. capitatum*.

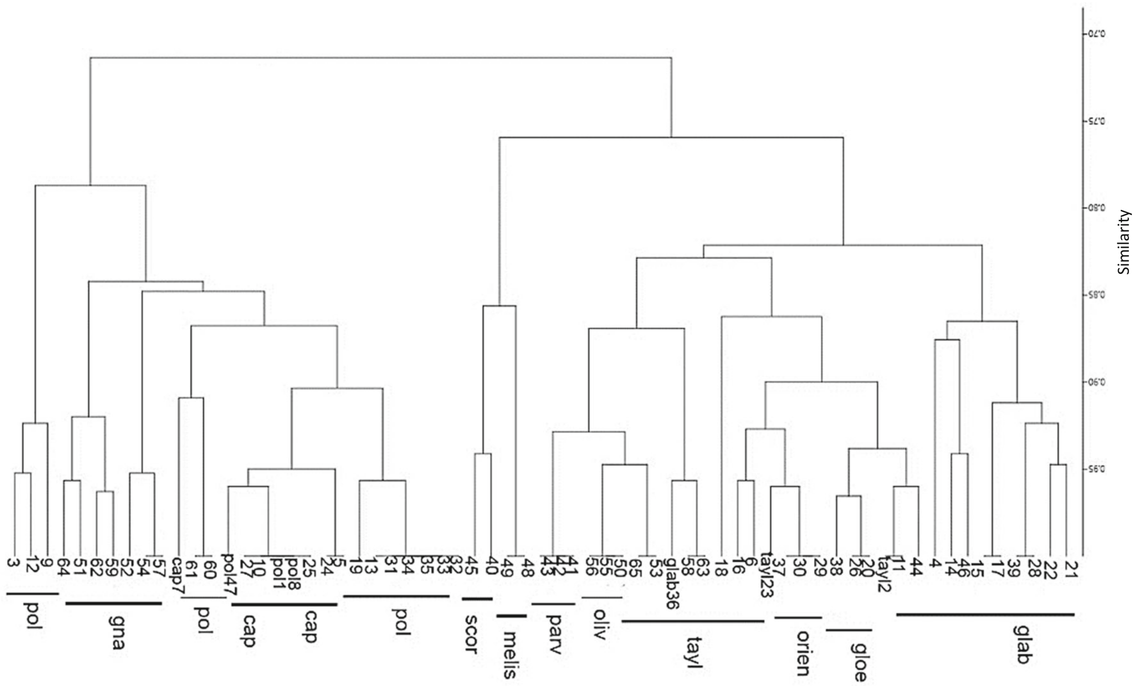

Fig. 6 Dendrogram of *Teucrium* species and their accessions using both morphological characters and flavonoid data. Pol: *T. polium,* cap: *T. capitatum*, gna: *T. gnaphalodes*, scor: *T. scordium* subsp. *scordioides*, melis: *T. melissoides*, gloeo: *T. orientale* subsp. *gloeotrichum*, tayl: *T. orientale* subsp. *taylori*, orien: *T. orientale* subsp. *orientale*, glab: *T. orientale* subsp. *glabrescens*, oliv: *T. oliverianum*, parv: *T. parviflorum*

With the purpose of discrimination, two morphological and flavonoid keys were provided for *Teucrium* species and its subspecies.

Morphological key to *Teucrium* species

1a—Leaf pinnatifid or lobed in apex, inflorescence panicle	2
b—Leaf simple, inflorescence terminal racem or racem with two or five flower	4
2a—Leaf apex with 3–5 lobed	*T. oliverianum*
b—Leaf deeply pinnatifid	3
3a—Leaf 34-44 mm, corolla lip in upper surface 1–2.1 mm, filament 3–3.5 mm	*T. parviflorum*
b—Leaf 7.1–30 mm, corolla lip in upper surface 5–12 mm, filament 7–18 mm	*T. orientale*
4a—Leaf linear, 7.1–19.3 mm	5
b—Leaf oblong and ovate-elliptic, 21–35 mm	7
5a—Bract up to 17.6 mm, calyx velutinous or lanate	*T. polium*
b—Bract less than 17.6 mm, calyx non-velutinous or non-lanate	6
6a—Indumentum of whole plant short, grayish	*T. capitatum*
b—Indumentum of whole plant long, white	*T. gnaphalodes*
7a—Stem 60–80 cm, inflorescence 33–53 cm, upper corolla lip 2–3 mm, corolla white, leaf base acute	*T. melissoides*
b—Stem 30–46 cm, inflorescence 5–26 cm, upper corolla lip 1.2 mm, corolla pink, leaf base sub-cordate	*T. scordium* subsp. *scordioides*

Morphological key to the subspecies of *T. oreintale*

1a—Stem non-glabrous	2
b—Stem glabrous	subsp. *glabrescens*
2a—Plant glandular trichome	subsp. *gloeotrichum*
b—Plant non-glandular trichome	3
3a—Stem non-dense indumentum, inflorescence up to 35 mm	subsp. *orientale*
b—Stem mostly dense indumentum, inflorescence up to 29 mm	subsp. *taylori*

Flavonoid key to *Teucrium* species

1a—Flavonoid classes include flavone (15, 18) and isoflavone (3, 13, 16)	2

b—Flavonoid classes include flavone (4–5, 12, 14, 16–17) and isoflavone (11, 14–15, 17)	4
2a-—Flavonid classes include flavone (18) and isoflavone (13)	*T. orientale*
b—Flavonoid classes non-flavone (18) and non-isoflavone (13)	3
3a—Flavonid with flavone (15), flavanone (2) and isoflavone (3, 16), non-fluorescent yellow spot	*T. oliverianum*
b—Non-flavone (15), flavanone (2) and isoflavone (3, 16), with fluorescent yellow spot	*T. parviflorum*
4a—Flavonoid classes include flavone (4–5, 12, 16) and isoflavone (11, 17)	5
b—Flavonoid classes include flavone (14, 17), isoflavone (14–15) and flavonol (10, 13)	6
5a—Flavonoid with flavone (4–5), without specific isoflavone and flavonol	*T. capitatum*
b—Flavonoid with flavone (12, 16), isoflavone (11) and flavonol (3–6, 8–9)	7
6a—Flavonoid classes include flavone (14), isoflavone (14–15) and non-flavonol	*T. melissoides*
b—Flavonoid classes include flavone (17), non-isoflavone and flavonol (10, 13)	*T. scordium* subsp. *scordioides*
7a—Flavonoid classes include flavone (16), isoflavone (17) and flavonol (8–9)	*T. polium*
b—Flavonoid classes include flavone (12), flavanol (3–6) and flavanone (1)	*T. gnaphalodes*

Flavonoid key to the subspecies of *T. orientale*

1a—Flavonoid with green spots	subsp. *orientale*
b—Flavonoid non-green spots	2
2a—Flavonoid with chalcone (3), flavone (18) and flavonol (11–12)	subsp. *taylori*
b—Flavonoid non-chalcone (3), with isoflavone (6–9, 13) or flavone (6–11), and flavonol (1–2)	3
3a—Flavonoid class includes isoflavone (13)	subsp. *gloeotrichum*
b—Flavonoid classes include isoflavone (6–9), flavone (6–11) and flavonol (1–2)	subsp. *glabrescens*

Regarding the literature, *Teucrium* species comprise of complex groups, which need to be more investigated (Salmaki et al. 2016; Ranjbar et al. 2017). There is a lack of chemotaxonomic study in the *Teucrium* species in worldwide. Therefore, other recent studies provided in *Teucrium* were discussed here.

Regarding the flavonoid and morphological information of the section *Teucris*, the subspecies of *T. orientale* were certainly separated. Eshratifar et al. (2009) specified that the transection of midrib and leaf was different in the subspecies of *T. orientale*. According to the data presented in the UPGMA dendrogram of flavonoid profiles and quantitative and qualitative morphological characteristics (Table 1; Figs. 4,5a), three subspecies were definitely separated. It was noted that flavonoid profiles in cluster and PCA analyses presented high potential to discriminate four subspecies. The chemotaxonomic status of *T. orientale* subsp. *orientale* and *T. orientale* subsp. *taylori* displayed distinctly different specification. Cakir et al. (2006) also reported different amounts of flavonoid and iridoids in *T. orientale* var. *orientale,* confirming our evidence.

This was interpreted that there was a relationship between *T. orientale* subsp. *taylori* and subsp. *glabrescens*. In some cases of flavonoid profiles clustering, a group was found in *T. orientale* subsp. *taylori* with accession number 2 and subsp. *glabrescens* with accession number 11. Similarly, a relationship was observed between *T. orientale* subsp. *taylori* with accession numbers 58 and 63 and subsp. *glabrescens* with accession number 36. Since there was a complexity in section *Teucris* members, in the present research, a separated analysis using flavonoid information provided for the subspecies of *T. orientale*. It is elucidated that its subspecies were distinctly classified. Morphological data were consistent with those earlier reported that the foliar trichome type (branched and dense trichome) was similar in *T. orientale* subsp. *taylori* and subsp. *glabrescens* (Ecevit Gen et al. 2015). However, the intraspecific levels of *T. orientale* included different attributes (Ecevit Gen et al. 2015). This was proposed that *T. orientale* subspecies could be introduced as specific levels, but further knowledge is required to obtain the current concept. It is shown that *T. orientale* subsp. *taylori* illustrates high polymorphism (Rechinger 1982). Moreover, considerable geographical races were proved in Iran and India, which involved in gradual variability of trichomes (Rechinger 1982). The evidence can possibly ascertain the presence of intermediate species.

In formerly published works, *T. orientale* subsp. *glabrescens* and subsp. *gloeotrichum* exhibited similarity in terms of the thickness of upper parenchyma, collenchyma cell, upper palisade parenchyma and rows of spongious parenchyma (Eshratifar et al. 2009). Moreover, subsp. *orientale* and subsp. *glabrescens* have the same unbranched multicellular trichomes (Eshratifar et al. 2009; Ecevit Gen et al. 2015). In morphological findings, few relations were also observed between subsp. *taylori* (accession nos. 18, 2 and 23), subsp. *glabrescens* and subsp. *gloeotrichum*. In their natural habitats, some accessions of sub-specific levels grow in the same ecological regions of Zagros such as Lurestan and Isfahan provinces. Eshratifar et al. (2011, 2009) presented the similarities of these taxa in terms of shape and cell wall of nutlet. It is of note that both morphological and flavonoid data mainly represent distinct groups of *T. orientale* subspecies.

Ecevit Gen et al. (2015) have stated that there was similarity of trichome between *T. orientale* and *T. parviflorum*. In our morphological and both morphological and flavonoid analysis, a few similarities were found. In flavonoid evaluations, there was no similarity between two species. It was previously considered that a high complexity was occurred between *T. orientale, T. oliverianum* and *T. parviflorum,* and taxonomic delimitation was not resolved (Rechinger 1982).

Following presented results, *T. parviflorum* and *T. oliverianum* showed different groups. In previously published reports, the two species were different in the type of trichomes including multicellular and unicellular, the presence of micropapilate trichomes, and the shape of midrib (Eshratifar et al. 2009, 2011). In some cases, the similarities of two species were previously described in terms of the thickness of nutlet, the presence of one celled and 2–5 celled trichomes (Eshratifar et al. 2009, 2011). Apparently, the nutlet characteristics were found to be similar in both species. However, flavonoid profiles and morphological results (qualitative and quantitative characters) led to consider differentiation of the two mentioned species (Tables 1, 2).

In section *Scordium*, *T. mellissoides* was discriminated from *T. scordium* subsp. *scordioides* in terms of cluster and PCA analyses using flavonoid and morphological characteristics. Two species were previously different in type of epidermal cell, parenchyma, leaf trichomes and nutlet surface (Eshratifar et al. 2009, 2011). Controversially, Eshratifar et al. (2011) evidenced some resemblances such as long clavate glandular trichome of leaf between *T. melissoides* and *T. scordium* subsp. *scordioides*. Discrimination was also found in the quantitative and qualitative morphological characteristics studied (Table 1). Morphologically, the differentiation of the two studied accessions of *T. scordium* was presented, including Isfahan and Kurdistan provinces. To support the present flavonoid results, no chemical variations were previously provided in *T. scordium* subsp. *scordioides* (Jurisic Grubesic et al. 2012).

In section *Polium*, clustering and PCA analysis of both flavonoid and morphological markers were performed. *T. polium* accessions were mostly isolated from other species in this section. Three members of this section were precisely identified, which were obtained from morphology (quantitative and qualitative characters) and flavonoid information (Tables 1, 2). It was recognized that the indumentum of calyx was known as the specific taxonomic value. From previous published reports, different indumentum of calyx were identified between *T. polium* and *T. capitatum* (Rechinger 1982), confirming the present findings. Some *T. polium* with accession numbers 1 and 8 were grouped with *T. capitatum* with accession numbers 10 and 27. There was also a correlation between the two studied species responsible for flavonoid profiles. In the previous results, *T. polium* was introduced as a non-independent taxon, which needs to be further revised (Rechinger 1982). Based on the previous information of indumentum characteristics, two different groups were recognized in *T. polium* (Rechinger 1982). Moreover, high variation in leaf form and its indumentum led to the present imbricate characteristics, which did not certainly separate them (Jamzad 2012). According to our flavonoid and morphological evidence, several groups were recognized in *T. polium*. Bosabalidis (2013) reported the seasonally dimorphic of *T. polium* in Greece. It affects the type of trichome on the surface of leaf. Moreover, biodiversity characteristic was described in *T. polium* of Saudi Arabia, but environmental conditions did not affect the trichome structure (Bukhari et al. 2015). Furthermore, Pavlova and Vasileva (2010) investigated the effect of edaphic conditions on variability of vegetative characteristics in *T. polium* in Bulgaria. Jurisic Grubesic et al. (2012) and Venditti et al. (2017) also stated that variability of metabolites was found in *T. polium*.

According to the findings, similar or different accessions of *Teucrium* species were clustered together or separated including *T. polium, T. scordium* subsp. *scordioides,* and *T. orientale* subsp. *taylori*. The differentiation of *T. polium* accessions was related to the length of stem, leaf, petiole, bract, bracteole, calyx and indumentum of stem, corolla lip, pedicel and form of leaf apex. The similarities of its accessions were assigned to the length of inflorescence, corolla tube, filament and indumentum of leaf, inflorescence, bract, bracteole, calyx, corolla tube and style, and form of leaf, leaf base, leaf margin and bracteole. Evidence from the present research displays the variability of *T. polium* confirmed by published reports (Rechinger 1982; Jurisic Grubesic et al. 2007; Pavlova and Vasileva 2010; Jamzad 2012; Bosabalidis 2013). Both *T. scordium* subsp. *scordioides* accessions were also differed by the length of stem, leaf, petiole, bract, bracteole and indumentum of stem, bract, pedicel and leaf form. In *T. orientale* subsp. *taylori*, the differentiation of some accessions was assigned to the length of stem, leaf, petiole, inflorescence, bract, bracteole and calyx, and indumentum of stem, leaf, petiole, inflorescence, bract, filament and style. Their similarities were also assigned to the indumentum of calyx, corolla lip, pedicel and form of leaf and bract.

According to the results obtained by Valant-Vetschera et al. (2003), some chemo diversities were attributed to *Teucrium* species. In fact, some flavonoid compounds in this genus were attributed to sectional and intraspecific levels.

Ranjbar et al. (2017) referred to some morphological characteristics in section *Polium; T. polium* including glandular and unbranched trichome at stem, tomentose and branched trichome at calyx, lanceolate, oblong-lanceolate of leaf form, sinuate–undulate of leaf margin, and length of inflorescence axis ranged from 3 to 15 cm. Moreover, *T. gnaphalodes* includes eglandular and glandular trichome on stem surface, tomentose and glandular trichome at calyx, triangular-oblong of leaf form, and dentate, crenate and lobed of leaf margin. It should be noted that our reports in both species were completely different.

It was identified that flavonoid classes certainly resolved the taxonomic complexity of the species in sections *Teucris* and *Polium*. The identified flavonoid classes in the *Teucrium* species included 18 flavones, 17 isoflavones, 13 flavonols, 3 flavanones, 3 chalcones, 2 dihydroflavonols and one aurone.

In previous reports, the flavone derivatives were identified, including apigenin-glucoside in *T. zanonii* Pamp. (Abdelshafeek et al. 2006; Jaradat 2015), mono-hydroxy-methoxylated flavone in *T. barbeyanum* Aschers. (Alwahsh et al. 2015), luteolin glucoside and glucopyranoside in *T. polium* and *T. chamaedrys* subsp. *sinuatum* (Celak.) Rech.f. (Cakir et al. 2006; Galstyan 2014; Ozer et al. 2018; Gecibesler et al. 2019), and diosmetin in different *Teucrium* species (Mitreskia et al. 2014; Venditii et al. 2017). The obtained information was identical to that of the UV-spectral properties of flavone nos. 2 (λmax 257, 349 nm), 5 (λmax 344 nm), 9 (λmax 340 nm), 12 (λmax 333 nm), and 17 (λmax 242, 349 nm).

Flavonols derivatives were previously reported, including patuletin and fisetin in *T. chamaedrys* L. (Vlase et al. 2014), penduletin, quercitrin, quercetagetin-methyl ether, in *T. polium* (Ozer et al. 2018), quercetin in *T. sandrasicum* O. Schwarz (Kaska and Mammadov 2019), and quercetin and kaempferol glucoside derivatives in the *Teucrium* species (Mitreskia et al. 2014) supported the UV-spectral properties of flavonols no. 1 (λmax 362 nm), 2 (λmax 333 nm), 3 (λmax 371 nm), 7 (λmax 350 nm), 9 (λmax 254, 340 nm), 12 (λmax 244, 350 nm) and 13 (λmax 271, 340 nm).

According to the former literature, formononetin was isolated in *T. polium* as an isoflavonoid class (Mirdeilami et al. 2011). Regarding our findings, isoflavone no. 15 with λmax 301 nm illustrates consistency with its derivatives.

It is has been confirmed that flavonoid classes such as chalcone nos. 1 (λmax 344 nm) and 2 (λmax 318 nm), isoflavone nos. 4 (λmax 353 nm) and 6 (λmax 328 nm), flavone nos. 1 (λmax 323 nm), 3 (λmax 364 nm), 4 (λmax 350 nm), 8 (λmax 313 nm), 10 (λmax 333 nm), and 13 (λmax 307 nm), flavonol nos. 4 (λmax 370 nm), and 10 (λmax 377 nm), flavanone nos. 1 (λmax 312 nm), and 2 (λmax 326 nm), and dihydroflavonol no. 2 (λmax 308 nm) accorded with other genera of Lamiaceae such as *Salvia* L., *Scutellaria* L., *Otostegia* Benth., and *Phlomis* L. species (Mamadalieva et al. 2011; Sadeghi et al. 2014; Kharazian 2014; Uritu et al. 2018; Sajjadi et al. 2018; Jafari Dehkordi and Kharazian 2019; Hajialyani et al. 2019). It was identified that flavonoid classes such as flavone no. 6, 7, 10, 11, 14–16, 18, 19, isoflavone no. 1, 3, 5, 7–14, 16, flavonol no. 5, 6, 8, 11, flavanone no. 3, aurone no. 1, and dihydroflavonol no. 1 were first described for the *Teucrium* species.

4 Concluding Remarks

In the present work, flavonoid and morphological markers were ascertained as valuable indicators to detect the taxonomic relationships of the *Teucrium* species. Moreover, the flavonoid information provided some intraspecific relations in *T. polium* and *T. capitatum*, and *T. orientale* subspecies. Thus, the kind of flavonoid classes can be designated as a powerful marker to identify the boundaries of sub-specific levels. Nevertheless, the more detailed flavonoid compound identification of *Teucrium* species is in progress.

Due to the presence of morphological and chemical diversities in the genus *Teucrium* from Zagros region, it is required to protect this valuable germplasm and its endemic species for saving natural vegetation in this area.

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Appendix

See Table 3.

Table 3 List of collected *Teucrium* species and its populations from their natural habitats with voucher numbers, locality, altitude and geographical position

Taxa/voucher number	Locality	Altitude (m)	Geographical position
T. polium 1	Kohgilouyeh va Boyer Ahmad-Kohgilouyeh, Higoune, Haji Mouri village	1043	54°38′9.44″E, 36°30′5.88″N
T. polium 3	Chaharmahal va Bakhtiari-Ardal, Helisad toward Sarkhoun	1456	50°32′56.5″E, 31°45′57.08″N
T. polium 8	Chaharmahal va Bakhtiari-Naghan, tang-e Dare Garm	2166	50°46′21.1″E, 31°54′36.7″N
T. polium 9	Chaharmahal va Bakhtiari-Chahar Tagh, Sabz-e Kouh	2166	50°54′12.03″E, 31°45′19.7″N
T. polium 12	Chaharmahal va Bakhtiari-Farokhshar, Kouh-e Baraftab	2175	51°07′15.6″E, 32°18′35.01″N
T. polium 13	Chaharmahal va Bakhtiari-Shahrekord, Sourak	2154	51°02′2.9″E, 32°32′51.5″N
T. polium 19	Lurestan-Azna, Dar-e Takht	1879	49°22′58.3″E, 33°22′29.3″N
T. polium 31	Isfahan-Semirom, Semirom water fall	1909	51°36′20.51″E, 31°25′57.79″N
T. polium 32	Chaharmahal va Bakhtiari-4 km to Soureshjan	2295	50°40′34.06″E, 32°19′0.04″N
T. polium 34	Chaharmahal va Bakhtiari-Farsan, Cheshme Ghalae	2182	50°34′23.8″E, 32°15′2.41″N
T. polium 35	Chaharmahal va Bakhtiari-Farsan	2163	50°34′53.37″E, 32°15′13.49″N
T. polium 46-47	Kurdestan-between Sanandaj and Kamyaran, Sou village	1800	46°54′34.09″E, 35°09′26.09″N
T. polium 60	Fars-Ghaemieh, Shiraz road	1121	51°34′36.3″E, 29°50′56.92″N
T. polium 61	Fars-Nourabad toward Masiri	973	51°31′26.65″E, 30°10′14.36″N
T. capitatum 5	Kohgilouyeh va Boyer Ahmad-Boyer Ahmad, Ludab, Kouh-e Nour	1620	50°48′18.43″E, 30°53′53.69″N
T. capitatum 7	Chaharmahal va Bakhtiari-Ardal, Emamzadeh Abdollah	1960	50°39′43.97″E, 32°0′54.98″N
T. capitatum 10	Chaharmahal va Bakhtiari-Ardal, Gel Sefid	1887	50°60′23.6″E, 31°09′41.3″N
T. capitatum 24	Lurestan-Doroud toward Saravand, Forest Park Shohada	1543	49°12′9.19″E, 33°44′18.57″N
T. capitatum 25	Lurestan-Khorramabad toward Poldokhtar, Shourab	1245	48°14′36.05″E, 33°46′27.27″N
T. capitatum 27	Lurestan-Khorramabad, Bisheh water fall	1724	48°87′10.63″E, 33°45′30.96″N
T. orientale subsp. *glabrescens* 4	Chaharmahal va Bakhtiari-Farsan	2151	50°34′56.38″E, 32°15′19.33″N
T. orientale subsp. *glabrescens* 11	Chaharmahal va Bakhtiari-Ardal, Gel Sefid	1887	50°60′23.6″E, 31°09′41.3″N
T. orientale subsp. *glabrescens* 14	Chaharmahal va Bakhtiari-Shahrekord, Dastgerd	2162	50°98′9.23″E, 32°09′28.0″N
T. orientale subsp. *glabrescens* 15	Isfahan-Fereydounshahr	2598	50°11′2.6″E, 32°51′46.3″N
T. orientale subsp. *glabrescens* 17	Lurestan-Aligoudarz, after Azad University	2048	49°07′43.9″E, 33°37′7.91″N
T. orientale subsp. *glabrescens* 21	Lurestan-road of Azna toward Doroud, economic region of Azna	1879	49°50′53.46″E, 33°44′12.78″N
T. orientale subsp. *glabrescens* 22	Lurestan-Oshtorankouh	1930	49°15′31.10″E, 33°14′17.75″N
T. orientale subsp. *glabrescens* 28	Chaharmahal va Bakhtiari-Soureshjan, Vanan	2196	50°63′42.03″E, 32°36′56.15″N
T. orientale subsp. *glabrescens* 39	Isfahan-Chadegan, Zayanderoud	2279	50°69′56.52″E, 32°72′27.89″N
T. orientale subsp. *glabrescens* 36	Isfahan-Bouein	2444	50°20′54.71″E, 33°03′38.80″N
T. orientale subsp. *glabrescens* 44	Kurdestan-Sarvabad, Houraman	2000	46°25′32.82″E, 35°26′12.42″N
T. orientale subsp. *gloeotrichum* 20	Lurestan-road of Azna toward Doroud, economic region of Azna	1879	49°50′53.46″E, 33°44′12.78″N
T. orientale subsp. *gloeotrichum* 26	Lurestan-Khorramabad toward Poldokhtar, Shourab	1245	48°14′36.05″E, 33°46′27.27″N
T. orientale subsp. *gloeotrichum* 38	Isfahan-Chadegan, Kalb Ali village	2396	50°69′34.95″E, 32°83′48.97″N
T. orientale subsp. *orientale* 29	Isfahan-Naghaneh toward Semirom	2338	51°42′53.42″E, 31°91′57.41″N
T. orientale subsp. *orientale* 30	Isfahan-Semirom toward Shahreza	2604	51°61′53.20″E, 31°54′18.81″N
T. orientale subsp. *orientale* 37	Isfahan-road of Daran toward Taraz	2406	50°45′27.75″E, 32°94′7.81″N

Table 3 (continued)

Taxa/voucher number	Locality	Altitude (m)	Geographical position
T. orientale subsp. *taylori* 2	Kohgilouyeh va Boyer Ahmad-Kohgilouyeh, Tang-e Higoun	1048	54°38′9.44″E, 36°30′5.88″N
T. orientale subsp. *taylori* 6	Kohgilouyeh va Boyer Ahmad-Boyer Ahmad, Garab toward Heydarabad	1490	50°04′57.50″E, 30°57′24.78″N
T. orientale subsp. *taylori* 16	Lurestan-Aligoudarz, after Azad University	2048	49°07′43.9″E, 33°37′7.91″N
T. orientale subsp. *taylori* 18	Lurestan-Azna, Dar-e Takht	1879	49°38′58.32″E, 33°43′29.31″N
T. orientale subsp. *taylori* 23	Lurestan-Oshtorankouh, Tiyan village	1835	49°28′52.05″E, 33°43′28.12″N
T. orientale subsp. *taylori* 53	Boushehr-road of Khormouj to Ahrom	47	51°38′11.70″E, 28°59′17.07″N
T. orientale subsp. *taylori* 58	Fars-Komarj village	857	51°47′51.26″E, 29°61′55.37″N
T. orientale subsp. *taylori* 63	Kohgilouyeh va Boyer Ahmad-Gachsaran, 14 km of Emamazade Jafar	817	51°09′10.46″E, 30°29′18.31″N
T. orientale subsp. *taylori* 65	Kohgilouyeh va Boyer Ahmad-Kohgilouyeh, Dehdasht, Pirbadami village	941	48°14′21.57″E, 35°56′12.37″N
T. scordium subsp. *scordioides* 40	Isfahan-Semirom, water fall	1909	51°36′20.51″E, 31°25′57.79″N
T. scordium subsp. *scordioides* 45	Kurdestan-Marivan, Zarivar	1250	46°13′37.89″E, 35°55′41.27″N
T. parviflorum 41-43	Kurdestan-Sarvabad, Houraman, Jivar village	1600-1900	46°20′30.48″E, 35°21′26.05″N
T. melissoides 48-49	Kermanshah-Dar-e Gamaleh, Nosoud	1700-1900	46°12′15.42″E, 35°10′40.24″N
T. gnaphalodes 51	Boushehr-Kangan, Taheri water fall	30	52°03′45.08″E, 27°86′17.39″N
T. gnaphalodes 52	Boushehr-Kangan, Siraaf	14	52°36′25.10″E, 27°65′46.27″N
T. gnaphalodes 54	Boushehr-road of Ahrom toward Borazjan, Chahkoutah	45	51°14′8.67″E, 29°08′42.73″N
T. gnaphalodes 59	Fars-Komarj village	855	51°47′51.26″E, 29°61′55.37″N
T. gnaphalodes 62	Kohgilouyeh va Boyer Ahmad-Kamarbandi of Basht	782	51°17′17.99″E, 30°35′10.89″N
T. gnaphalodes 64	Kohgilouyeh va Boyer Ahmad-Gachsaran, Emamzadeh Ghotbeddin	456	50°47′50.12″E, 30°49′18.36″N
T. gnaphalodes 57	Boushehr-Borazjan toward Dalaki	84	51°21′27.43″E, 29°29′34.11″N
T. oliverianum 50	Boushehr-12 km from Dalaki toward Borazjan	108	51°26′15.10″E, 29°34′30.41″N
T. oliverianum 55	Boushehr-Borazjan, Abolfirouz village	60	51°20′40.13″E, 29°20′46.63″N
T. oliverianum 56	Boushehr-2 km from Borazjan toward Dalaki	91	51°21′20.24″E, 29°30′17.86″N

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