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The Quantitative Comparison of Essential Oil Composition of an Iranian Endemic Plant [*Leonurus cardiaca* L. subsp. *persicus* (Boiss.) Rech. F.] vs. Previous Studied Population

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ABSTRACT

The essential oil obtained from the aerial parts *Leonurus cardiaca* L. subsp. *persicus* (Boiss.) Rech. F., endemic to Iran, was analyzed by using GC and GC-MS. *L. cardiaca* subsp. *persicus* with a yield of 0.25% (w/w), consist of forty six compounds representing 90.03 % of the essential oil. The major components in the latter one included Thymol (35.25%), Germacrene D (7.62%), Borneol (6.69%), trans-Caryophyllene (4.83%), γ -Guaiol acetate (4.43%), Phytol (4.10%) and β -Phellandrene (2.85%). The differences between the populations of the current study and the previous studied ones may be because of the time of collecting the specimens, chemotypes, drying conditions, mode of distillations, geographical and climatic factors.

Key words: Lamiaceae, Volatile oil, Endemic species, *Leonurus cardiaca*, Iran.

INTRODUCTION

The *Lamiaceae* plant family is one of the largest families among the dicotyledons, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil [1].

Two new phenylethanoid glycosides 1 and 2 named leonoside E and leonoside F, and one new sesquiterpene glycoside (3) identified as 7 α (H)-eudesmane-4,11 (12)-diene-3-one-2 β -hydroxy-

13-β-d-glucopyranoside, together with seven known glycosides (4-10), were isolated from the aerial part of *Leonurus japonicus* Houtt. [2]. Liang *et. al.* studied protective effects of alkaloid extract from *Leonurus heterophyllus* Sw. on cerebral ischemia reperfusion injury by middle cerebral ischemic injury (MCAO) in rats [3]. *Leonurus cardiaca* was first described in medicinal literature in the 10th century as a remedy for healing nervous and functional cardiac disorders. *L. cardiaca* is utilized for healing cardiac diseases till now in Germany, France, Russia, Hungary, Lithuania and some other countries. Different meaning of *L. cardiaca* names and its different uses in healing in various countries may be caused by a quantitative variation of the constituents in the plant. *L. cardiaca* herbs biosynthesise flavonoids, alkaloids, iridoids, diterpenoids, cardenolids such as glycosides, tannins and other constituents in lower amounts. Among other compounds, 0.01–0.05% belong to essential oils. Some authors proposed that the main healing power of *L. cardiaca* depends on the content of flavonoids. The content of these compounds, expressed as hyperoxide, was determined in herbs according to European Pharmacopoeia. Qualitative analysis of iridoids by thin layer chromatography is recommended beside the quantitative determination of flavonoids [4]. Mockute *et. al.* analyzed the essential oils of wild *L. cardiaca* collected at full flowering in six habitats in Vilnius district. About the half of the oils were consisted of sesquiterpene hydrocarbons (48.8-62.2%). The oils from fresh dried plants were of the Germacrene D (26.6-35.1%) chemotype. The other main constituents were β-caryophyllene (5.8-9.0%) and α-humulene (6.4-9.2%). Forty-nine components were identified representing 73.1-84.8% of the oils [5]. Total Phenolic and Flavonoid Contents of *L. cardiaca* in Compare with Antioxidant Activity was investigated by Jafari *et. Al.* in 2010 [6]. Miłkowska-Leyck *et. al.* detected Lavandulifolioside for the first time in *L. cardiaca* var. *vulgaris* [Moench] Briquet [7]. Najafpour Navaei and Mirza isolated the essential oil of *L. cardiaca* by water steam distillation (Clevenger) and analyzed by a combination of capillary GC and GC/MS [8].

The aim of this research is a comparative study of the chemical composition essential oil of *Leonurus cardiaca* L. subsp. *persicus* (Boiss.) Rech. F. that is endemic to Iran [9 & 10].

MATERIALS AND METHODS

material: The aerial part of *Leonurus cardiaca* subsp. *persicus* from Damavand to Tar Road (N 35°43', E 52°13'), in June 2011 (IRAN-56840). The plant was identified by following Flora Iranica [11], and the voucher specimens are preserved in the herbarium of the Ministry of Jihad-e-Agriculture ("IRAN") at the Iranian Research Institute of Plant Protection (Tehran, Iran).

Isolation of the volatile oils: The air-dried aerial parts of the plant were subjected to hydrodistillation for four hour using a Clevenger-type apparatus. The oil was kept at 4°C in the sealed brown vials until required.

Gas chromatography-Mass spectroscopy: Analytical gas chromatography was carried out using a Thermoquest 2000 GC with capillary column DB-5 (30 m. 0.25 mm i.d., 0.25 μm film Thickness); carrier gas, He; split ratio, 1:25; and using a flame ionization detector. The column temperature was programmed at 50°C for 1 min. and then heated to 265°C at a rate of 2.5°C/min. and then kept constant at 265°C for 20 min. GC-MS was performed on a Thermoquest 2000 with a quadruple detector, on capillary column DB-5 (GC); carrier gas, He; flow rate, 1.5 ml/min. the column was held at 50°C for 1 min. and programmed up to 265°C at rate of 2.5°C/min, then kept

constant at 256°C for 20 min. The MS operated at 70 eV ionization energy. Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oils were identified by comparison of their mass spectra and retention indices with Wiley library and those published in the literature [12].

Table 1- Chemical composition (%) of the essential oil of *Leonurus cardiaca* subsp. *Persicus*

No.	Component	%	KI	Rt
1	α -Pinene	1.58	942	9.63
2	Camphene	0.17	957	10.11
3	2- β -Pinene	0.15	982	11.26
4	3-Octanone	0.15	989	11.42
5	1-Octen-3-ol	0.34	995	11.61
6	α -Terpinene	0.28	1020	13.12
7	<i>o</i> -Cymene	1.55	1026	13.30
8	1,8-Cineole	1.78	1036	13.56
9	dl-Limonene	0.18	1041	13.67
10	γ -Terpinene	1.79	1092	15.03
11	cis- β -Terpineol	0.18	1106	16.36
12	Nonanal	0.19	1119	16.78
13	Linalool L	0.89	1128	17.05
14	Camphor	1.19	1148	18.17
15	Phellandral	0.25	1157	18.72
16	trans-Verbenol	0.21	1162	18.88
17	Borneol	6.69	1171	19.88
18	β -Phellandrene	2.85	1182	20.39
19	cis-Dehydrocarvone	0.15	1189	20.83
20	α -Terpineol	0.27	1197	21.03
21	Carvacrol methyl ether	0.32	1225	23.35
22	Bornyl acetate	0.71	1278	25.12
23	Thymol	35.25	1292	26.46
24	α -Copaene	0.30	1349	29.57
25	β -Bourbonene	0.41	1358	29.86
26	trans-Caryophyllene	4.83	1408	31.25
27	α -Humulene	1.18	1435	32.6
28	β -Ionone	0.57	1459	33.46
29	α -Amorphene	0.30	1467	33.56
30	Germacrene D	7.62	1478	33.71
31	Isolatedene	0.27	1492	34.01
32	Germacrene A	0.68	1506	34.29
33	β -Bisabolene	0.82	1511	34.96
34	1S-cis-Calamene	0.15	1523	35.11
35	δ -Cadinene	0.67	1537	35.36
36	cis- β -Elemene	0.35	1564	36.54
37	Caryophyllene Oxide	1.77	1589	37.29
38	β -Oplophenone	0.40	1604	37.64
39	α -Cadinol	0.55	1656	39.71
40	Valerianol	0.56	1668	40.06
41	Pentadecanone	0.74	1764	47.16
42	α -Eudesmol acetate	0.70	1785	48.27
43	Palmitic acid	0.98	1796	51.49
44	γ -Guaiaol acetate	4.43	1811	53.90
45	cis-Farnesyl acetate	0.53	1828	54.86
46	Phythol	4.10	2300	62.53
Total		90.03		

Rt = Retention time; KI= Kovats Index, %= Relative percentage obtained from peak area

Table 2- Comparison the essential oil composition (%) from two populations of *Leonurus cardiac*

No.	Component	Damavand to Tar (%)	Damavand Region ^[8] (%)
1	α -Pinene	1.58	0.1
2	Camphene	0.17	
3	2- β -Pinene	0.15	
4	3-Octanone	0.15	
5	1-Octen-3-ol	0.34	
6	α -Terpinene	0.28	
7	o-Cymene	1.55	
8	1,8-Cineole	1.78	0.8
9	dl-Limonene	0.18	
10	γ -Terpinene	1.79	0.1
11	cis- β -Terpineol	0.18	
12	Nonanal	0.19	0.3
13	Linalool L	0.89	
14	Camphor	1.19	
15	Phellandral	0.25	
16	trans-Verbenol	0.21	
17	Borneol	6.69	
18	β -Phellandrene	2.85	
19	cis-Dehydrocarvone	0.15	
20	α -Terpineol	0.27	
21	Carvacrol methyl ether	0.32	
22	Bornyl acetate	0.71	
23	Thymol	35.25	
24	α -Copaene	0.30	0.6
25	β -Bourbonene	0.41	1.8
26	trans-Caryophyllene	4.83	
27	α -Humulene	1.18	15.3
28	β -Ionone	0.57	2.2
29	α -Amorphene	0.30	
30	Germacrene D	7.62	20.9
31	Isoledene	0.27	
32	Germacrene A	0.68	
33	β -Bisabolene	0.82	
34	1S-cis-Calamene	0.15	
35	δ -Cadinene	0.67	
36	cis- β -Elemene	0.35	
37	Caryophyllene Oxide	1.77	6.0
38	β -Oplopenone	0.40	
39	α -Cadinol	0.55	2.9
40	Valerianol	0.56	
41	Pentadecanone	0.74	
42	α -Eudesmol acetate	0.70	
43	Palmitic acid	0.98	
44	γ -Guaiol acetate	4.43	
45	cis-Farnesyl acetate	0.53	
46	Phythol	4.10	
47	β -Pinene		0.1
48	ρ -Cymene		0.4
49	Limonene		0.8

50	(Z)- β -Ocimene	0.1
51	(E)- β -Ocimene	0.1
52	Terpinolene	0.3
53	α -Campholenal	0.2
54	trans-Pinocarveol	0.2
55	Myrtenal	0.5
56	Decanal	0.2
57	Carvacrol	2.4
58	β -Caryophyllene	13.8
59	β -Gurjunene	0.5
60	β -Guaiene	1.3
61	allo-Aromadendrene	0.7
62	γ -Muuroolene	2.0
63	Bicyclogermacrene	3.7
64	γ -Cadinene	3.0
65	δ -Cadinene	3.3
66	Elemol	1.8
67	Germacrene B	2.5
68	Spathulenol	2.1
69	Viridiflorol	1.2
70	Cubenol	0.9
71	α -Muurolol	2.3
72	β -Eudesmol	3.0
Total		90.03
		98.4

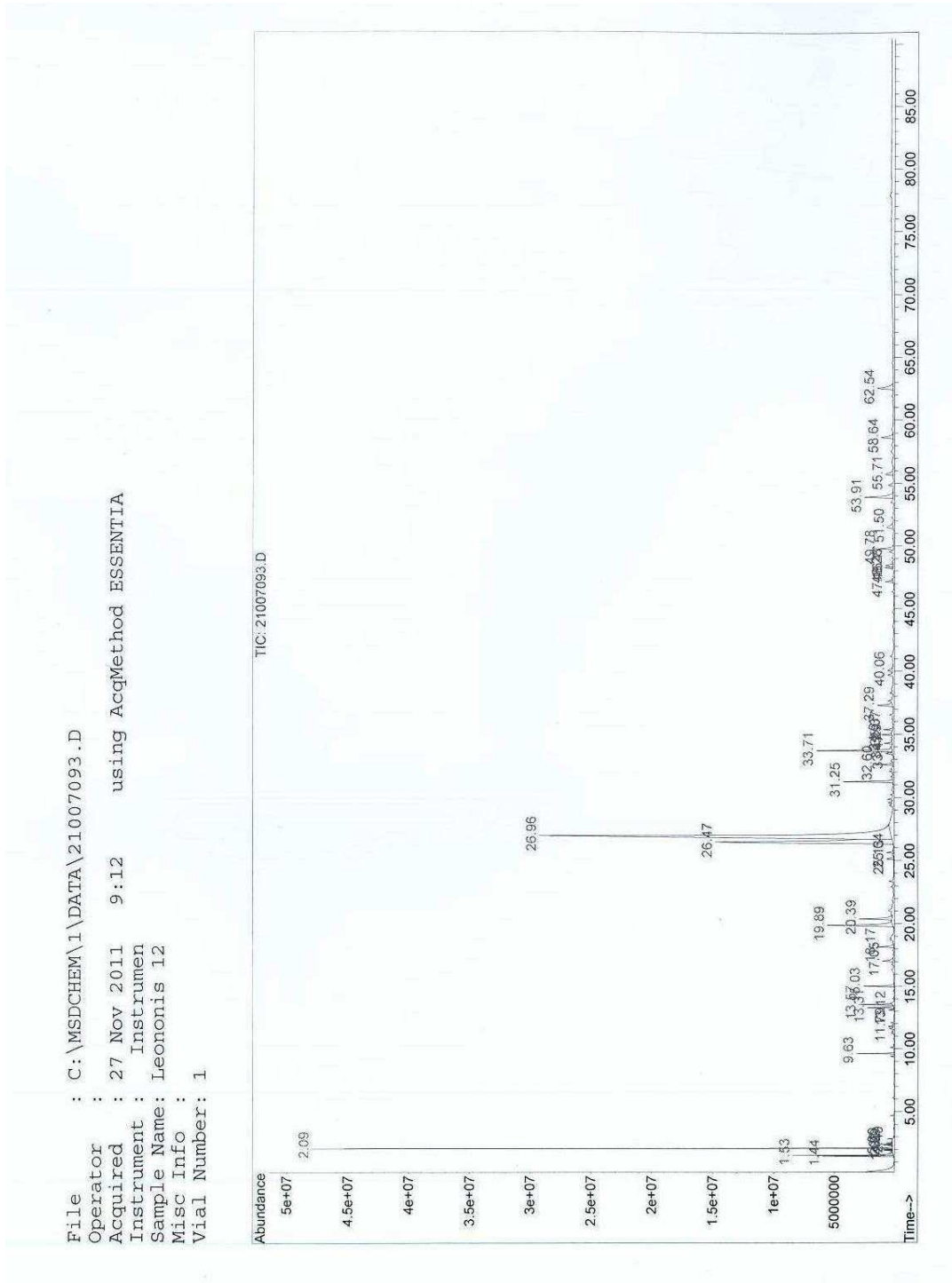
Table 3- The Percentage of Class compositions of *Leonurus cardiaca* subsp. *persicus* Essential Oils

Terpenes	%
Nonterpene	1.42
Monoterpene hydrocarbons	8.55
Oxygenated monoterpene	48.46
Sesquiterpene hydrocarbones	17.58
Oxygenated sesquiterpenes	8.94
Oxygenated Diterpene	5.08
Total	88.61

Table 4- The Fraction of Class Compositions Percentage of *Leonurus cardiaca* subsp. *persicus* Essential Oils

Terpenes	%
Monoterpene Fraction	57.01
Sesquiterpene Fraction	26.52
Diterpene Fraction	5.08

Figure 1- Chromatogram of *Leonurus cardiaca* subsp. *persicus*



RESULTS AND DISCUSSION

The hydrodistillation of the aerial parts of *Leonurus cardiaca* L. subsp. *persicus* (Boiss.) Rech. F. gave pale yellow oil with a distinct sharp odor in the yield of 0.25% (w/w), basis on dry weight. Forty six compounds representing 90.03 % of the oil were identified. The major components were Thymol (35.25%), Germacrene D (7.62%), Borneol (6.69%), trans-Caryophyllene (4.83%), γ -Guaial acetate (4.43%), Phytol (4.10%) and β -Phellandrene (2.85%) (Table 1). Figure 1 shows the chromatogram of *Leonurus cardiaca* subsp. *persicus*. Table 2 indicates the comparison (%) of the essential oil composition (%) from two populations of *Leonurus cardiaca* from Damavand to Tar Road population chemical components and Damavand region population. The major components of the latter population were dominated by Germacrene-D (20.9%), α -humulene (15.3%) and β -caryophyllene (13.8%). There are 11 common chemical compositions between these two populations. Table 3 shows the percentage of class compositions of *Leonurus cardiaca* subsp. *persicus* essential oils. In particular, oxygenated monoterpenes were the most abundant compound group of the oil (48.86%), but the amount of sesquiterpene hydrocarbons was notable (17.58%). The most amount fraction is monoterpenoid one (57.01) (Table 4).

The qualitative and quantitative differences between the oils compositions of the populations of the current studies and the previous ones may be as a result of the collection time, chemotypes, drying conditions, mode of distillations, geographical and climatic factors.

CONCLUSION

Based on current study, it may be concluded that the aerial parts of *Leonurus cardiaca* subsp. *persicus* can be utilized for separation of the essential oil and a source of oxygenated monoterpenes (48.86%), and sesquiterpene hydrocarbons (17.58%).

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