Acta Bot. Croat. 77 (2), 119–125, 2018
DOI: 10.2478/botcro-2018-0006

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Application and limitation of molecular data and essential oil content in identification of *Leutea elbursensis* Mozaff in northern Iran

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Abstract – In this paper, the internal transcribed spacer (ITS) sequences, genetic structure and the chemical composition of essential oils of four populations belonging to *Leutea elbursensis* and *Leutea petiolaris*, two species endemic to northern Iran, are analyzed. Phylogenetic analysis based on the ITS data showed that all accessions of *L. elbursensis* formed a monophyletic clade, and *L. elbursensis* was a sister to the rest of *Leutea* species. Results of amplified fragment length polymorphism (AFLP) analysis performed on the total genome showed that all individuals presented in the study belonged to two different genetic clusters. The individuals belong to *L. petiolaris* had a different genetic structure and yielded no traceable amount of essential oils. The essential oil obtained from the ripe fruits of *L. elbursensis* yielded 0.5–0.6% of volatile essential oils. In total, 15–29 volatile natural components were identified on the basis of their mass spectra characteristics and retention indices, in which α-pinene (33.18–43.22%), β-pinene (32.4–40.9%) were the major constituents. Our results indicate that *L. elbursensis* is a distinct species, segregated from the other species based on morphology, ITS data and AFLP profile. In addition, despite the relatively uniform genetic structure of *L. elbursensis*, the chemical composition of essential oil could be highly affected by different factors.

Keywords: chemotaxonomy, Iran, phylogeny, α-pinene, β-pinene

Introduction

Plants are an important component of traditional food, but are also central to healthy diets of the modern urban population (Benjak et al. 2005, Ercisli 2009, Ercisli et al. 2010, Rop et al. 2014, Canan et al. 2016, Zorenc et al. 2016). Essential oils obtained from plants have applications in food, chemistry, pharmacy, medicine and perfumery (Hay and Waterman 1993, Mehregan and Ghannadi 2013). Members of the family Apiaceae (Umbelliferae) are well known for the production of essential oils with high pharmaceutical and economic value (Olivier and van Wyk 2013). Based on the most recent researches, the plant family Apiaceae with about 430 genera and 3800 species has a worldwide distribution, especially in the northern hemisphere (Stevens 2012). Apiaceae has about 110 genera and 400 species distributed in Iran (Mozaffarian 2007). The genus *Leutea* Pimenov belongs to the "*Ferula* group" including *Ferula* L., *Dorema* L. and *Leutea* (Kurzyna-Młynik et al. 2008). The genus *Ferula* includes more than 170 species distributed in central and southwestern Asia, and the Mediterranean region including northern Africa (Pimenov and Leonov 1993, Kurzyna-Młynik et al. 2008). It has about 30 aromatic species in Iran (Mozaffarian 2007). The genus *Dorema* has seven species in Iran (Mozaffarian 2007). The taxonomic status of *Leutea* is more complex. The genus *Leutea* with its few species is limited to SW Asia (Pimenov 1987). The taxonomy of the group has changed considerably, especially since publication of Flora Iranica, volume 162 (Pimenov 1987). The type of the genus, i.e. *Leutea petiolaris* was first effectively published as part of the genus Ferula as *F. petiolaris* DC. (de Candolle 1830). Boissier (1872) integrated *L. petiolaris* with other species of the genus *Peucedanum* L. sect. *Juncea*. He described two other new species, which are today known to be part of *Leutea*. Pimenov (1987) transferred all six known species of the group to the new genus named *Leutea* Pimenov. Spalik and Downie integrated the whole *Leutea* species into the genus *Ferula* (see Kurzyana-Mlynik et al. 2008). Recent works of Panahi et al. (2015) suggested that species of *Leutea* should be re-established from *Ferula* again.

Leutea elbursensis Mozaff. (syn.: *Ferula elbursensis* (Mozaff.) Spalik et. S. R. Downie), an endemic to northern Iran,

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is a glabrous perennial tough herb, 1.5 to 3 m high, with compound pinnate leaves with tubular lobes. It has compound umbels consisting of yellow 5-merous flowers. The fruits are schizocarp, compressed, elliptic and up to 10 × 4 mm in size (Mozaffarian 2007, Kanani et al. 2013). *L. elbursensis* can be distinguished from the other species by its narrow pedicels, ovate-elliptic fruits, greenish-yellow petals, and large and strong habits (Mozaffarian 2007). Despite the recent publication date of its name, many populations of *L. elbursensis* have been known for a long time. Material of *L. elbursensis* was first considered part of *L. cupularis* (Pimenov 1987), and was later segregated from it by Mozaffarian in 2003 (Kurzyna-Młynik et al. 2008). Mozafarian (2007) identifield two different species in the north of Iran (Tehran province) i.e. *L. petiolaris* and *L. elbursensis*. These taxonomic complexities have resulted in ambiguity attaching to those works already performed on three species *L. elbursensis, L. cupularis* and *L. petiolaris* (Masoudi et al. 2004, Kurzyna-Młynik et al. 2008, Alipour et al. 2015).

Members of the family Apiaceae in Iran are widely studied for their essential oil contents (Olivier et al. 2013, Safaeian et al. 2015). Most of those studies are single records based on the material collected from a single locality. Chemical composition of the essential oils depends on many factors such as collection time, geographical locality and genetic structure (Munoz-Bertomeu et al. 2007). Therefore, a single report of essential oil composition for a given species could not be generalized for all of its populations. Looking for their chemotaxonomic value, Kanani et al. (2011) studied chemical composition of the essential oils for 18 *Ferula* species from Iran.

Since its introduction in 1995, amplified fragment length polymorphism (AFLP) has become a popular research tool for detecting genetic structure of populations as well as differences at intra-species level (Vos et al. 1995).

In this paper we try to find out if different populations growing wild in the north of Iran (Tehran province) belong to the same species. Material from the western part of the province was previously identified as *L. elbursensis* by Mozaffarian (2007). We used analysis of ITS (internal transcribed spacer) of the nuclear genome to find the phylogenetic placement of those plants. In addition, AFLP fingerprinting techniques are used for distinguishing the genetic structures of different populations. We also aim to study the possible correlation between the genetic structure and the essential oil profile of *L. elbursensis* populations collected from different localities in northern Iran (Tehran province), using GC/MS and AFLP fingerprinting techniques. Finally, we will try to clarify the taxonomic position of *L. elbursensis*.

Materials and methods

Between August and September 2013, plants from three populations of *L. elbursensis* and one population of *L. petiolaris* were collected from northern Iran, the province of Tehran (Tab. 1). Plant materials were identified by authors after Mozaffarian (2007) and vouchers were deposited in the herbarium of Islamic Azad University, Science and Research branch, Tehran, Iran (IAUH).

Fresh leaf fragments from at least six individuals from each population (24 individuals in total) were taken and gradually dried in silica-gel pearls. Total DNA was extracted for each individual sampled using NucleoSpin® Plant II kit after the manufacturer's manual (Machery-Nagel, Dueren, Germany). The complete internal transcribed spacer (ITS) region of the DNA was amplified using the primer pair AB101 (5′- ACG AAT TCA TGG TCC GGT GAA GTG TTC G – 3′) and AB102 (5′-TAG AAT TCC CCG GTT CGC TCG CCG TTA C – 3′) (Douzery et al. 1999), in a PCR reaction under the following conditions: a pretreatment of 5 minutes at 95 °C, 35 cycles of 30 seconds at 95 °C, 30 seconds at 50 °C, and 1 minute 30 seconds at 72 °C, and a final extension of 7 minutes at 72 °C. The complete ITS region was sequenced on an ABI 3730 sequencer machine (Applied Biosystems, Waltham, Massachusetts, USA). Sequences were visually checked and edited with Sequencher 4 (Gene Codes Corporation, Ann Arbor, MI USA), and then aligned using MacClade 4.08 (Maddison and Maddison 2000), alongside additional sequences taken from the GeneBank. *Ferula violacea* Korovin and *F. olivacea* (Diels) H. Wolff. were chosen as outgroup taxa after Panahi et al. (2015). Maximum parsimony (MP) analysis of the ITS dataset was performed with PAUP* (Swofford 2002). Bayesian analysis (BA) of the ITS dataset was performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001).

The AFLP™ procedure for this work followed Vos et al. (1995), and Scalone et Albach (2012) with the following modifications: three primer combinations used for selective PCR were E38-^{HEX} labelled (5′ – GAC TGC GTA CCA ATT CAC T – 3′) combined with M57 (5′- GAT GAG TCC TGA GTA ACG G – 3′), E45-^{FAM} labelled (5′-GAC TGC GTA CCA ATT CAT G – 3′) combined with M54 (5′ – GAT GAG TCC TGA GTA ACC T – 3′), and E40-^{NED} labelled (5′- GAC TGC GTA CCA ATT CAG C – 3′) with M55 (5′-GAT GAG TCC TGA GTA ACG A – 3′). PCR products of each sample were combined equally, and 2 µl of this multiplex product was run with 7.75 µl HiDi formamide (Applied Biosystems) and 0.25 µl internal size standard GeneScan ROX (Applied Biosystems) on an ABI 3730 automated capillary sequencer. Fragments were analyzed and scored using GeneMarker 2.4.1 (SoftGenetics). Structure 2.3.4 (Pritchard et al. 2000) was used for analyzing the genetic structure of populations. An analysis of molecular variance (AMOVA) test was performed using GenAlEx 6.5 (Peakall and Smouse 2012).

100 g of the ripened and dried fruits of the plant for each population were chopped in distilled water and a hydro-distilled fraction of it was isolated by hydrodistillation for 3 hours. We used a Hewlett Packard 5972A mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (50 m × 0.25 mm, film thickness 0.32 µm) for gas chromatography-mass spectrometry (GC-MS) analysis. The following GC operating conditions were used: carrier gas, helium with a flow rate of 1 mL min^{-1}; column

Tab. 1. Populations of *Leutea elbursensis* from northern Iran included in the molecular and phytochemical analysis with voucher information and GenBank accession numbers.

No.	Species	Locality	Herbarium number	Genbank number
1	*Leutea elbursensis* Mozaff.	Iran: Tehran, Emamzadeh-Davoud, 35.898, 51.288, 2400 m	Emami 14391 (IAUH)	KP793684
2	*Leutea elbursensis* Mozaff.	Iran: Tehran, Morad-Abad, 35.796, 51.322, 1800 m	Emami 14393 (IAUH)	KP793683
3	*Leutea elbursensis* Mozaff.	Iran: Tehran, Hesarak 35.796, 51.305, 1700 m	Emami 14394 (IAUH)	KP793686
4	*Leutea petiolaris* (DC.) Pimenov	Iran: Tehran, Dizin, 36.043, 51.441, 3400 m	Emami 14392 (IAUH)	KP793685
5	*Leutea elbursensis* Mozaff.	Iran: Tehran, Karaj, 35.933, 51.067, 1600 m	Valiejo-Roman et al., 526 (MW)	AY941276 AY941304
6	*Leutea elbursensis* Mozaff.	Iran: NW of Tehran, Souleghan	Mozaffarian & Jamzad 33570 (TARI)	KJ660832
7	*Leutea glaucopruinosa* (Rech. fil.) Akhani & Salimian	Iran: Mazandaran	Termeh & Zargari 040483-E (W 0023608)	KJ660833
8	*Leutea petiolaris* (DC.) Pimenov	Iran: Azarbayejan	Mozaffarian & Massoumi 78152 (TARI)	KJ660836
9	*Leutea petiolaris* (DC.) Pimenov	Iran: Tehran, 35.767, 51.950, 2400 m	Valiejo-Roman et al. 63 (MW)	AY941278 AY941306
10	*Leutea rechingeri* (Leute) Pimenov	Iraq: Suleymanieh, Mt. Algurd	Rechinger 11416 (W 05825)	KJ660838
11	*Leutea cupularis* (Boiss.) Pimenov	Iran: SW, Dena Mts., 2900-3100 m	Valiejo-Roman et al. 235 (MW)	AY941277 AY941305
12	*Leutea cupularis* (Boiss.) Pimenov	Iran: SW, Dena Mts., 3500-3900 m	Assadi & Mozaffarian. 31236 (TARI)	KJ660831
13	*Leutea polyscias* Pimenov	Iran: Manjil, 1650 m	Mozaffarian 64227 (TARI)	KJ660837
14	*Leutea gracillima* Pimenov	Iran: NE, Golestan Park.	Akhani 12060 (W 1999-03655)	KJ660834
15	*Leutea nematoloba* (Rech.f.) Pimenov	Iran: N, Chalus Valley.	Rechinger & Rechinger 6668 (W 02835)	KJ660835
16	*Ferula violaceae* Korovin	SW Asia	Valiejo-Roman et al., 119899 (MW)	AF077891
17	*Ferula olivacea* (Diels) H. Wolff.	China	Chamberlain, Ming, Yuan & al. 229 (E)	EF560691

temperature, 60 °C with 7 °C temperature increase per minute, up to 230 °C; injector and detector temperatures, 280 °C; volume injected, 0.1 μL of the oil; split ratio, 1:25. In addition, the following MS operating parameters were used: ionization potential, 70 ev; resolution, 1000; ion source temperature, 200 °C. Components in the oil were identified based on GC retention indices relative to n-alkanes and computer matching with the Wiley 275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams 1995, Masoudi et al. 2004, Alipour et al. 2015).

SPSS v. 20 (IBM corporation) was used to perform cluster analyses based on the chemical components of essential oils with Ward's method (Ward 1963).

Results

Figure 1 shows the phylogeny of different species of *Leutea* based on the Bayesian analysis (BA) of the ITS region. Two species of *Ferula* i.e. *F. violacea* and *F. olivacea*, were included in the tree as outgroups. Posterior probabilities (PP) are indicated by numbers above each clade. Bootstrap supports (BS) for those clades also retrieved in the maximum parsimony (MP) analysis are indicated by the numbers below each clade (Fig. 1). As seen on the phylogenetic tree, species of the genus *Leutea* are mainly grouped into two clades: clade A) a firmly supported clade including all samples of *L. elbursensis* with posterior probability (PP) = 1.00 and bootstrap support (BS) = 100%, and clade B) another well supported clade including a polythomy of eight species with PP = 1.00 and BS = 85%. *Leutea elbursensis* is sister to rest of species (Fig. 1). All our three accessions of *L. elbursensis* together with another two samples taken from the Genbank (both from NW of Tehran) formed a monophyletic clade. All four samples are taken from a relatively small locality in N Iran (central Elburz) with distances of less than 100 km among them. The monophyletic clade B presents a polythomy of different species. These include three samples of *L. petiolaris*, two samples of *L. cupularis*, and one sample of each species *L. galucopruinosa*, *L. rechingeri*, *L. polyscias*, *L. avicennae*, *L. gracillima* and *L. nematoloba* (Fig. 1). Two samples of *L. cupularis* collected from the Dena mountain ranges (SW Iran) formed a monophyletic clade with strong support (PP = 1, BS = 85%). Different samples of *L. petiolaris* did not form a monophyletic clade. The results showed that our samples from the area clearly belong to two different species. Phylogenetic relationships within this clade are not essentially resolved. This clade consists of samples collected from a larger region involving distances of hundreds of kilometres.

In the selective PCR of AFLP analysis, the E38-M57 primer combination yielded 62 bands, the E45-M54 primer combination 36 bands, and the E40-M55 primer combina-

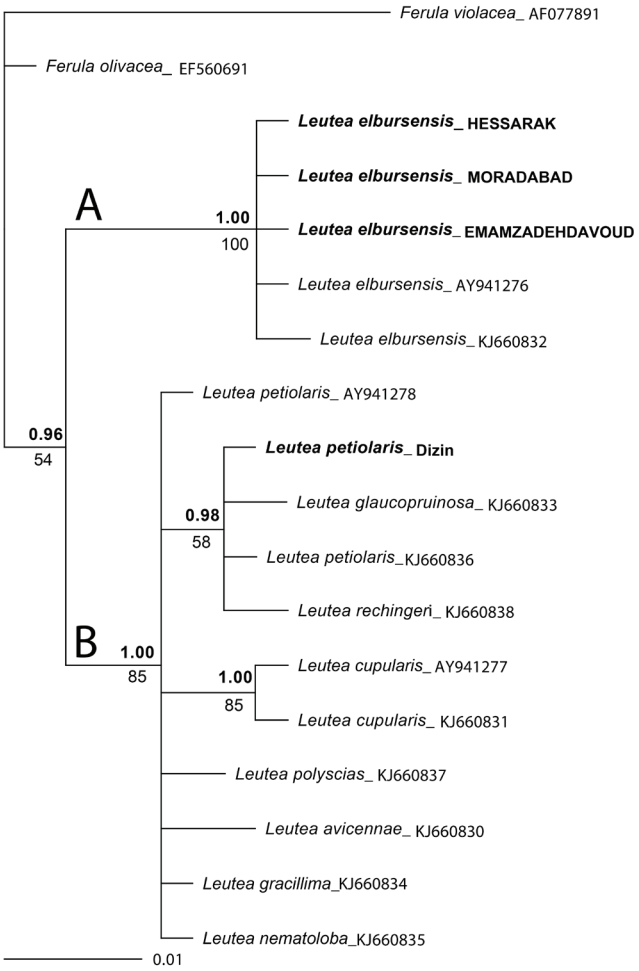

Fig. 1. Phylogenetic tree obtained from the Bayesian analysis of the internal transcribed spacer region of different species of *Leutea* alongside two *Ferula* species as outgroup (total characters = 442; constants = 299; parsimony informatives = 60. Model of evolution = SYM +G; A-C constitution rate = 1.7930; A-G constitution rate = 2.2561; A-T constitution rate = 2.0384; C-G constitution rate = 0.4189; C-T constitution rate = 5.9481; G-T constitution rate = 1.0000. Gamma distribution rate = 0.6465). Numbers above clades are posterior probabilities. Numbers below clades are the bootstrap supports (100 replicates) for those clades retrieved in the maximum parsimony analyses (bootstrap supports less than 50% are not shown).

tion 36 bands. The AMOVA test results showed that of 100% total variation, 34% was among regions, 6% was among populations and 60% was within populations (Tab. 2). Analysis of the populations using Structure 2.3.4 software showed that all individuals belonged to two different clusters showed here with different colours (Fig. 2). As seen in Figure 2, individuals of *L. petiolaris* from "Dizin" population are homogenous in their genetic structure (cluster "a"). All six individuals collected from "Dizin" (*L. petiolaris*) had genetic structures 90–100 % consisting of cluster "a" alleles. Genetic profiles of three populations of *L. elbursensis* mainly consist of a different cluster (cluster b). Genetic structures of all six individuals from the "Emamzadeh-Davoud" population consisted 100 % of alleles from cluster b. Except for one individual from the "Hessarak" population and another one individual from the "Morad-Abad" population, all other individuals from those two populations were 97-100% made by alleles from cluster "b". AFLP profiles of two species are clearly different. This clearly shows that two different species are distributed in the area N and NW of Tehran.

This is the first report of chemical composition of essential oils extracted from the ripened fruits of *L. elbursensis*. The essential oils obtained from dried fruits were clear, pale yellow liquids. The essential oil content of *L. elbursensis* was between 0.5% (w/w; in the Emamzadeh-Davoud population) – 0.6% (w/w; in Hessarak and Moradabad populations). Between 15 to 29 natural compounds were identified, accounting for 98.7 – 100% of the oils (Tab. 3). Ripened fruits of *L. petiolaris* collected for two successive years did not yield

Tab. 2. AMOVA test results showing the variations of *Leutea elbursensis* from northern Iran: within populations, among populations (pops) and among regions. Df – degrees of freedom; SS- sum of the squares; MS – mean squares; Est. Var. – estimation of variance.

Source	Df	SS	MS	Est. Var.	%
Among regions	1	124.972	124.972	10.602	34
Among pops	2	59.111	29.556	1.798	6
Within pops	20	375.333	18.767	18.767	60
Total	23	559.417	173.295	31.167	100

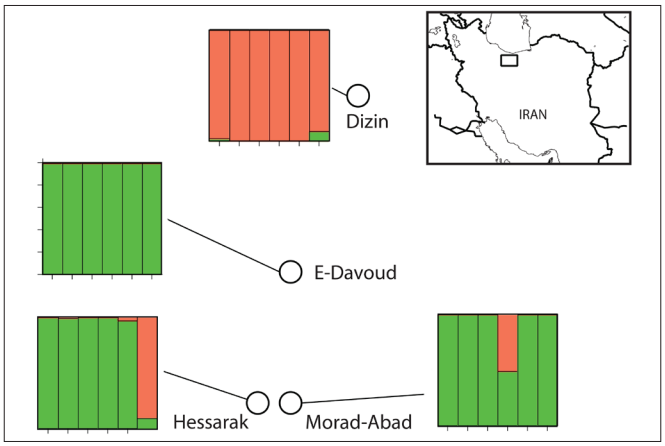

Fig. 2. Amplified fragment length polymorphism finger printing results showing the genetic profile of 24 individuals collected from northern Iran (*Leutea elbursensis* from Hessarak, Morad-Abad and Emamzadeh (E-) Davoud; *L. petiolaris* from Dizin).

Tab. 3. Composition of the essential oils of different populations of *Leutea elbursensis* from northern Iran. KI – Kovats index.

Number	Component name	KI	Percentage of components in each population		
			Morad-Abad	Hessarak	Emamzadeh-Davoud
1	α-Thujene	930	1.38	0.54	1.2
2	α-Pinene	939	39.77	33.18	43.22
3	Camphene	954	1.32	0.57	1.24
4	Thuja-2,4 (10)-diene	960	0.14	0	0
5	Sabinene	975	2.05	0.71	1.9
6	β-Pinene	979	36.1	32.4	40.9
7	Myrcene	990	0	1	0
8	P-Cymene	1024	0.35	0	0
9	Limonene	1029	3.14	1.9	3.5
10	β-Phelandrene	1029	0.91	0.64	0
11	Cineol	1031	0.78	0	0.8
12	z-β-Ocimene	1037	0.18	0	0
13	γ-Terpinene	1059	0.19	0	0
14	α-Campholenal	1126	0.41	0.53	0
15	Trans-Pinocarveol	1139	3.5	0	1.3
16	Trans- Verbenol	1144	0.16	5.62	0
17	Pinocarvone	1164	0.9	1.52	0.8
18	α-Terpineol	1188	0.33	0.78	0.46
19	Myrtenol	1195	1.34	5.6	1.2
20	Verbenone	1205	0.19	0	0
21	endo-Fenchyl acetate	1220	1.32	3.02	0.8
22	Isobornyl acetate	1285	1.36	4.95	1.73
23	cis-Pinocarvyl acetate	1312	0.25	0.75	0
24	Myrtenyl acetate	1326	0.57	2.12	0.45
25	Neryl acetate	1361	0.26	2.01	0.5
26	Daucene	1381	0.52	0.81	0
27	α-Trans Bergamotene	1434	0.1	0	0
28	z-β-Farnesene	1442	0.15	0	0
29	Pentadecane	1500	0.2	0	0
30	Trans-β-Guaiene	1502	0.2	0	0
31	α-Copaen-11-01	1539	0	0.8	0
Total			98.07	99.45	100

any traceable essential oil. The highest number of components was identified in the Moradabad population (29 components). We also identified 20 components for the "Hessarak" population. The smallest number of components was 15 and was identified in the "Emamzade-Davoud" population. The main components identified in our three populations were α –pinene (33.18 – 43.22%) and β-pinene (32.4 – 40.9%). There were few other important but less abundant components (ca. 5% or more) i.e. myrtenol (5.6%), transverbenol (5.6%) and isobornyl acetat (4.95%) which were identified only in the Hesarak population.

Discussion

Different populations of *L. elbursensis* had identical ITS sequences and relatively similar genetic structures. Its segregation from *L. petiolaris* was supported by ITS and AFLP data. This segregation is also supported by morphological and geographical evidence. *Leutea elbursensis* has unique tall flowering stems 1.5–3 m high, while other species are clearly shorter (up to 1.5 m high). *Leutea* species have an allopatric distribution. *Leutea elbursensis* is endemic to N and NW Tehran, especially the rocky slopes of Souleghan valley (Mozaffarian 2007). Despite the similarities in morphology, ITS sequence and genetic structure, the essential oil contents of our three populations were highly variable.

The results showed that *Leutea* plants distributed in the region N and NW of Tehran do not belong to a single species. Pimenov (1987) mistakenly identified all herbarium material collected from the area under *L. cupularis*. We showed here that they belong to two different species. Our results are in agreement with Mozaffarian (2007), who identified the material from "central Elburz" under two different species i.e. *L. elbursensis* and *L. petiolaris*. These results indicate that *F. petiolaris* and *F. elbursensis* are two different entities. Regardless of the disputable taxonomic position of the genus *Leutea*, our results clearly showed that *L. elbursensis* is a distinct species sister to the rest of *Leutea* species (Fig. 1).

The genus *Ferula* s. l. (including *Leutea*) in Iran is widely studied chemically. We compared our results to one of the most comprehensive studies, that performed by Kanani et al. (2011) on 18 different populations belonging to different species of *Ferula* (Fig. 3). Our analysis showed that the essential oil profile of our three samples was most similar to that of a sample from *F. stenocarpa* Boiss. & Hausskn. All these four samples formed a distinct group "rich" and "balanced" of α – Pinene (33.18 – 48.8%) and β-Pinene (30.1 – 40.9%), and this group is also related to another group consisted of *F. gummosa* Boiss. and *F. galbaniflua* Boiss. & Buhse with a higher amount of β-Pinene (26.8 – 69.2%) and a lower amount of α– Pinene (1.4–33.9%) (Ghannadi and Amree 2002, Ghasemi et al. 2005, Jahansouz et al. 2008, Talebi Kouyakhi et al. 2008, Kanani et al. 2011). The chemical composition of essential oils of *Leutea glaucopruinosa* Rech.f. Yassa et al. (2003) showed no close similarity to our samples ("LG" in Figure 3). The chemical composition of *Dorema glabrum* Fisch. & C. A. Mey. Delnavazi et al. (2015) is similar to that of some *Ferula* species. Comparing the dendrogram shown in Figure 3 with the *Leutea* phylogeny (Fig. 1) and the phylogeny obtained by Kurzyna-Mlynik et al. (2008), it becomes clear that similarities in chemical composition of the essential oils of the "*Ferula* group" does not reflect the phylogenies based on the molecular data.

Regarding their uniform ITS sequences and similar AFLP profiles, the essential oil profiles of different populations of *L. elbursensis* were very variable, most probably as the result of different ecological conditions. The chemical composition of essential oils in the family Apiaceae can be very variable, especially according to the plant parts the oils are extracted from (Kanani et al. 2011). Alipour et al. (2015) reported different chemical compositions for the essential oils extracted from different parts of *L. cupularis*. They found following major components in different parts of the plant: ð-2-Carene (15.81%) and DL-Limonene (25.04%) in flowers; β-Pinene (13.87%), β-Ocimene (9.05%), Bornyl angelate (6.55%) and allo-Ocimene (6.08%) in leaves; and ð-3-Carene (8.38%), α-Terpinyl isobutyrate (8.69%) and Bornyl angelate (7.45%) in stems. In addition, the chemical composition of essential oil can be highly affected by ecological conditions and genetic structure (Munoz-Bertomeu et al. 2007). Therefore, we here suggest that a standard method should be used for analyzing and interpreting the data obtained from essential oils. As mentioned above, different parts of the plants might yield essential oils with different contents. Ripened fruits of the members of the family Apiaceae could be an appropriate source for obtaining essential oils. Regarding those issues, we here suggest that the chemical composition of essential oils cannot be used as a trusted taxonomic tool, at least in case of the "*Ferula*-group".

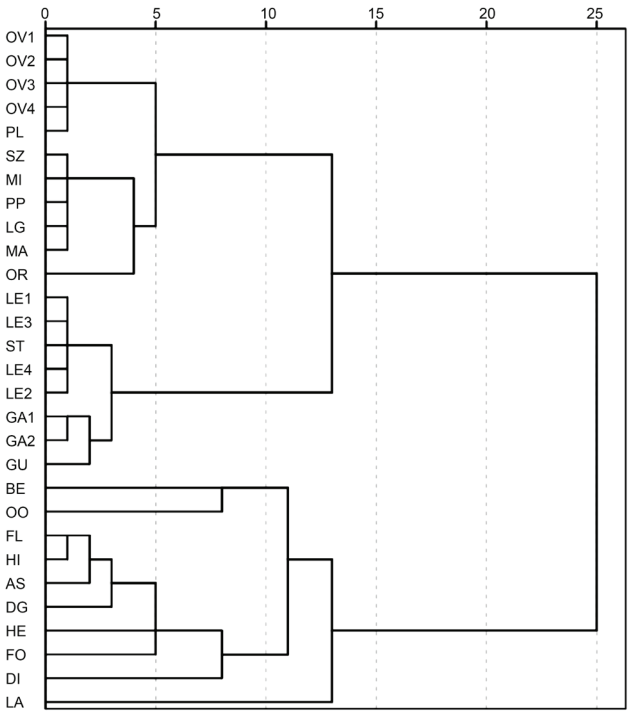

Fig. 3. Dendrogram showing classification of different populations of *Ferula* spp. based on the similarities in essential oil composition. Amount of components for *Leutea* populations are shown in Table 3 and for other populations are presented in Kanani et al. (2011). Numbers on the x-axis represent values of dissimilarity. LE1 – *Leutea elbursensis,* Moradabad; LE2 – *L. elbursensis*, Hessarak; LE3 – *L. elbursensis*, Emamzadeh-Davoud); LE4 – *L. elbursensis*, Karaj; OV1-4 – *Ferula ovina*; SZ – *F. szowitsiana*; MI – *F. microcolea*; PP – *F. persica* var. *persica*; PL – *F. persica* var. *latisecta*; ST – *F. stenocarpa*; GA1-2 – *F. galbaniflua*; GU – *F. gummosa*; BE – *F. behboudiana*; OO – *F. oopoda*; HI – *F. hirtella*; MA – *F. macrocolea*; FL – *F. flabelliloba*; AS – *F. assa-foetida*; OR – *F. orientalis*; HE – *F. hezarlalehzarica*; FO – *F. foetida*; DI – *F. diversivittata*; LA – *F. latisecta*); LG – *L. glaucopruinosa*; DG – *Dorema glabrum*.

Acknowledgments

The authors would like to thank Mrs R. Amini for her help in collecting part of the material.

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