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Research Article

Chemical composition of the essential oils isolated from *Phlomis olivieri* Benth (Lamiaceae) in four western provinces in Iran

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Abstract: *Phlomis olivieri* Benth is a valuable medicinal plant in the flora of Iran and can be collected in different parts of the country. To date, no comprehensive phytochemical research has been done on it in different parts of Iran. In this research, the essential oils of this medicinal plant were investigated in eight locations of western provinces of Iran. For this, aerial parts of the plant were collected in its natural habitats, dried under the shade condition (approximately 25°C), and then powdered. The essential oil was isolated by Clevenger apparatus and chemically analyzed by a Gas Chromatography (6890N)-Mass Spectroscopy (5973N) device in Payame Noor University (PNU), Hamedan, Iran. Except for some cases (EC, TNV, and K) there were no significant differences in the characteristics of the soil of the investigated areas. In the chemical structure of this plant, 17 and 11 constituents were identified in A1 and A2, 17 and 18 in B1 and B2, 17 and 15 in C1 and C2, and 21 and 15 in D1 and D2 locations of four western provinces in Iran. The results showed that caryophyllene (A1, A2, B1 and B2), 1H-cyclopenta [1, 3] cyclopropa [1, 2] benzene (C1), naphthalene, decahydro-4a–methyl (C2), estra-1, 3, 5(10)-trian-17a-ol (D1), and n-hexadecanoic acid (D2) were dominant constituents. Therefore, this valuable medicinal plant has diverse chemical constituents in the studied locations in Iran which should be considered from different aspects.

1. INTRODUCTION

Various medicinal plants are used by people in developed and developing countries all over the world. For this reason, they have great therapeutic value. People around the world use 50,000 to 80,000 flowering plants for medicinal purposes. In other words, medicinal and aromatic plants are consumed by 70%-80% of the world's population (Uritu *et al.*, 2018). *Phlomis* L. is a large genus in the Lamiaceae, with over 100 species distributed throughout Euro- Asia and North Africa continents. This genus represented by 17 species, witch 10 species are endemic in Iran (including *P. olivieri*). *P. olivieri* grows wildly in north, west and center of Iran . In differend countries, they have various uses for human health. This plant is generally used as an herbal tea to treat gastrointestinal troubles and promote good health by protecting the liver, kidney, bone and cardiovascular systems (Amor *et al.*, 2009). Many of them are fragrant in all

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parts and contain widely used medicinal herbs including basil, mint, rosemary, sage, savory, marjoram, oregano, hyssop, thyme, lavender and perilla. Some species of this family are shrubs and trees and rare of them are vines (Raja, 2012).

Phlomis olivieri (Figure 1) is an herbaceous and perennial plant with height between 25-65 cm. Its stem is multiple and branched with a white or yellow coating. The leaves are simple, opposite, and their base has a more or less broad petiole up to 10 cm long on a shorter stem. The basal and middle leaves are ovate or oblong with a cordate base or a cordate cut with a flat tip. The density of hair is higher on the lower surface of the leaves, the color of which is variable, and the upper leaves are pointed and slightly curved at the edge. The terminal leaves are small, short or wedge-shaped at the base and have reticulated veins. Its inflorescence is a spike and includes flower cycles with multiples of 2 to 10. The lower leaves of the plant are longer than the flowers. The length of the leaves is 5 to 9 mm. The calyx is tubular, swollen with prominent veins with 14-20 mm long. In calyx the length of the teeth is 4-6 mm. The calyx has two yellow edges and 25-35 mm long. The upper edge of the flower cup has two lobes and the lower one has three. The outer surface of the flower cup is covered with star-shaped felt hairs on the upper lip, and inside of them covered with a ring of hairs. The fruit of this medicinal plant has four seeds with a three-sided and round tip (Ghassemi *et al.*, 2001; Mohammadifar *et al.*, 2015).

Figure 1. General appearance of *P. olivieri*.

Essential oils are concentrated hydrophobic liquid contains volatile plant chemical compounds that evaporate easily at normal temperatures. The terpene derivatives present in essential oils are essentially hemiterpenes, monoterpenes, and sesquiterpenes which can be little or very volatile and thermolabile and may be easily oxidized or hydrolyzed depending on their respective structure (Turek & Stintzing, 2013). An essential oil contains aroma of plants whose properties are derived. Essential oils are generally isolated by hydro-distillation method often by using steam. Other processes include expression, solvent extraction, sumatra, absolute oil extraction, resin tapping, wax embedding, and cold pressing (Hanif *et al.*, 2019). Essential oils have been produced in plants for many years and are also called as plant secondary metabolites. These compounds have contact, fumigant, repellent, and anti-nutritional effects (Asadi *et al.*, 2019). For years, plants have used these compounds during co-evolution to repel invading insects. Plant essential oils can have changes in different conditions. One of the factors that can be very involved in this field is the type of weather and region (Figueiredo *et al.*, 2008; Mehalaine *et al.*, 2021). Soil characteristics are influential in this field, and in rich soils, the number and variety of secondary compounds is greater (Khalid & Ahmed, 2021). These factors have significant effects on the quality and quantity of plant essential oils. For this purpose, this

study was conducted in different western provinces of Iran in order to investigate the changes of secondary compounds in this important medicinal plant.

2. MATERIAL and METHODS

2.1. Identification of Species

Identification of the species for the medicinal plant *P. olivieri* was done by sending its mature sample to one of the botany specialists in the Herbarium of Payame Noor University (PNU), Hamedan, Iran.

2.2. Soil Sampling

The soil of different localities from natural habitats of *P. olivieri* was removed from a depth of 30 cm. After mixing the samples with each other and separating the pebbles, finally 2 kg sample was separated from each area and sent to the soil science laboratory of research center and natural resources of Hamadan province (Carter & Gregorich, 2007). In this way, the physical and chemical characteristics of the soil from each locality were determined.

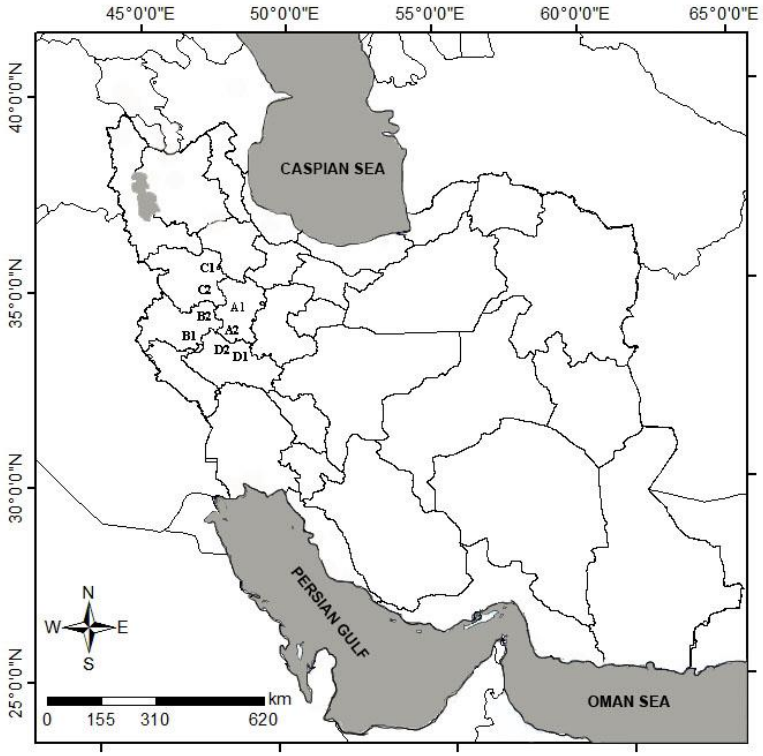

Figure 2. Different localities of studied regions in western of Iran.

2.3. Isolation of Essential Oil

In this research, the aerial parts of *P. olivieri* were collected from its natural habitats in eight localities of four western provinces in Iran, during 2022 (Figure 2) (see Table 1). After drying the collected plants at a temperature of 25°C, they were transferred to the laboratory and their essential oils were isolated. For this purpose, the collected samples were powdered. Then 50 g of their powder was added along with 500 ml of the deionized water inside balloon of the Clevenger apparatus (1 liter) (Figure 3) (Babaee Ghaghelestany *et al.*, 2020). About four hours after starting the heating, the essential oils formed as a light green layer on top of water. Na_2So_4 (sodium sulfate compound) was used to remove water and purify the essential oils (Asadi, 2022). Finally, the purified essential oil was stored in special 5 ml microtubes covered with aluminum foil inside a refrigerator (about 4 °C) until GC-MS analysis (Asadi *et al.*, 2018; Negahban *et al.*, 2007; Parsia Aref & Valizadegan, 2015; Samsam Shariat 2007).

Table 1. List of localities which *P. olivieri* collected of them.

Country	Locality	Latitude	longitude	Altitude (m)	Voucher
Iran	A1	34° 45' 46" N	48° 26' 22" E	2500	Asgari (HPNU) 35140
	A2	34° 11' 18" N	48° 22' 37" E	2200	Asgari (HPNU) 35141
	B1	34° 37' 43" N	47° 32' 28" E	1800	Asgari (HPNU) 35142
	B2	34° 28' 52" N	47° 41' 36" E	1380	Asgari (HPNU) 35143
	C1	35° 17' 47" N	46° 57' 18" E	2300	Asgari (HPNU) 35144
	C2	34° 58' 42" N	47° 54' 53" E	2450	Asgari (HPNU) 35145
	D1	33° 26' 58" N	49° 12' 50" E	1540	Asgari (HPNU) 35147
	D2	33° 51' 51" N	48° 15' 45" E	1750	Asgari (HPNU) 35146

Figure 3. Essential oil isolation by Clevenger apparatus.

2.4. Chemical analysis

Quantitative and qualitative components of the essential oils were detected by Agilent technology gas chromatography (6890N)-Mass Spectroscopy (5973N) (made in the USA) with the following specifications (Figure 4):

Program:
- Mode: Splitless
- Gas: Helium
- Heater: 220
- Split ratio: 0

Column:
- Mode: Const Flow
- Detector: MSD
- Flow:1ml/min

Oven:
- C/min (--), Next C (60), and holdmin (3)
- C/min (20), Next C (220), and holdmin (3)

Aux:
- Setpoint: 280

MS:
- Mode: Scan 2-800

Identification of the components was based on comparison of their mass spectra with those of internal Wiley GC-MS spectral library, or with published mass spectra (Adams *et al.*, 2001).

Figure 4. GC-MS analyzer device in Razi University of Kermanshah, Iran.

3. RESULTS

3.1. Soil features

In examining the soil of different regions, the differences were observed which are given in Table 2. In the case of EC, the difference between A2 location and the rest was evident, while there was no significant difference about this parameter in the other seven regions. In terms of pH, there was no clear difference among eight studied locations. About TNV, the highest and lowest percentages were determined in D2 and B1 locations with 47% and C2 (2%) respectively. In organic compound (OC), the highest and lowest values were observed in C1 and A2. The highest soil phosphorus (P) was determined in C2 with 51 ppm and D2 with 9 ppm. In the case of potassium (K), the highest and lowest values were determined as 512 ppm and 222 ppm in C2 and D2. About the total nitrogen (N), there was no difference among the locations studied. On the soil texture, except for two localities D1 and D2, the rest had clay-loam texture.

Table 2. Soil characteristics of the investigated localities.

Locality	EC (ds/m)	pH	%TNV	%OC	P (ppm)	K (ppm)	Total N (%)	Texture
A1	2.70	8.02	5	0.31	11	246	3	Clay loam
A2	22.2	7.98	7	0.20	16	282	2	Clay loam
B1	1.93	8.16	47	0.62	10	246	6	Clay loam
B2	2.44	8.07	39	0.23	18	307	2	Clay loam
C1	2.40	8.00	14	0.7	27	331	7	Clay loam
C2	2.64	7.95	2	0.51	51	512	5	Clay loam
D1	0.80	8.05	24	0.31	18	464	3	Clay
D2	2.63	7.94	47	0.35	9	222	3	Clay

3.2. Localities A1 and A2

In locality of A1, 17 constituents were identified (Table 3) while caryophyllene was dominant (peak 4, retention time of 9.585 min, 24.093% of total). Also, 12-methyl-E, E-2, 13-octadecadien-1-ol (peak 16, retention time of 11.447 min, 0.290% of total) was minimum constituent. Furthermore, 11 constituents were detected in locality of A2 (Table 4). Accordingly, caryophyllene was determined as main constituents (peak 2, retention time of 9.585 min, 30.371% of total). In opposite, Azulene, 1, 2, 3, 3a, 4, 5, 6, 7-octahydro in peak 6

with retention time of 10.047 min and 2.327% on total was detected as minimum of them. According to this, caryophyllene was a dominant constituent in two localities of A1 and A2. Their chromatogram is shown in Figure 5.

Table 3. Chemical compounds in essential oil of *P. olivieri* collected from locality A1.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	Decane, 2, 4, 6-trimethyl-	5.810	189111	1.223
2	Heptadecane, 2, 6, 10, 14-tetramethyl-	6.861	121985	0.836
3	1H-Indene, 1-ethyloctahydro-7a-methyl	8.105	110468	0.514
4	Caryophyllene	9.585	3648417	24.093
5	(E)-â-Famesene	9.689	3648417	18.445
6	Humulene	9.823	432464	3.551
7	Germacrene D	9.997	2295870	15.489
8	1S,2E,6E,10R)-3, 7, 11, 11-Tetramethylbicyclo	10.091	951466	8.382
9	1-Isopropyl-4, 7-dimethyl-	10.223	201134	3.057
10	Formic acid, 3, 7, 11-trimethyl-1	10.456	104357	1.079
11	Dodecanoic acid	10.531	557774	6.195
12	n-Hexadecanoic acid	10.575	323764	2.989
13	Caryophyllene oxide	10.769	599228	6.370
14	1-Heptatriacotanol	11.212	100059	1.541
15	Cholestan-3-ol, 2-methylene	11.333	99966	2.099
16	12-Methyl-E,E-2,13-octadecadien-1-ol	11.447	29663	0.290
17	Tetradecanoic acid	12.191	260947	3.848

Table 4. Chemical compounds in essential oil of *P. olivieri* collected from locality A2.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	â-Ylangene	9.322	181133	2.921
2	Caryophyllene	9.585	2762424	30.371
3	cis-â-Farnesene	9.687	172618	3.030
4	Humulene	9.822	506214	5.282
5	Germacrene D	9.997	2086704	21.329
6	Azulene, 1, 2, 3, 3a, 4, 5, 6, 7-octahydro	10.047	231596	2.327
7	(1S, 2E, 6E, 10R)-3, 7, 11, 11-Tetramethylbicyclo	10.091	238751	2.381
8	1S, 2S, 5R-1, 4, 4-Trimethyltricyclo	10.537	342454	6.130
9	n-Hexadecanoic acid	10.651	412835	14.526
10	Caryophyllene oxide	10.768	460229	8.787
11	9, 12-Octadecadienoic acid	12.461	33581	2.915

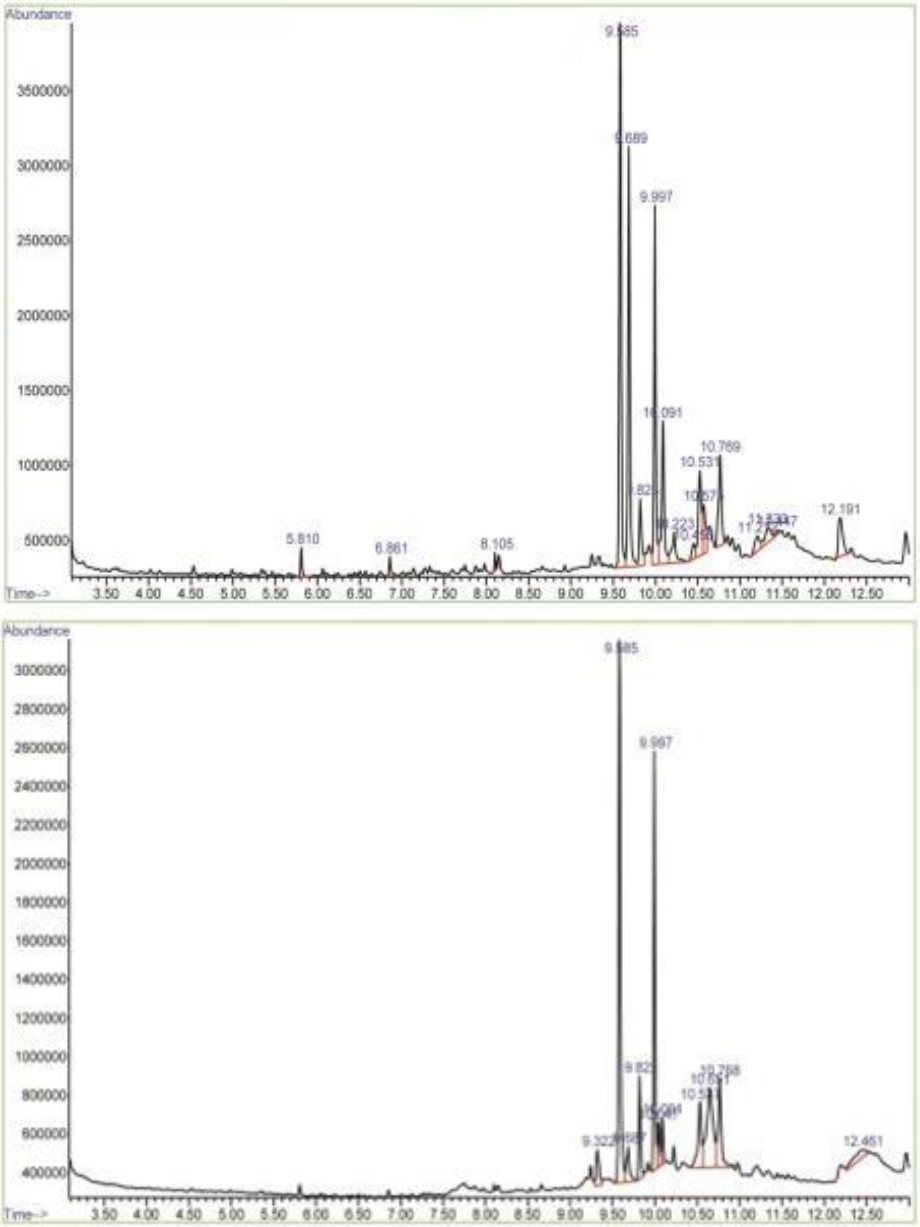

Figure 5. Chromatogram of chemical compounds from *P. olivieri* in localities of A1 and A2.

3.3. Localities B1 and B2

Totally, 17 constituents were identified in locality of B1 (Table 5), among which caryophyllene was dominant (peak 6, retention time of 9.583 min, 18.550% of total). Also, Heptadecane, 2, 6-dimethyl in peak 2 with retention time of 6.860 min and 1.244% on total being minimum constituent. Futhermore, 18 constituents were detected in locality of B2 (Table 6), among which caryophyllene being a dominant constituent (peak 7, retention time of 9.582 min, 25.905% of total) while tetradecane, 2, 6,10-trimethyl detected in peak 10 with retention time of 9.891 min and 1.720% on total was minimum of them. Accordingly, caryophyllene was dominant compound in two locations of B1 and B2. The chromatogram of these two localities is also shown in Figure 6.

Table 5. Chemical compounds in essential oil of *P. olivieri* collected from B1.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	Decane, 2, 5, 6-trimethyl	5.809	137139	1.685
2	Heptadecane, 2, 6-dimethyl	6.860	95155	1.244
3	1H-Indene, 1-ethyloctahydro-7a-methyl	8.105	112105	1.437
4	2(1H)-Naphthalenone, octahydro-8a-methyl	8.149	102825	1.730
5	Tetradecane, 2, 6, 10-trimethyl	9.252	115980	1.354
6	Caryophyllene	9.583	1482750	18.550
7	cis-â-Farnesene	9.688	1160083	14.703
8	Humulene	9.823	188731	3.070
9	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl	9.995	1000903	12.902
10	(1S,2E,6E,10R)-3, 7, 11, 11-Tetramethylbicyclo[	10.090	428373	7.287
11	Phenol, 3,5-bis(1,1-dimethylethyl)-	10.199	142961	4.456
12	Estra-1, 3, 5(10)-trien-17â-ol	10.578	217113	3.545
13	n-Hexadecanoic acid	10.646	157284	5.633
14	Caryophyllene oxide	10.767	378527	9.048
15	Olean-12-ene-3,28-diol, (3â)-	11.180	90074	3.179
16	7-Hexadecenal, (Z)-	11.352	111279	4.764
17	Octadecane, 3-ethyl-5-(2-ethylbutyl)	11.447	136044	3.137

Table 6. Chemical compounds in essential oil of *P. olivieri* collected from B2.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	Decane, 2, 5, 6-trimethyl	5.811	164674	3.301
2	Decane, 2, 4, 6-trimethyl	6.861	103008	2.010
3	1H-Indene, 1-ethyloctahydro-7a-methyl- (1á,3aâ,7aá)	8.105	113156	2.253
4	1,7-Dodecadiene	8.150	101600	2.712
5	Tetradecane, 2,6,10-trimethyl	9.252	133034	3.348
6	(2E,4S,7E)-4-Isopropyl-1, 7-dimethylcyclodeca-2,7-dienol	9.322	73671	2.740
7	Caryophyllene	9.582	1172705	25.905
8	Formic acid, 3, 7, 11-trimethyl-1,6,10-dodecatrien-3-yl ester	9.687	90132	4.386
9	Humulene	9.822	202530	4.676
10	Tetradecane, 2, 6, 10-trimethyl	9.891	56082	1.720
11	Germacrene D	9.994	913151	18.429
12	á-aAcorenol	10.046	91238	1.851
13	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo	10.090	96865	2.515
14	Ledol	10.537	146094	4.342
15	Caryophyllene oxide	10.768	154314	4.643
16	Olean-12-ene-3, 28-diol	11.178	55743	2.034
17	Isopropyl linoleate	11.368	67368	5.312
18	Tetradecane, 2, 6, 10-trimethyl	11.447	90529	3.937

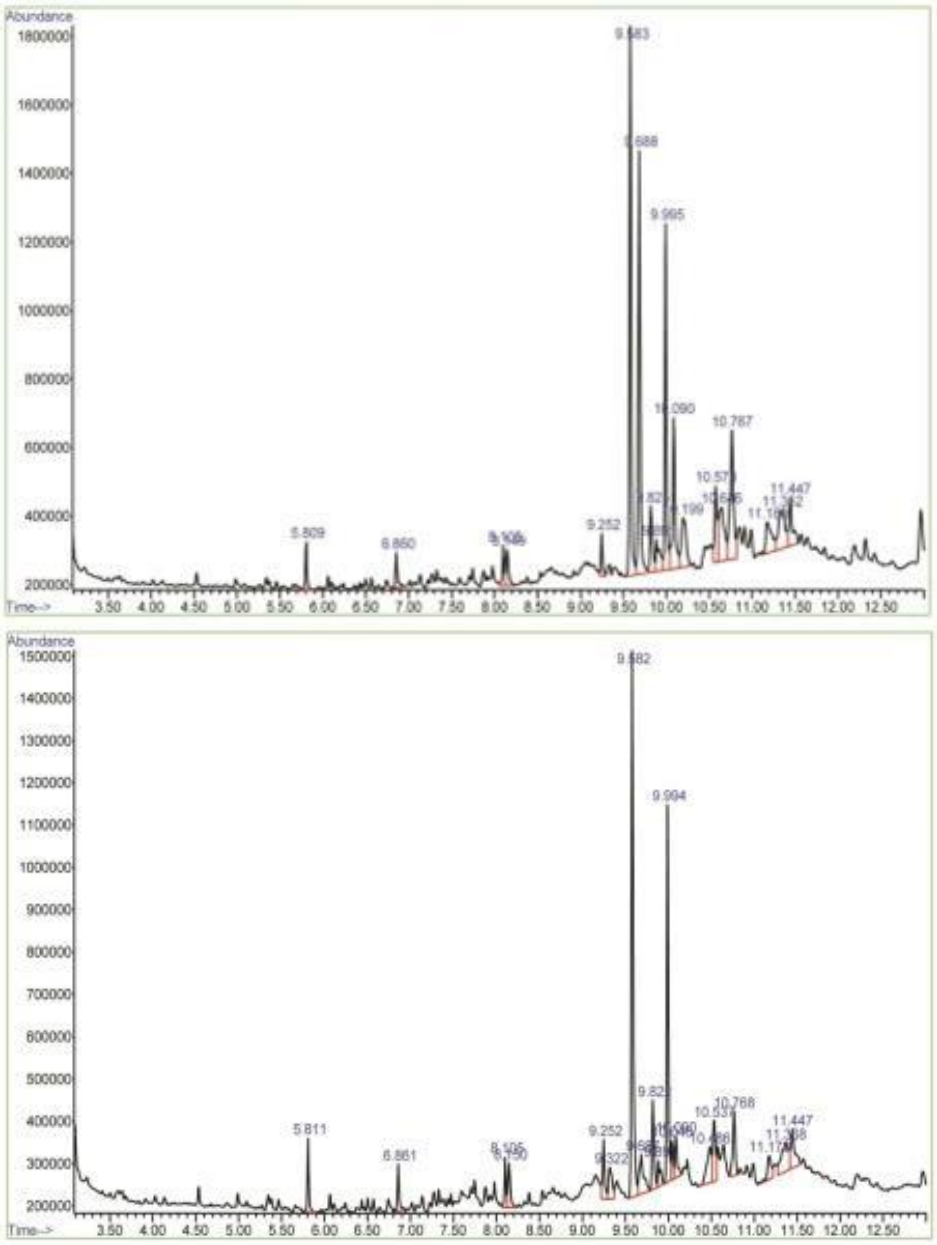

Figure 6. Chromatogram of chemical compounds from *P. olivieri* in localities of B1 and B2.

3.4. Localities C1 and C2

In total, 17 constituents were identified in locality of C1 (Table 7), among them 1H-cyclopenta [1, 3] cyclopropa [1, 2] benzene, octahydro-7-methyl was determined as dominant constituent (peak 12, retention time of 9.994 min, 16.137% from total). Also, 2(1H)-naphthalenone, octahydro-8a-methyl-trans in peak 3 with retention time of 8.106 min and 1.044% of total being a minimum compound. Futhermore, 15 compounds were detected in locality of C2 (Table 8), among which naphthalene, decahydro-4a-methyl-1-methylene-7 being a dominant (peak 8, retention time of 10.000 min, and 19.990% on total) when tetradecane, 2, 6, 10-trimethyl in peak 2 with retention time of 9.253 min and 1.200% on total was determined as minimum constituent. Their chromatogram is shown in Figure 7.

Table 7. Chemical compounds in essential oil of *P. olivieri* collected from C1.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	Hydroxylamine, O-decyl	5.811	101504	1.602
2	Z-2-Dodecenol	7.752	92234	2.684
3	2(1H)-Naphthalenone, octahydro-8a-methyl	8.106	60509	1.044
4	Tetradecane, 2, 6, 10-trimethyl	9.252	89011	2.318
5	Isocaryophillene	9.488	526353	11.223
6	Caryophyllene	9.583	580657	12.726
7	cis-â-Farnesene	9.634	176649	4.538
8	(E)-â-Famesene	9.688	171517	4.413
9	Humulene	9.749	87983	1.956
10	Nerolidyl acetate	9.822	118973	2.775
11	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl	9.938	659231	15.084
12	1H-Cyclopenta [1, 3] cyclopropa[1,2]benzene	9.994	734154	16.137
13	(1S,2E,6E,10R)-3, 7, 11, 11-Tetramethylbicyclo	10.042	191288	4.641
14	Geranyl isovalerate	10.566	97546	7.082
15	Estra-1, 3, 5(10)-trien-17â-ol	10.641	65326	2.857
16	Caryophyllene oxide	10.739	128211	5.353
17	Heptadecane, 9-octyl	11.442	110011	3.566

Table 8. Chemical compounds in essential oil of *P. olivieri* collected from C2.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	2-Pentadecanone, 6, 10, 14-trimethyl-	9.003	157379	1.919
2	Tetradecane, 2, 6, 10-trimethyl-	9.253	212731	1.200
3	6-epi-shyobunol	9.328	213388	1.502
4	Caryophyllene	9.586	3685264	16.993
5	cis-â-Farnesene	9.689	1119730	9.005
6	Humulene	9.824	722638	4.715
7	Tetradecane, 2, 6, 10-trimethyl-	9.894	305912	3.340
8	Naphthalene, decahydro-4a-methyl-1-methylene	10.000	4313200	19.990
9	(1S, 2E, 6E,10R)-3, 7, 11, 11-Tetramethylbicyclo [8.1.0]	10.050	596229	2.749
10	(3R, 3aR, 3bR, 4S, 7R, 7aR)-4-Isopropyl	10.093	838145	5.417
11	Dodecanoic acid	10.225	324779	4.647
12	Caryophyllene oxide	10.534	576183	6.817
13	Germacrene D	10.769	644367	5.724
14	n-Hexadecanoic acid	11.663	501210	13.678
15	Tetradecanoic acid	12.194	213502	2.304

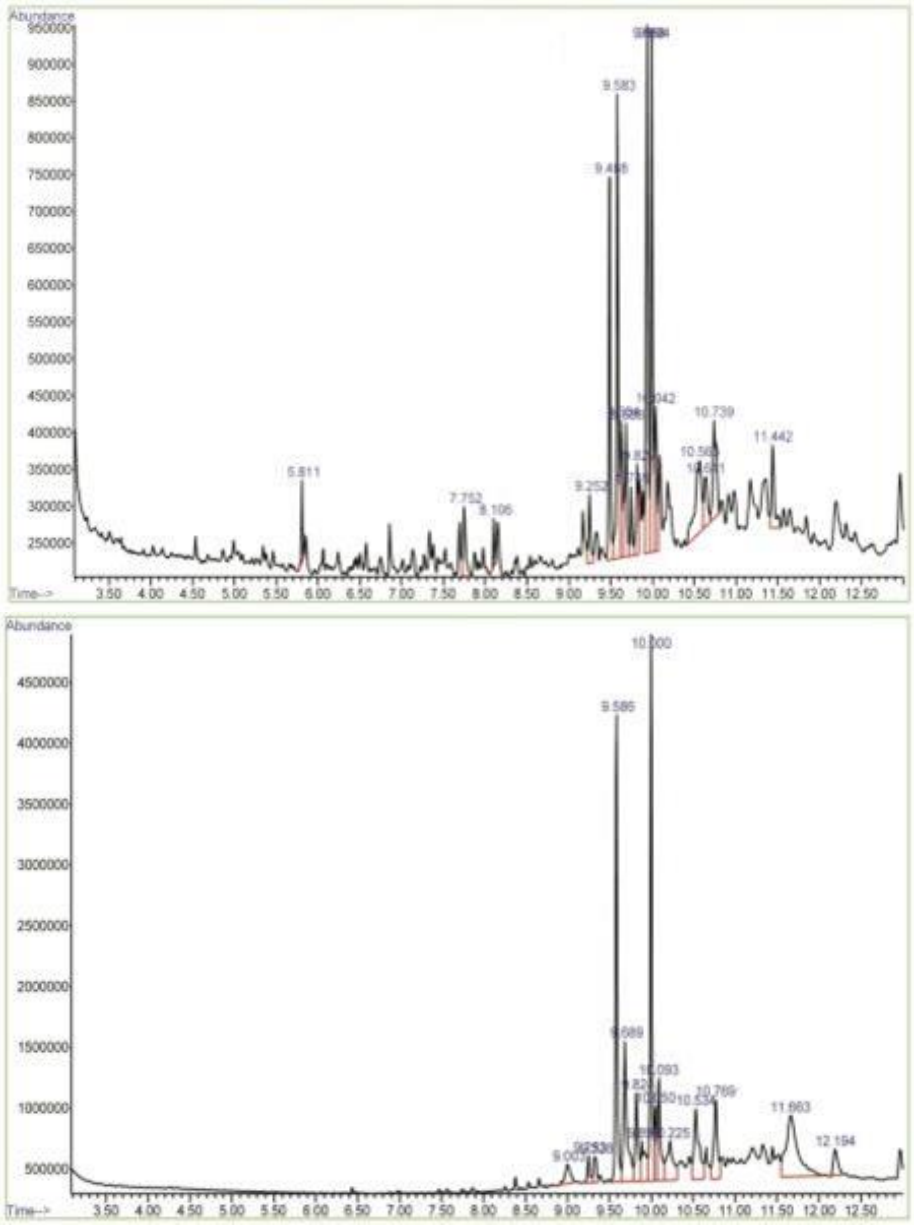

Figure 7. Chromatogram of chemical compounds from *P. olivieri* in localities of C1 and C2.

3.5. Localities D1 and D2

In locality of D1, 21 compounds were detected (Table 9), among which estra-1, 3, 5 (10)-trien-17â-ol being dominant compound (peak 17, retention time of 10.639 min, 18.320% of total) when chloromethanesulfonyl-dichloromethanesulfonyl chloride detected in peak 1 with retention time of 4.538 min and 1.075% on total being minimum of them. In locality of D2, 15 constituents were identified (Table 10), among which n-hexadecanoic acid being dominant compound (peak 12, retention time of 10.646 min, and 29.363% of total) when ã-elemene (peak 6, retention time of 9.615 min, and 1.668% of total) was minimum of them. The chromatogram of these two localities is also shown in Figure 8.

Table 9. Chemical compounds in essential oil of *P. olivieri* collected from D1.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	Chloromethanesulfonyl-dichloromethanesulfonyl chloride	4.538	35939	1.075
2	Decane, 2, 4, 6-trimethyl	5.811	129700	3.727
3	Hydroxylamine, O-decyl	6.861	80224	2.468
4	2(1H)-Naphthalenone, octahydro-8a-methyl	7.878	45319	2.014
5	1H-Indene, 1-ethyloctahydro-7a-methyl	7.979	50343	2.331
6	Hexen-1-ylcyclohexane	8.106	89321	2.471
7	2(1H)-Naphthalenone, octahydro	8.151	79469	2.699
8	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	8.602	47227	4.852
9	Octadecane, 6-methyl	9.164	42513	4.394
10	Tetradecane, 2, 6, 10-trimethyl	9.252	75446	2.547
11	Hexadecanoic acid, methyl ester	9.418	38221	2.806
12	7-epi-cis-sesquisabinene hydrate	9.582	75308	1.923
13	Formic acid, 3, 7, 11-trimethyl-1,6,10-dodecatrien-3-yl ester	9.615	72468	2.526
14	Heptadecane, 2, 6, 10, 15-tetramethyl	9.668	57578	3.123
15	â-Copaene	9.993	432025	11.297
16	á-Acorenol	10.089	83730	2.993
17	Estra-1, 3, 5(10)-trien-17â-ol	10.639	154200	18.320
18	Methyl 16-hydroxy-hexadecanoate	10.813	129032	8.483
19	1-Ethyl-3-propyl-5-(propene-1-yl)adamantine	11.174	129032	1.956
20	Tetradecane, 2, 6, 10-trimethyl	11.447	35115	1.051
21	Tetradecanoic acid	12.204	45712	2.894

Table 10. Chemical compounds in essential oil of *P. olivieri* collected from D2.

Peak	Compound	Retention time (min)	Peak height	Percentage of total
1	Decane, 2 ,6, 7-trimethyl	5.811	154663	2.668
2	Decane, 2, 4, 6-trimethyl	6.862	92784	1.723
3	2(1H)-Naphthalenone, octahydro-8a-methyl-, trans	8.106	108447	1.855
4	1H-Indene, 1-ethyloctahydro-7a-methyl	8.150	101381	2.309
5	Tetradecane, 2, 6, 10-trimethyl	9.252	114629	2.033
6	ã-Elemene	9.615	94262	1.668
7	Tetradecane, 2, 6, 10-trimethyl	9.667	179329	3.036
8	Germacrene D	9.995	1493761	26.558
9	7-epi-cis-sesquisabinene hydrate	10.050	123410	1.956
10	(1S,2E,6E,10R)-3, 7, 11, 11-Tetramethylbicyclo	10.090	285307	5.291
11	á-Acorenol	10.538	245616	9.656
12	n-Hexadecanoic acid	10.646	434435	29.363
13	Estra-1,3,5(10)-trien-17â-ol	10.744	434435	4.884
14	9,12-Octadecadienoic acid	11.367	64427	4.153
15	7-Hexadecenal	11.444	80542	2.846

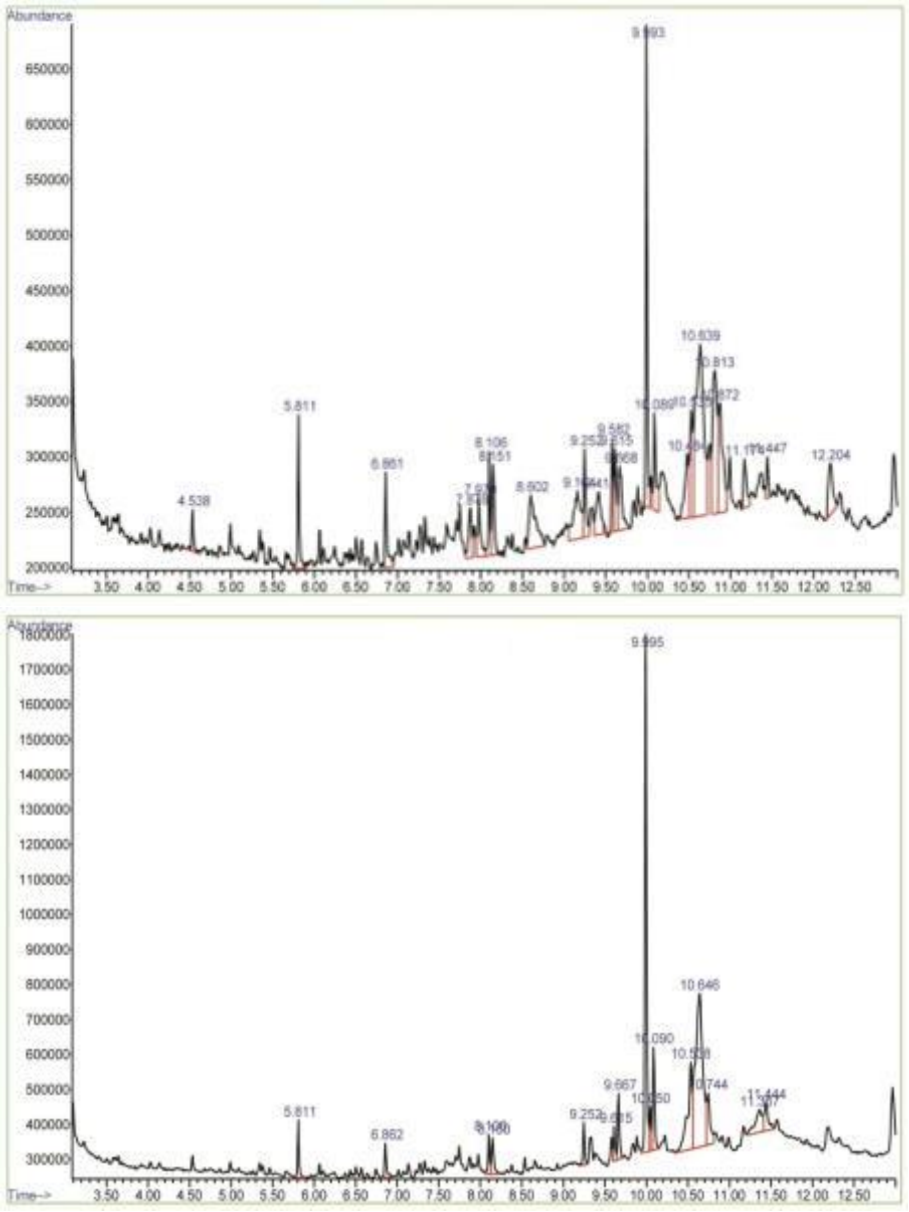

Figure 8. Chromatogram of chemical compounds from *P. olivieri* in localities of D1 and D2.

4. DISCUSSION and CONCLUSION

Regarding the investigation on this valuable medicinal plant, various studies have been conducted in Iran. We indicate some of them in the following and explain their results in comparison with ours. Mirza and Baher Nik (2003) studied the essential oil of *P. olivieri* in Iran and among 15 identified constituents, germacrine D (28.1%), β-caryophyllene (16.1%), α-pinene (11.7%), and β-seleline (10.2%) were dominant constituents. Similarities among the dominant compounds can be confirmed in two studies, which indicate similar conditions in production of secondary compounds. Sarkhail *et al.*, (2006) found that 22 constituents from this plant essential oil made up 93.6% of total volume when main constituents were germacrene D (66.1%), β-seline 5.1%, β-caryophyllene (4.2%), and α-pinene (4.2%). The results of previous studies in comparing the constituents of essential oil of *P. olivieri* in different regions showed that germacrene D and β-caryophyllene were main compounds, that our studies confirm it. In our investigation, caryophyllene had a significant percentage in majority of essential oils isolated from different localities shows that the essential oils of this plant in the northern regions of Iran have similarities with western regions. Although, quality of collected medicinal plant,

method of isolation, and analyzer device of essential oil are also effective agents in this direction. β-caryophyllene (BCP) is a natural bicyclic sesquiterpene and a common constituent of the essential oils of numerous spice and food plants. Anti-inflammatory agents that are non-steroidal in nature. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions (Amor *et al.*, 2009).

The reaction mechanism of all sesquiterpene synthases starts with the ionization of farnesyl diphosphate. The resulting carbocation can undergo a range of cyclizations. The 11,1-closure yields the 11-membered ring humulyl cation. Then, deprotonation followed by cyclobutane ring formation leads to b-caryophyllene production. Otherwise, the 10,1-closure originates the germacrenyl cation, which can be converted to germacrene D. Thus, the precursors flow toward one of these constituents can reduce the amounts of the other (Degenhardt *et al.*, 2009).

Tajbakhsh *et al.*, (2007) investigated the essential oils isolated from leave, flower, stem, and root of *P. olivieri*. They identified thirteen components in the oil of leaf that germacrene D (58%), α-pinene (10.4%), and bicycle germacrene (4.4%) were major components of them. In flowers, 10 compounds were identified and germacrene D (48%), α-pinene (19.7%), and β-selinene (10.1%) made up its majority. Moreover, 10 compounds characterized stem essential oil and germacrene D (57%), α-pinene (8.6%), and 2-pentadecanone (6.5%) were dominant constituents. In the essential oil of root, germacrene D (35%), α-pinene (12.6%), and spathulenol (6.6%) were major. Different parts of the plants can have differences in terms of chemical compounds. In our study, we examined the aerial parts including leaves and stem and flowers in a mixed form , which have similarities with this study; but, there are obvious differences in other parts, and it is not possible to make a direct examination. Javidnia *et al.*, (2010) studied essential oil composition of two species from the genus *Phlomis* including *P. aucheri* Boiss. and *P. elliptica* Benth. in Iran. They found 39 compounds of *P. aucheri* and 58 from *P. elliptica* containing 91.2% and 90.1% of total. Moreover, caryophyllene-type compounds contained 63.8% of *P. aucheri* and the main of them were caryophyllene oxide (33.5%), β-caryophyllene (27.0%) and β-selinene (10.2%). In apposite for *P. elliptica*, essential oil structure included aliphatic hydrocarbons with hexadecanoic acid (19.1%), linoleic acid (10.2%) and β-selinene (9.9%) as main constituents. One of the important factors in modifying the composition of plant essential oils is the studied species. However, in the species within the same genus, the differences in secondary metabolites are insignificant. In this study, minor changes were determined compared to the species under our study, the differences were not overall. In different genus, variation among the compounds will definitely be a lot of.

Jamzad *et al.*, (2013) studied the essential oil from leaves and flowers of *P. persica* Boiss. and *P. olivieri* from Iran and concluded that in *P. persica*, 98.5 % of essential oil obtained from the leaves and 89.8 % from flowers. Also, in *P. olivieri*, 98.9 % of the essential oil isolated from leaves and 99.2 % from flowers. The essential oils were rich in sesquiterpenes and germacrene D being major constituent in both (26.5 %, 19.5 %) and (37.6 %, 19.5 %), in the leaves and flowers of *P. persica* and *P. olivieri*. Bicyclo germacrene was abundant in leaves and flowers of two species (18.7%, 20.4%) and (8.0%, 5.7%). In another study, Mohammadifar *et al.*, (2015) studied chemical analysis of *P. olivieri* and *P. persica* essential oils and found that 46 compounds were available in both essential oils. Among them, β-caryophyllene (25.7%) and germacrene D (19.5) in *P. olivieri* and germacrene D (17.2%) and γ-elemene (15.4%) in *P. persica* were dominant. Essential oils of *P. olivieri* and *P. persica* being rich of sesquiterpens. The chemical constituents of the analyzed essential oils were also found to be different from those reported in other regions of Iran, which may be due to the existence of possible different chemotypes between populations of these two species.

Mohammadifar *et al.*, (2015) studied constituents of *P. olivieri* essential oil and found that one caffeoylquinic acid derivative, chlorogenic acid (1), one iridoid glycoside, ipolamiide (2),

two phenylethanoid glycosides, phlinoside C (3), and verbascoside (5), along with two flavonoids, isoquercetin (4), and naringenin (6) were identified from this medicinal plant. Their results study indicated that *P. olivieri* is a medicinal plant with suitable biological and pharmacological properties. In this study, type of analyzer device was different from ours, and for this reason, some differences were observed in the results. Therefore, the method of analysis is one of the influential factors in secondary metabolites and plant volatiles, which should be carefully considered. Bajalan *et al.,* (2017) studied the essential oil of wild populations from *P. olivieri* and concluded that 27 compounds containing 90.52- 98.51% of total. Results indicated that major constituents were germacrene D (26.54-56.41%), bicycle germacrene (6.38-30.55%), β-caryophyllene (5.32-24.52%), and α-pinene (1.29-15.53%). The results of their study showed new insights for cultivation and industrial uses of this medicinal plant in Iran; because, this valuable medicinal plant contains numerous compounds with abundant medicinal properties that have received little attention.

Khalilzadeh *et al.,* (2005) investigated essential oils of *P. persica* and *P. olivieri* from Iran when found that *P. persica* contained germacrene D (38.2%), bicycle germacrene (16.3%), and α-pinene (13.3%) as major of them. The oil of *P. oliveri* was also characterized also by higher amount of germacrene D (26.4%) and bicycle germacrene (12.7%). Both oils consisted mainly of sesquiterpene hydrocarbons. There are no obvious differences in secondary compounds in closely related species, and the results of this study confirm this subject.

Fattahi and Najjari (2022) studied the essential oil composition from three species of *Phlomis olivieri*, *P. persica*, and *P. herba-venti* collected from northwest of Iran. They found that 38 compounds were available in each essential oil when the main compounds of *P. olivieri* included as dioctyl phthalate (69.71%), gamma-almen (7.93%), and germachron-di (7.44%). Also, napthalactone (45.24%), bis(2-ethylhexyl) phthalate (32.72%), and Germacron-D (5.59%) was major in *P. herba-venti*, Moreover, in essential oil of *P. persica*, dioctyl phthalate (49.74%), alpha-murolene (16.18%), and hexa hydrofarnesyl acetone (7.82%) being dominant. Comparing the constituents identified in the essential oil of three species showed a higher percentage of dioctyl phthalate in *P. olivieri* (69.71%) compared to the other species. The highest amount of total phenol (136.34 mg of gallic acid per gram of dry weight), total flavonoid (49.29 mg of quercetin per dry weight), and antioxidant activity (48.68%) related to *P. persica*. Different species within the same genus have differences in the essential oil structure, which should be taken into account in different studies. However, due to the differences in these compounds, occurrence of biological differences is a natural subject. Regarding the species in two studies, *P. olivieri*, a certain difference in the dominant compounds and total number of them observed when weather can be considered an effective factor for the differences.

Salehi and Kalvandi (2022) investigated essential oil of different *P. olivieri* populations in Hamedan province and found that 31 compounds were identified when caryophyllene, germacrene D, and (E)-b-farnesene had the highest percentage compared to the other essential oil constituents. Also, the highest essential volume of essential oil was obtained in the Koohani population which had the lowest altitude among other populations. In our study, caryophyllene in the essential oil of both regions had the highest percentage in essential oil, which confirms each other. Although, we have investigated A1 and A2 regions in this province. Despite the presence of these regions within the same province and slight differences in climate, no drastic changes were observed in the secondary compounds. Salehi and Kalvandi (2023) investigated chemical structure of essential oils from *P. olivieri* when found that variation was estimated in samples collected from 11 different regions of Hamedan province. The major constituents contained sesquiterpenes including germacrene D, (E)-β-caryophyllene, and (E)-β-farnesene. Germacrene D differed from 5.3% to 36.9%, (E)-β-caryophyllene from 6.3% to 61.9%, and (E)-β-farnesene from 5.1% to 18.4%, suggesting occurrence of three chemotypes in this province. Ghavam (2023) studied essential oil analysis from of *P. olivieri* in Iran when the main

compounds included sesquiterpenes such as germacrene D (26.43%), β-caryophyllene (20.72%), elixene (6.58%), β-trans-farnesene (6.17%), β-cyclo germacrane (5.04%), germacrene B (4.73%), α-humulene (4.22%), and monoterpene α-pinene (3.22%).

There are very limited studies about the effect of ecological factors on the changes of plant essential oils. In this direction, Mahdavi *et al.*, (2014) investigated these factors on the essential oil of *Phlomis cancellata* Bunge in Mazandaran province (Iran). They found that the efficiency was high in 2400 m when percentage of main component was higher at altitude of 2000 m. Due to the high efficiency, large number of components, and valuable amount of main constituents, the essential oil isolated from height of 2000 m has the most favorable quality for use in various industries especially pharmaceutical industries. The results of their study are consistent with ours; although the examined species was different. In the area where this study was conducted, since the ecological conditions were better in terms of altitude, the variety and number of secondary compounds were identified and the difference between dominant constituent with the others was obvious. Unfortunately, there is no direct study on other parameters including soil characteristics of each region on the changes of these compounds. The current research is considered as new step in this field and must be considered.

In general, it can be said that this plant has valuable medicinal effects with good diversity and proper distribution in different parts of Iran; but, it has received less attention. With supplementary and comprehensive studies, we can obtain localities that that this medicinal plant has more compounds with better quality. There are many studies about this valuable medicinal plant; but, the effects of its various medicinal compounds have not been comprehensively investigated. The authors of this article encourage other researchers to conduct more studies on this valuable plant and to examine the effects of the various factors on it. Later, if the compounds of this medicinal plant are suitable, it is possible to isolate and commercialize the compounds contained in it for medicinal usages.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Mahtab Asgari Nematian: Investigation, Resources, Visualization, and Software. **Behjat Bahramynia**: Formal Analysis and Writing original draft. **Zahra Baghaeifar**: Methodology, Supervision, and Validation.

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