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CHEMICAL COMPOSITION AND *IN VITRO* ANTIBACTERIAL ACTIVITIES OF THE OIL OF *Ziziphora clinopodioides* AND *Z. capitata* SUBSP. *capitata* FROM IRAN

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The genus *Ziziphora* is represented in the flora of Iran by four species [1]. In Iranian and Turkish folk medicine, *Ziziphora* species have been used as sedative, stomachic, and carminative [2].

The composition of the essential oils of *Z. pamiralaica, Z. denticulata, Z. Tenuior* [3], *Z. bungeana, Z. clinopodioides* [4], *Z. vychodceviana , Z. pedicelcata,* and *Z. persica* [5] grown under different ecological conditions in Russia was studied by gas chromatography. Pulegone and isomenthone were the major constituents of these oils. Also the antibacterial activity and composition of the essential oil of *Z. persica* in Turkey have been documented [6]. (+)-Pulegone (79.3%), limonene (6.78%), and piperitenone (4.2%) were the predominant compounds. The major constituent found in the oil of *Z. tenuior* L. has been reported to be pulegone (87.1%) [7]. The essential oil of Turkish endemic *Z. taurica* subsp. *clenioides* was found to contain pulegone (81.9%), limonene (4.5%), and piperitenone (2.3%) [8]. The antibacterial test results showed that the oil had antibacterial activity against a number of bacteria tested. In the previous study on the essential oil and antibacterial activity of *Z. clinopodioides* subsp. *rigida* (Boiss.) from Iran, pulegone (45.8%), piperitenone (17.4%), *p*-menth-3-en-8-ol (12.5%), and thymol (8.0%) were found as the main components [9].

The composition of the oils of *Z. clinopodioides* Lam. from two different locations and *Z. capitata* L. subcp. *capitata* are listed in Tables 1 and 2, respectively, in which the percentage and retention indices of components are given. Twenty components were identified in the oil of *Z. clinopodioides*, sample A, which represented about 100% of the total composition of the oil. Thymol (53.6%), *p*-cymene (10.5%), carvacrol (8.7%), γ-terpinene (6.7%), and 1,8-cineole (5.4%) were the major components of the oil of the plant.

Twenty- six components were identified in the oil of *Z. clinopodioides,* sample B, making up 96.2% of total composition. 1,8-Cineole (21.6%) and terpinen-4-ol (18.2%) were the major components in this oil followed by linalool (7.9%), pulegon (7.7%), and α-terpineol (5.3%).

Thus the oil of *Z. clinopodioides*, sample A, consists of eight oxygenated monoterpenes (72.1%), nine monoterpene hydrocarbons (24.5%), and three sesquiterpenes (3.4%), while sample B consists of 12 oxygenated monoterpenes (72.1%), 10 monoterpene hydrocarbons (14.1%), three sesquiterpenes (9.1%), and one aliphatic alcohol (0.9%).

As can be seen from the above, both samples are rich in oxygenated monoterpenes (72.1%). In the previous investigation, oxygenated monoterpenes (93.3%) were the predominant portion of the oil of *Z. clinopodioides* from another location in Iran, with pulegone (45.8%), piperitenone (17.4%), *p*-menth-3-en-8-ol (12.5%), and thymol (8.0%) as the main constituents [9].

In comparison with the present study, pulegone in sample A cannot be found and, in sample B, pulegone is present in low concentration. Also thymol, which is the main component in sample A, does not exist in sample B and is not even found in high amount in the previous study on the oil of *Z. clinopodioides* [9]. The oil obtained from *Z. clinopodioides* grown in Russia was also rich in oxygenated monoterpenes: pulegone (57.0%) and isomenthone (28.6%) [4].

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TABLE 1. Percentage Composition of Two Oils of *Ziziphora clinopodioides* and *Z. capitata*

Compound	RI	*Z. capitata*	Z. clinopodioides		Compound	RI	*Z. capitata*	Z. clinopodioides	
			A[a]	B[b]				A[a]	B[b]
α-Thujene	929		1.2	0.7	α-Terpineol	1179		0.3	5.3
α-Pinene	934	0.5	1.1	1.8	Pulegone	1230		-	7.7
Camphene	949		-	0.6	Bornyl acetate	1280		-	3.3
Sabinene	972		-	3.9	Thymol	1283		53.6	-
β-Pinene	978	0.7	0.7	2.5	Menthyl acetate	1288		-	0.1
Myrcene	989		1.9	0.6	Carvacrol	1292		8.7	-
3-Octanol	991		-	0.9	Geranyl formate	1296		-	1.1
α-Phellendrene	1001		0.3	-	α-Copaene	1366	0.6		
α-Terpinene	1012		2.0	-	β-Bourbonene	1375	2.0		
p-Cymene	1020		10.5	-	β-Cubebene	1382	0.5		
Limonene	1026	7.8			β-Elemene	1384	2.3		
1,8-Cineole	1027		5.4	21.6	β-Caryophyllene	1410	6.1		
(*Z*)-β-Ocimene	1037	15.4	-	1.1	α-Humulene	1447	1.1	2.7	4.5
(*E*)-β-Ocimene	1046	9.7	-	1.2	Germacrene D	1473	31.1	-	4.0
γ-Terpinene	1056	1.0	6.7	1.5	Bicyclogermacrene	1485	5.2	-	0.6
cis-Sabinene hydrate	1062		0.3	2.2	β-Bisabolene	1501		0.2	-
Terpinolene	1080		0.1	0.2	δ-Cadinene	1518	0.5		
Linalool	1098		1.8	7.9	Spathulenol	1565	2.5		
p-Menth-3-en-8-ol	1140		-	2.7	Caryophyllene oxide	1573	4.2	0.5	-
Menthone	1148		-	0.8	α-Cadinol	1648	1.7		
Borneol	1160		0.9	1.2	Hexadecanoic acid	1969	5.9		
Terpinen-4-ol	1172		1.1	18.2	Total		100	100	96.2

[a]Oil from Lorestan; [b]oil from Qom.

TABLE 2. Antibacterial Activity of the Essential Oils of *Z. clinopodioides* and *Z. capitata* Oils

Microorganisms	Gram +/-	*Z. clinopodioides*[a]		*Z. clinopodioides*[b]		Gentamicin
		IZ	MIC	IZ	MIC	
Staphylococcus aureus PTCC 1113	+	23.0	1.15	15	1.9	12
Staphylococcus epidermidis PTCC 1349	+	35.5	0.3	22	1.2	20
Staphylococcus saprophyticus PTCC 1379	+	22.5	1.2	17	1.1	15
Shigella flexneri PTCC 1234*	-	30.0	0.3	16	2.2	12
Salmonella typhi PTCC 1185	-	28.5	0.6	18	1.2	14
Escherichia coli PTCC 1330	-	35	0.3	21	1.0	15
Pseudomonas aeruginosa PTCC 1310	-	14.0	9.1	9	9.5	15

[a]Oil from Lorestan (Sample A); [b]Oil from Qom. (Sample B). IZ - inhibition zone (mm); MIC - minimum inhibitory concentration (mg/mL); **Z. capitata*: IZ - 14.5, MIC - 9.6.

Although we can see biochemical convergence among the *Z. clinopodioides* from different locations, due to the frequent occurrence of chemotypes in the family Lamiaceae and environmental factors, different patterns in the composition of the oils are common.

As is shown in Table 1, 19 components were identified in the oil of *Z. capitata,* which represented about 98.8% of the total composition of the oil.

Germacrene D (31.1%) and (*Z*)-β-ocimene (15.4%) were the major components in this oil, followed by (*E*)-β-ocimene (9.7%), limonene (7.8%), β-caryophyllene (6.1%), hexadecanoic acid (5.9%), and bicyclogermacrene (5.2%). Thus the oil of

Z. capitata consists of six monoterpene hydrocarbons (35.1%), nine sesquiterpene hydrocarbons (49.4%), three oxygenated sesquiterpenes (8.4%), and one aliphatic acid (5.9%). In the oil of *Z. capitata,* in contrast to *Z. clinopodioides* oils, sesquiterpenes (57.8%) predominated over monoterpenes (35.1%).

Antibacterial Activity. The results of the antibacterial screening of the oils (zones of growth inhibition and minimal inhibitory concentration) are shown in Table 2. The antibacterial assays showed that the oils of sample A and B from *Z. clinopodioides* inhibited the growth of all bacteria. Previous antibacterial study on *Z.clinopodioides,* with the main compounds of pulegone (nearly half of the total oil, 45.8%), piperitenone (17.4%), *p*-menth-3-en-8-ol (12.5%), and thymol (8.0%), has shown it to have it moderate activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteria compared to our results from sample A, where as, sample B, in which pulegone exists, show the same results for both Grampositive bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* as in previous study of *Z.clinopodioides.* As thymol has considerable antibacterial activity against Gram±bacteria, [9], the remarkable results of the antibacterial activity of *Z.clinopodioides,* sample A, in which thymol is the main constituent (53.6%), is explainable.

On the other hand, *Z. capatita* oil was insensitive against Gram-positive and Gram-negative bacteria except against *Shigella flexneri.* Gram-negative bacteria with moderate activity with MIC of value 9.6 mg/mL was observed. From our results obtained, it is clear that the activity of the above oils can be associated mainly with significant monoterpene compounds. In sample A, we can see that thymol is the main compound (about half of the total oil, 53.6%) while in sample B 1,8- cineole (21.6%) and terpinen-4-ol (18.2%), linalool (7.9%), and also pulegone in an amount of just 7.7% are found as the main compounds. It may be concluded that some compounds such as 1,8-cineole, terpinen-4-ol, and linalool in sample B contribute to the antibacterial activity of the oil beyond pulegone. In addition, both sample A and B are rich in monoterpenes, while the oil of *Z. capitata* is rich in sesquiterpenes, and we could not see any sensitivity against Gram- positive and Gram- negative bacteria except against *Shigella flexneri* bacteria.

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