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Professor Gerald Blunden
On the Occasion of his 72nd Birthday**

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Chemical Diversity of *Ziziphora clinopodioides*: Composition of the Essential oil of *Z. clinopodioides* from Tajikistan

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The chemical composition of the essential oils of *Ziziphora clinopodioides* Lam. from the aerial flowering parts, collected during two different years, were obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. Forty-five components representing 100% and 94.7% of the total oil were identified. The main constituents of the essential oils were pulegone (72.8 and 35.0%), neomenthol (6.5 and 23.1%), menthone (6.2 and 13.3%), *p*-menth-3-en-8-ol (1.7 and 3.5%), piperitenone (2.6 and 1.1%) and piperitone (0.7 and 1.2%). A cluster analysis was carried out on the essential oil compositions of *Z. clinopodioides*.

Keywords: *Ziziphora clinopodioides*, essential oil composition, pulegone, neomenthol, menthone, cluster analysis.

The genus *Ziziphora* belongs to the Lamiaceae and consists of about 30 species that are distributed in southern Siberia, central Asia and the Mediterranean [1]. *Z. clinopodioides* Lam. is an edible medicinal plant, which is widely distributed in Tajikistan. The leaves, flowers and stem of the plant are frequently used as a wild vegetable or as an additive to foods. The plant has been used since ancient times in traditional herbal medicines for the treatment of colds and cough [2]. *Z. clinopodioides* oil has been evaluated for insecticidal activity [3], antibacterial activity [4], antifungal activity [5], and antioxidant activity [6]. Previous studies on the chemical constitutions of *Z. clinopodioides* growing in Iran [4a,c,e,f,5a,6,7], Turkey [4d,8], Kazakhstan [9], Altai Republic (Russia) [10], and Urumqi (China) [11], have been reported. To our knowledge, this is the first report of the essential oil composition of *Z. clinopodioides* from Tajikistan.

The chemical compositions of the essential oils of *Ziziphora clinopodioides* from the aerial flowering parts were obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. A total of 49 compounds were identified in the two samples (Table 1). The major components in the 2008 sample were pulegone (72.8%), neomenthol (6.5%), and menthone (6.2%). The 2010 sample, on the other hand, contained less pulegone (35.0%), but increased concentrations of neomenthol (23.1%) and menthone (13.3%). In addition, the 2010 sample contained 2.4% of isoevernin aldehyde, which had previously been reported as a fungal metabolite [12].

In order to provide some insight into the chemotaxonomy of *Z. clinopodioides*, a cluster analysis was carried out on 24 different *Z. clinopodioides* essential oil samples reported in the literature as well as the current samples from Tajikistan (see Figure 1). The cluster analysis reveals at least 22 pulegone-rich samples, three 1,8-cineole-rich samples, and a thymol-rich sample. The pulegone-rich group can be further subdivided into 10 different chemotypes; a “pure” pulegone chemotype and a number of pulegone “mixed” chemotypes. The Tajikistan 2008 sample clearly belongs to a “pure” pulegone chemotype, along with samples from Russia (#4), Iran (#5, ssp. *bungeana*), and Kazakhstan. The Tajikistan 2010 sample, on the other hand, is a pulegone/neomenthol/menthone “mixed” chemotype.

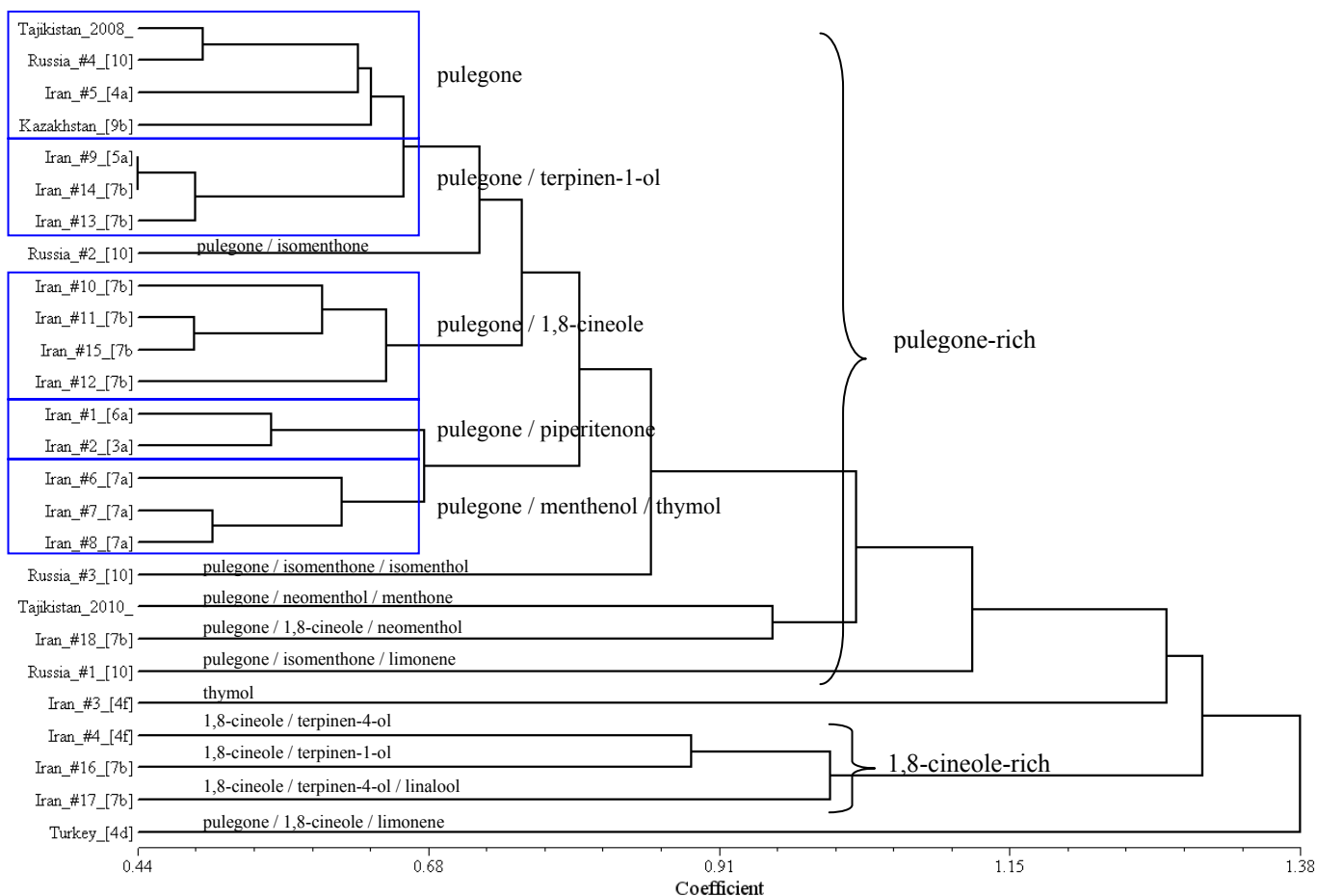
The major components in *Z. clinopodioides* (pulegone, 1,8-cineole, or thymol), regardless of the particular chemotype, account for the traditional uses of this plant to treat coughs and colds and also account for the observed bioactivities. Pulegone, 1,8-cineole, and thymol have exhibited antimicrobial [13] and insecticidal [14] activities.

Experimental

Plant Material: The aerial parts of *Z. clinopodioides* were collected during its flowering stage in July 2008 and 2010 from the Chormaghzak village, Yovon region of Tajikistan, (38.417502 N, 69.172175 E, 1300 m above sea level) and identified by F.S. Sharopov. A voucher specimen (TJ2010-041) has been deposited in the herbarium of the Chemistry Institute of the Tajikistan Academy of

Table 1: Composition of *Ziziphora clinopodioides* essential oil from Tajikistan.

RI	Compound	Composition		RI	Compound	Composition	
		2008	2010			2008	2010
941	α -Pinene	0.4	---	1227	Coahuilensol methyl ether	0.2	0.7
953	Camphene	0.1	---	1237	Pulegone	72.8	35.0
954	3-Methylcyclohexanone	---	0.2	1249	8-Hydroxymenthone	---	3.1
975	Sabinene	0.2	---	1254	Piperitone	0.7	1.2
978	β -Pinene	0.5	0.1	1263	Unidentified	---	1.9
981	1-Octen-3-ol	0.2	---	1266	8-Hydroxyisomenthone	---	0.5
992	Myrcene	0.5	0.2	1285	Isobornyl acetate	---	0.2
996	3-Octanol	0.3	0.3	1288	8-Hydroxy- <i>p</i> -menth-4-en-3-one	0.2	1.2
1004	α -Phellandrene	0.1	---	1292	Thymol	0.8	0.5
1024	<i>p</i> -Cymene	0.5	0.4	1294	Menthyl acetate	---	0.2
1028	Limonene	0.3	---	1301	Carvacrol	1.2	1.6
1030	1,8-Cineole	0.4	0.3	1303	Unidentified	---	1.1
1036	Santolina alcohol	0.1	---	1340	Piperitenone	2.6	1.1
1048	(<i>E</i>)- β -Ocimene	0.1	---	1349	Unidentified	---	0.6
1058	γ -Terpinene	0.2	---	1359	(<i>2E</i>)-Undecenal	---	0.3
1069	<i>p</i> -Mentha-3,8-diene	0.1	---	1366	Piperitenone oxide	0.1	---
1105	α -Thujone	0.1	---	1419	(<i>E</i>)-Caryophyllene	0.6	---
1116	β -Thujone	0.3	---	1466	(<i>2E</i>)-Dodecenal	0.3	0.2
1149	<i>p</i> -Menth-3-en-8-ol	1.7	3.6	1481	Germacrene D	0.1	---
1152	Menthone	6.2	13.3	1497	Mintlactone	---	1.8
1165	neo-Menthol	6.5	23.1	1529	Isomintlactone	---	0.3
1172	Menthol	---	0.4	1556	Unidentified	---	1.7
1175	<i>cis/trans</i> -Isopulegone	1.2	1.0	1583	Caryophyllene oxide	0.1	0.3
1188	Isomenthol	0.2	1.2	2316	Isosovermin aldehyde	---	2.4
1191	Neoisomenthol	0.3	---		Total identified	100	94.7

**Figure 1:** Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Ziziphora clinopodioides* samples, based on the correlation and using the unweighted pair-group method with arithmetic average (UPGMA).

Sciences. The air-dried samples were crushed and hydrodistilled for 3 h to give the essential oils, 0.7-0.8% yield.

Gas Chromatography – Mass Spectrometry: The essential oils of *Z. clinopodioides* were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was 200°C and interface temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C; increased 2°/min to 220°C. A 1 % w/v solution of the sample in CH₂Cl₂ was prepared and 1 µL was injected using a splitless injection technique.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [15] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system

(G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The essential oil compositions of *Z. clinopodioides* are summarized in Table 1.

Numerical Cluster Analysis: Twenty-six *Ziziphora clinopodioides* samples were treated as operational taxonomic units (OTUs). The percentage composition of the 28 major essential oil components [pulegone, 1,8-cineole, neomenthol, thymol, piperitenone, isomenthone, menthone, terpinen-1-ol, *p*-menth-3-en-8-ol, limonene, terpinen-4-ol, neomenthyl acetate, β-pinene, α-terpineol, isobornyl acetate, sabinene, *p*-cymene, isomenthol linalool, menthol, α-pinene, carvacrol, *p*-mentha-3,8-diene, piperitone, germacrene D, γ-terpinene, *cis*-sabinene hydrate, and *p*-mentha-1,3-dien-7-al] was used to determine the chemical relationship between the different *Z. clinopodioides* essential oil samples by cluster analysis using the NTSYSpc software, version 2.2 [16]. Correlation was selected as a measure of similarity, and the unweighted pair group method with arithmetic average (UPGMA) was used for cluster definition.

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