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Essential Oil Composition of *Mentha longifolia* from Wild Populations Growing in Tajikistan

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Keywords: Carvone, *cis*-piperitone epoxide, horse mint, menthone, piperitenone oxide, pulegone, thymol, wild mint.

ABSTRACT

Mentha longifolia selections, collected from three different sites in south-central Tajikistan, were analyzed to determine essential oil constituency. Essential oils were extracted by hydrodistillation of the plants and subsequently analyzed by gas chromatography – mass spectrometry. A total of 82 compounds were identified, representing 84.5-99.0% of total oil composition. Although qualitatively similar, the Tajikistan *M. longifolia* samples did show quantitative differences. The major components and their percentage of the oil were *cis*-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), pulegone (0.3-5.4%), menthone (0.0-16.6%), thymol (1.5-4.2%), β-thujone (0.2-3.2%), carvacrol (0.0-2.7%), and (*E*)-caryophyllene (0.9-2.5%).

INTRODUCTION

The mints, *Mentha* species belonging to the family Labiatae (Lamiaceae), are widely distributed in Eurasia, Australia, and South and North Africa (Gulluce *et al.*, 2007; Lange and Croteau, 1999). Various species of *Mentha* have been used as folk remedies for treatment of bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints, due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharral activities (Al-Bayati, 2009; Džamić *et al.*, 2010; Gulluce *et al.*, 2007; Hajlaoui *et al.*, 2010; Hussain, 2009; Mimica-Dukić *et al.*, 1991; Mkaddem *et al.*, 2009; Oyedeji and Afolayan, 2006; Rasooli and Rezaei, 2002; Viljoen *et al.*, 2006). The active virtues of the mints depend on the abundant volatile oils that contain a wide variety of terpenes and terpenoids.

The mint species, *Mentha longifolia* (L.) Huds., has been commonly used as a kitchen and medicinal plant for centuries. Known as wild mint and horse mint, the plant can reach to 1.5 m high in favorable conditions. The plant has a strong aroma.

The objectives of this study were to analyze the composition of the oil of *Mentha longifolia* growing wild in different areas of Tajikistan. In this report, the essential oil compositions of five samples collected at three sites in south-central Tajikistan. To our knowledge, no previous reports on *M. longifolia* essential oil in this area have been made.

MATERIALS AND METHODS

Plant material. Aerial parts of *M. longifolia* were collected from the three regions of Tajikistan. Samples numbered 1, 2, and 4 were gathered in the area of Korvon village, Dushanbe (38.506044N, 68.751535E, 800 m above sea level), on 25 April 2010. Sample number 3 was gathered in the area of Khonaobod village, Muminobod region (38.107547 N, 69.966431 E, 1200 m above sea level), on 7 May 2010, and sample number 5 was gathered in the Chormaghzak village area, Yovon region, (38.417502 N, 69.172175 E, 1300 m above sea level), on 25 July 2010. The plants were identified by F. S. Sharopov, and a voucher specimens (TJ2010-031) have been deposited in the herbarium of the Chemistry Institute of the Tajikistan Academy of Sciences.

From the collected plant samples, 300 g of each were air dried, crushed into smaller pices, and hydrodistilled for 3 h, producing yellow colored essential oils at a yield of 0.5-0.9%. The oils were dried over sulfuric acid and subsamples were taken for analysis of the oil constituents.

Essential oil analysis. The essential oils were analyzed using an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/s). A fused silica capillary column (HP-5 ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 µm phase thickness) was used in the gas chromatography, the carrier gas was helium with a flow rate of 1 mL/min, and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 min at 40°C before ramping to 200°C at 3°C/min, and then to 220°C at 2°C/min. The interface temperature was 280°C.

A 1% w/v solution of each oil was prepared in CH_2Cl_2 was prepared, and 1 µL of the samples was used in the analysis. The sample was injected into the gas chromatograph using a splitless injection. Identification of the oil components was based on their retention indices as determined by reference to a homologous series of *n*-alkanes (C_9-C_{30}), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007), 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 essential oil component are reported as raw percentages based on total ion current without standardization.

Numerical cluster analysis. For comparison of the essential oil constituency, the 47 samples of *Mentha longifolia* were treated as operational taxonomic units (OTUs). The percentage of the 26 major essential oil components (carvone, piperitenone oxide, menthofuran, menthone, pulegone, *cis*-piperitone epoxide, 1,8-cineole, *trans*-piperitone epoxide, *cis*-carveol, menthol, limonene, piperitone, (*E*)-caryophyllene, β-pinene, *trans*-dihydrocarvone, isomenthone, diosphenol, germacrene D, borneol, myrcene, α-pinene, piperitenone, rotundifolone, thymol, *cis*-dihydrocarvone, and menthyl acetate) were used to determine the chemical relationship between the different *M. longifolia* essential oil samples by cluster analysis using the NTSYSpc software, version 2.2 (Rohlf, 2005). Correlation was selected as a measure of similarity, and the unweighted pairgroup method with arithmetic average (UPGMA) was used for cluster definition.

RESULTS

A total of 82 compounds were identified in the essential oils extracted from *M. longifolia* plants collected in Tajikistan (Table 1). The identified oil compounds represented 84.5-99.0% of the total oil compositions. The major components of Tajikistan *M. longifolia* oil were *cis*-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), menthone (0.0-16.6%), thymol (1.5-4.2%), pulegone (0.3-5.4%), β-thujone (0.2-3.2%), (*E*)-caryophyllene (0.9-2.5%), myrcene (0.3-2.5%), carvacrol (0.0-2.7%), borneol (0.9-1.8%), and *p*-cymene (0.2-1.9%).

Although qualitatively similar, the Tajikistan *M. longifolia* oils showed notable quantitative differences. For example, *cis*-piperitone epoxide was relatively abundant in all samples, ranging from a low of 7.8% in sample #1 from Dushanbe to 77.6% in sample #5 from Yovon. Similarly, piperitenone oxide had the lowest concentration in the Yovon sample (1.5%), but highest in Dushanbe #1 (49.1%). Neither carvone nor menthone were detected in Yovon #5, but were both present in the other oil samples.

DISCUSSION

M. longifolia essential oils from other geographical locations have been extensively studied. The species has demonstrated a great degree of morphological diversity (Gobert *et al.*, 2002), and the Missouri Botanical Garden lists some 276 subspecies, varieties, and forms (Missouri Botanical Garden, 2011).

Table 1. Chemical composition of *Mentha longifolia* essential oils from Tajikistan.

RI	Oil constituent[1]	Sample number				
		1	2	3	4	5
		(% of total oil)				
854	(2*E*)-Hexenal	---	0.1	---	---	0.1
867	(2*E*)-Hexenol	---	0.1	---	---	---
907	Santolina triene	---	0.1	0.1	0.1	---
935	α-Thujene	---	tr[3]	0.1	---	tr
941	α-Pinene	0.1	0.2	0.1	0.2	0.4
953	Camphene	---	0.1	0.1	---	0.2
963	Benzaldehyde	---	tr	tr	---	---
976	Sabinene	---	0.1	0.1	0.3	0.3
978	β-Pinene	---	0.1	0.1	0.2	0.6
981	1-Octen-3-ol	---	tr	0.1	---	---
992	Myrcene	0.8	2.2	1.9	2.5	0.3
996	3-Octanol	0.2	0.6	0.5	0.7	0.9
1004	α-Phellandrene	0.2	0.3	0.2	0.3	0.1
1016	α-Terpinene	0.1	0.1	0.1	0.1	0.1
1024	*p*-Cymene	0.6	1.6	1.8	1.9	0.2
1028	Limonene	0.1	0.3	0.2	0.6	0.9
1030	1,8-Cineole	0.2	0.4	0.4	0.5	0.2
1036	Santolina alcohol	0.3	0.3	0.5	0.5	---
1038	(*Z*)-β-Ocimene	---	---	---	---	0.1
1043	Phenylacetaldehyde	---	tr	---	---	0.1
1048	(*E*)-β-Ocimene	---	tr	tr	---	---
1058	γ-Terpinene	0.3	0.4	0.4	0.6	0.2
1066	*cis*-Sabinene hydrate	---	---	0.1	---	---
1088	Terpinolene	---	---	---	---	0.1
1097	3-Nonanol	---	tr	---	---	0.1
1097	*trans*-Sabinene hydrate	---	---	0.1	---	---
1100	Linalool	0.1	0.1	0.1	0.1	0.4
1105	α-Thujone	0.5	0.8	1.1	0.8	0.3
1116	β-Thujone	1.5	2.6	3.2	2.5	0.2
1121	*cis*-*p*-Menth-2-en-1-ol	---	tr	0.1	---	---
1125	3-Octyl acetate	---	0.2	0.1	0.1	---
1144	Camphor	---	0.1	0.1	---	0.2
1152	Menthone	2.8	16.6	4.1	2.1	---
1161	Pinocarvone	---	---	---	---	0.1
1166	Borneol	1.6	1.8	1.0	0.9	1.4
1171	Menthol	0.2	0.1	0.1	---	---
1175	*cis/trans*-Isopulegone	---	0.4	0.4	0.7	---
1176	Terpinen-4-ol	0.2	---	---	---	0.2
1184	*p*-Cymen-8-ol	0.3	0.1	0.4	0.5	---
1189	α-Terpineol	0.3	0.2	0.2	0.2	0.2
1195	Myrtenal	0.1	0.1	---	---	0.1
1195	*cis*-Dihydrocarvone	---	---	0.3	0.6	---
1220	Coahuilensol methyl ether	0.3	0.1	0.2	0.2	0.1
1224	Citronellol	0.5	---	---	---	---
1226	Unidentified	---	0.1	0.2	0.5	1.4
1229	Thymol methyl ether	0.1	---	---	---	---
1237	Pulegone	1.6	1.6	2.6	5.4	0.3
1243	Carvone	0.8	1.1	10.2	21.5	---
1254	*cis*-Piperitone epoxide	7.8	27.1	25.0	23.1	77.6
1262	*cis*-Chrysanthenyl acetate	---	---	---	0.5	---
1262	Unidentified	0.7	---	---	---	---
1278	*trans*-Carvone oxide	---	---	0.1	---	---
1286	Bornyl acetate	0.1	0.2	0.2	---	0.4
1287	Dihydroedulan I	0.1	---	---	0.2	---
1292	Isothymol	0.4	---	0.3	---	---
1293	Thymol	4.2	1.5	3.1	3.5	3.0
1298	Diosphenol	---	---	---	0.1	0.7
1302	Unidentified	0.6	---	---	---	---
1303	6-Hydroxy-6-isopropyl-3-methylcyclohex-2-enone	---	0.7	0.9	---	3.2
1305	Carvacrol	2.7	0.8	2.5	1.7	---
1340	Piperitenone	0.6	0.3	0.3	0.4	---
1368	Piperitenone oxide	49.1	29.4	28.2	20.4	1.5
1386	β-Bourbonene	---	0.1	0.2	0.2	---
1388	Unidentified	1.3	---	---	---	---
1392	4a-α,7-β,7a-α-Nepetalactone	---	0.1	0.2	0.1	---
1392	Unidentified	0.7	---	---	---	---
1394	β-Elemene	---	0.1	---	---	---
1400	(*Z*)-Jasmone	---	0.1	0.1	---	---
1419	(*E*)-Caryophyllene	1.4	2.1	1.7	2.5	0.9
1436	α-*trans*-Bergamotene	---	0.1	---	---	---
1441	Unidentified	6.4	0.3	---	---	---
1454	α-Humulene	0.2	0.2	0.2	0.2	
1455	Unidentified	0.1	---	---	0.2	1.5
1458	(*E*)-β-Farnesene	0.1	0.1	0.2	0.2	---
1462	Unidentified	0.5	---	---	---	---
1475	Unidentified	0.8	0.2	0.1	0.2	---
1482	Germacrene D	0.3	0.6	0.3	0.7	0.1
1487	(*E*)-β-Ionone	---	---	0.1	---	---
1497	Bicyclogermacrene	---	0.1	---	---	---
1509	β-Bisabolene	0.3	0.3	0.5	0.1	---
1524	δ-Cadinene	---	---	0.1	---	---
1565	(*E*)-Nerolidol	0.1	0.1	---	---	---
1566	Unidentified	---	---	0.6	---	---
1578	Spathulenol	0.9	0.5	0.5	0.3	---
1584	Caryophyllene oxide	1.6	0.9	1.4	0.7	---
1610	Unidentified	---	0.3	0.9	---	---
1635	Isospathulenol	0.2	0.1	0.1	---	---
1642	(2*S*,5*E*)-Caryophyll-5-en-12-al	0.2	---	---	---	---
1654	α-Cadinol	0.1	---	---	---	---
1669	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	0.2	---	---	---	---
1685	Germacra-4(15),5,10(14)-trien-1-α-ol	0.1	---	---	---	---
1758	14-Hydroxy-α-Muurolene	0.1	---	---	---	---
1991	Manool oxide	0.1	---	---	---	---
	Total constituents identified	84.5	98.2	97.0	99.0	95.6

[1]Size of text in RI 1303 = 6-Hydroxy-6-isopropyl-3-methylcyclohex-2-enone, 1392 = 4a-α,7-β,7a-α-Nepetalactone, 1642 = (2*S*,5*E*)-Caryophyll-5-en-12-al, 1669 = 14-Hydroxy-9-*epi*-(*E*)-caryophyllene, and 1685 = Germacra-4(15),5,10(14)-trien-1-α-ol was reduced to fit table. Constituent identification based on RI & MS matching using Adams (2007) and NIST database. tr = trace (<0.01%).

With the extent of morphological diversity in *M. longifolia*, a great degree of chemical variation in the species might be expected as well. Indeed, results from previous studies on several wild and cultivated *M. longifolia* have produced a number of chemotypes (Table 2). Identified chemotypes of *M. longifolia* include those dominated by piperitenone oxide (Baser *et al.*, 1999; Gulluce *et al.*, 2007; Hussain, 2009; Maffei, 1988; Mastelic and Jerkovic, 2002; Rezaei *et al.*, 2000; Sharipova *et al.*, 1983; Venskutonis, 1996; Viljoen *et al.*, 2006), piperitone epoxide (Baser *et al.*, 1999; Fleisher and Fleisher, 1998; Fraisse *et al.*, 1985; Hussain, 2009; Karousou *et al.*, 1998; Kokkini and Papageorgiou, 1988; Vidal *et al.*, 1985; Viljoen *et al.*, 2006), carvone (Banthorpe *et al.*, 1980; Fraisse *et al.*, 1985; Kokkini *et al.*, 1995; Lawrence, 1978; Lawrence, 2007; Mastelic and Jerkovic, 2002; Monfared *et al.*, 2002; Vidal *et al.*, 1985; Younis and Beshir, 2004), menthone (Fraisse *et al.*, 1985; Hajlaoui *et al.*, 2010; Mimica-Dukić *et al.*, 2003; Oyedeji and Afolayan, 2006; Vidal *et al.*, 1985), pulegone (Fleisher and Fleisher, 1991; Gulluce *et al.*, 2007; Hajlaoui *et al.*, 2010; Mkaddem *et al.*, 2009; Oyedeji and Afolayan, 2006), piperitone (Džamić *et al.*, 2010; Ghoulami *et al.*, 2000; Rasooli and Rezaei, 2002; Rezaei *et al.*, 2000), *trans*-dihydrocarvone (Džamić *et al.*, 2010; Matovic and Lavadinovic, 1999; Mimica-Dukić *et al.*, 1991), isomenthone (Mimica-Dukić *et al.*, 1991; Mimica-Dukić *et al.*, 2003; Mkaddem *et al.*, 2009), menthofuran (Mimica-Dukić *et al.*, 1991; Viljoen *et al.*, 2006), menthol (Al-Bayati, 2009; Hajlaoui *et al.*, 2010), 1,8-cineole (Fleisher and Fleisher, 1998; Oyedeji and Afolayan, 2006), isopiperitenone (Rezaei *et al.*, 2000), piperitenone (Ghoulami *et al.*, 2000), and borneol (Hussain, 2009).

Comparing the results obtained for our *M. longifolia* plant samples with those reported for the same species from other locations of the world reveals profound differences in essential oil composition. A cluster analysis (Figure 1) illustrated the numerous different chemotypes of *M. longifolia* and showed that the essential oil of Tajikistan samples labeled #1, #2, #4 (Dushanbe region), and #3 (Muminobod region) form a cluster (rich in piperitenone oxide and *cis*-piperitone epoxide) distinct from other *M. longifolia* samples. The essential oil of Tajikistan sample #5 (Yovon region), dominated by *cis*-piperitone epoxide, is separate from the cluster formed by the other samples from Tajikistan.

Kokkini and co-workers (Karousou *et al.*, 1998; Kokkini *et al.*, 1995) observed analogous chemical differences between samples from western Crete compared with those from the eastern end of the island. Other notable clusters in this analysis include a piperitenone oxide cluster (India and S. Africa #9), a *trans*-piperitone epoxide cluster (Crete #5 and #6), a menthone/menthol cluster (Tunisia samples #4, #5, #6, and #7), a *cis*-carveol cluster (Iran samples #3, #4, and #5), a menthofuran cluster (S. African samples #5, #6, #7, #10, #11, and #12), a carvone cluster (Iran #1, Sudan, Crete #1, Crete #2, and Greece #1), a menthone/pulegone/1,8-cineole cluster (S. Africa #1, #2, and #3) as well as a pulegone/menthone/1,8-cineole cluster (Tunisia #2 and #3).

Viljoen and co-workers (2006) had reported the clustering of their South African samples (#5, #6, #7, #10, #11, and #12), distinct from two other samples in their study (#8 and #9), and these are all chemically distinct from other samples from South Africa reported by Asekun and co-workers (2007). Seasonal variation in essential oil does occur (Hussain, et al., 2010) and geographical location and environmental factors (climate/weather, soil/nutrition, herbivory/disease) undoubtedly play a large role in the morphological and chemical differentiation of *Mentha longifolia*.

Table 2. Main constituents in Mentha longifolia samples collected at various locations.

Country	Main essential oil constituents	Reference
Crete	Carvone (56-66%), 1,8-cineole (2-13%), limonene (3-11%), trans-dihydrocarvone (1-33%)	(Kokkini *et al.*, 1995)
Croatia	Carvone, piperitenone oxide, limonene and β-caryophyllene	(Mastelic and Jerkovic, 2002)
France	Chemotype I: Menthone (60%), pulegone (10%) and 1,8-cineole (9%); Chemotype II: Piperitone oxide isomer (60%), piperitenone oxide (15%), α-muurolol (6%) and 1,8-cineole (3%); Chemotype III: Carvone (57%), 1,8-cineole (13%) and limonene (7%)	(Fraisse *et al.*, 1985; Vidal *et al.*, 1985)
Greece	Piperitone oxide	(Kokkini and Papageorgiou, 1988)
Greece	Chemotype 1: Carvone (55%), limonene (20%) Chemotype 2: cis-Piperitone epoxide (33%), 1,8-cineole (25%), trans-piperitone epoxide (17%)	(Koliopoulos *et al.*, 2010)
India	Piperitenone oxide (54%), *trans*-piperitone epoxide (20%)	(Singh *et al.*, 2008)
Israel	1,8-cineole (29%), *cis*-piperitone oxide (15%) and piperitone (14%)	(Fleisher and Fleisher, 1998)
Iran	Piperitone (68%), 1,8-cineole (12%)	(Jaimand and Rezaei, 2002)
Iran	Carvone (62%), limonene (19%)	(Monfared *et al.*, 2002)
Iran	Piperitone (44%), limonene (14%) and *trans*-piperitol (13%)	(Rasooli and Rezaei, 2002)
Iran	Isopiperitenone (12-58%), piperitenone oxide (20-34%), piperitone (8-44%)	(Rezaei *et al.*, 2000)
Iraq	(-) Menthol	(Al-Bayati, 2009)
Italy	Piperitenone oxide (77%)	(Maffei, 1988)
Jordan	Pulegone (70%)	(Fleisher and Fleisher, 1991)
Kazakhstan	Piperitenone oxide (52%)	(Sharipova *et al.*, 1983)
Lithuania	Piperitenone oxide (44-57%), 1, 8-cineole (8-15%), myrcene (6-10%)	(Venskutonis, 1996)
Morocco	Piperitenone and piperitone	(Ghoulami *et al.*, 2000)
Netherlands	Carvone (66%)	(Lawrence, 1978)
Pakistan	Piperitenone oxide (40-65%), piperitone (2-16%), and borneol (2-13%)	(Hussain, 2009)
Serbia	*trans*-Dihydrocarvone (24%), piperitone (17%), *cis*-dihydrocarvone (16%)	(Džamić *et al.*, 2010)
Serbia	*trans*-Dihydrocarvone (16%-31%)	(Matovic and Lavadinovic, 1999)
Serbia	Chemotype A: *trans*-Dihydrocarvone (18%), isomenthone (12%), piperitone (8%) Chemotype B: Isomenthone (42%), methone (12%) Chemotype C: Menthofuran (38%), 1,8-cineole (10%), (*E*)-caryophyllene (11%)	(Mimica-Dukić *et al.*, 1991)
Serbia	Menthone and isomenthone	(Mimica-Dukić *et al.*, 2003)
South Africa	Menthone (31-48%), pulegone (18-35%), 1,8-cineole (13-17%)	(Asekun *et al.*, 2007)
South Africa	Menthone (51%), pulegone (19%),1,8-cineole (12%)	(Oyedeji and Afaloayan, 2006)
South Africa	Menthofuran (51-62%), *cis*-piperitone oxide (15-36%), piperitenone oxide (15-66%)	(Viljoen *et al.*, 2006)
Sudan	Carvone (77%)	(Banthorpe *et al.*, 1980)
Sudan	Carvone (67%), limonene (14%)	(Younis and Beshir, 2004)
Tunisia	Pulegone (54%), isomenthone (12%)	(Mkaddem *et al.*, 2009)
Tunisia	Menthol (33%), menthone (21%), pulegone (18%)	(Hajlaoui *et al.*, 2010)
Turkey	Piperitone oxide (65%), piperitenone oxide (12%),	(Baser *et al.*, 1999)
Turkey	*cis*-Piperitone epoxide (18%), pulegone (16%), piperitenone oxide (15%)	(Gulluce *et al.*, 2007)

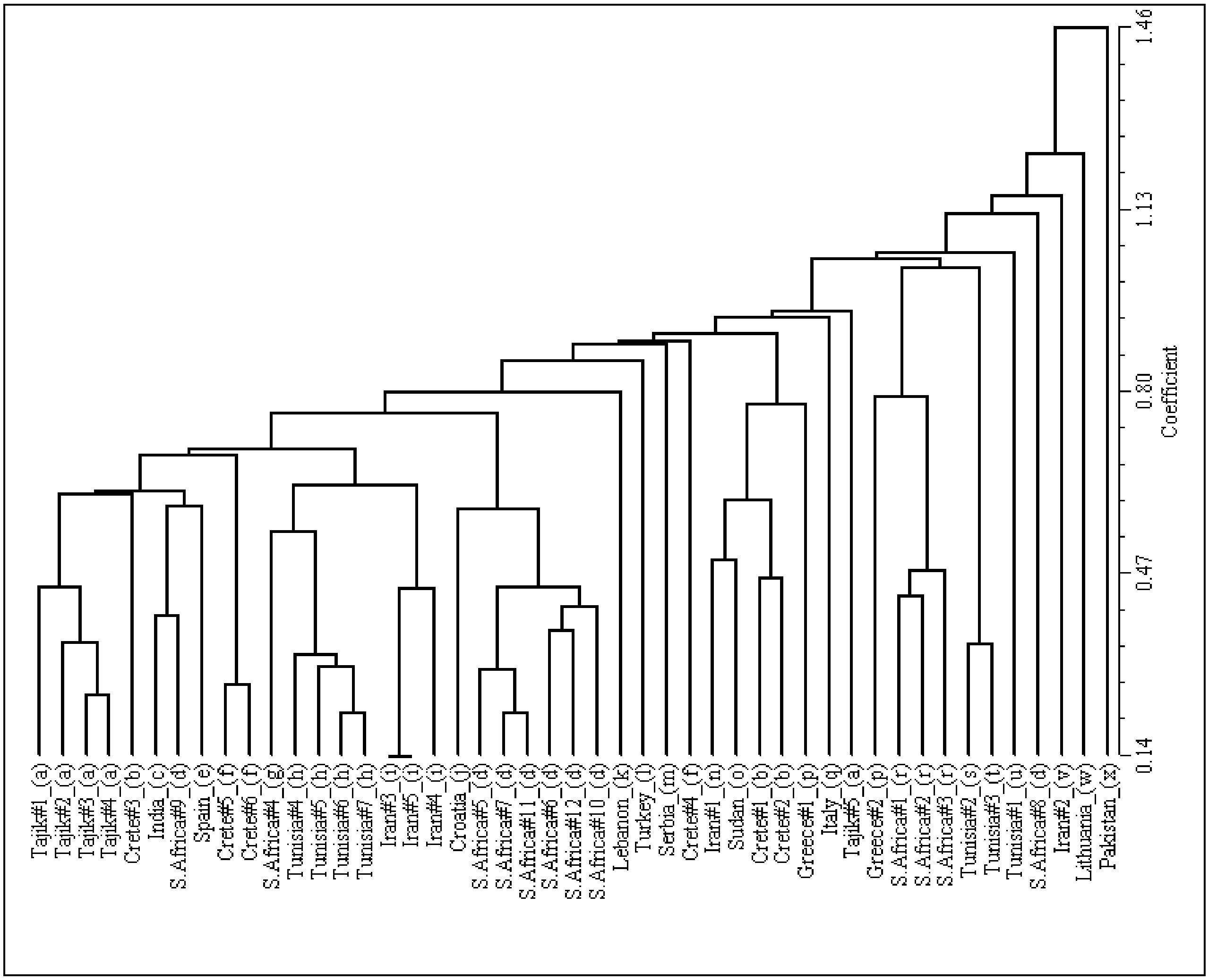

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Mentha longifolia* samples. Results are based on correlation and use of the unweighted pair-group method with arithmetic average (UPGMA). Data from: (a) current study; (b) Kokkini *et al.*, 1995; (c) Singh *et al.*, 2008; (d) Petkar, 2006; (e) Pérez Raya *et al.*, 1990; (f) Karousou *et al.*, 1998; (g) Oyedeji and Afolayan, 2006; (h) Hajlaoui *et al.*, 2008; (i) Zeinali *et al.*, 2005; (j) Mastelic and Jerkovic, 2002; (k) Hilan *et al.*, 2006; (l) Gulluce *et al.*, 2007; (m) Džamić *et al.*, 2010; (n) Monfared *et al.*, 2002; (o) Younis and Beshir, 2004; (p) Koliopoulos *et al.*, 2010; (q) Maffei, 1988; (r) Asekun *et al.*, 2007; (s) Snoussi *et al.*, 2008; (t) Hajlaoui *et al.*, 2009; (u) Mkaddem *et al.*, 2009; (v) Jaimand and Rezaei, 2002; (w) Venskutonis, 1996; (x) Hussain, 2009.

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