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Individual sampling approach to study the chemodiversity of volatile and semivolatile compounds of *Mentha longifolia* L. growing wild in Jiloca basin, Spain

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Abstract

Volatile and semivolatile composition of 47 individuals of *Mentha longifolia* L. collected from five locations in Jiloca basin (Teruel, Spain) was investigated to give insight into sources of chemical variability. It was determined by Simultaneous Distillation Extraction using a Likens-Nickerson apparatus and subsequent analysis by GC/MS and GC/FID. The statistical analysis of results allowed to identify four chemotypes: (a) pulegone (63.94 %) + isomenthone (16.67 %); (b) piperitone oxide (15.53 %) + piperitenone oxide (50.32 %); (c) α-terpineol acetate (39.81 %) + carvone acetate (33.84 %) and (d): (*Z*)-sabinenhydrate (41.15 %) + 1-terpinen-4-ol (13.99 %). The last two chemotypes have never been reported so far. As the sampling locations were selected with similar environmental conditions, the co-occurrence of different chemotypes in the same population turned out to be very noticeable. These results support the advisability of individual sampling instead of mixing material from randomly selected individuals, in order to identify and make available selected chemotypes for their propagation. It may be particularly useful for applied purposes based on relationships between chemical composition and biological activity.

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Introduction

Aims and justification *Mentha longifolia* L. (Lamiaceae), also known in Spain as "wild mint" or "mentastro", is a perennial plant growing in wet places, close to water streams. Besides its traditional applications, mainly against respiratory and digestive diseases and for culinary purposes, recent researches are focused on the antimicrobial and antioxidant activity of its essential oil related with its use in food preservation technology (Dudai *et al.*, 2005; Viljoen *et al.*, 2006; Gulluce *et al.*, 2007; Hussain *et al.*, 2008, 2009; Hajlaoui *et al.*, 2009; Mkaddem *et al.*, 2009; Dzamic *et al.*, 2010; Hafedh*et al.*, 2010; Ahmad *et al.*, 2011; Niksic *et al.*, 2012; Iqbal *et al.*, 2013; Pajohi *et al.*, 2013; Stanisavljevic *et al.*, 2014). Moreover, its main components are oxygenated monoterpenes, which are characterized by their allelopathic potentiality (Duke, 2003; Kordali *et al.*, 2007; Young and Bush, 2009), so that *M. longifolia* may also be a promising source of new and useful bioactive compounds.

Given that this plant has asexual reproduction via rhizome, the propagated plants keep the essential oil composition and wild individuals belonging to particularly useful chemotypes can be horticulturally conserved (Segev *et al.*, 2012). This way, sampling and analytical methods based on processing individual plants can allow having chemically homogeneous crops with particularly useful biological activities. This way, since the genetic sources of chemical variability are controlled, this work methodology can be also useful for conducting more reliable researches concerning the influence of environmental factors on essential oil chemical profiles.

So, the key motivation of this study is to emphasize the advisability for different purposes of individual sampling instead of methods based on gathering and mixing of leaves (or other plant materials) from randomly selected individuals in a population. Likewise, this work is also aimed to extend the knowledge of *M. longifolia* chemodiversity with data coming from Spain. For this purpose, individual plants belonging to five close populations with similar environmental conditions were analyzed. Their chemical profiles were subsequently defined and statistically compared with those coming from the literature.

In order to justify the above mentioned aims, after summarizing the reported data about essential oil chemodiversity in *M. longifolia*, some results of previous studies conducted in the same populations will be described to support the proposal of individual sampling.

Chemodiversity in M. longifolia essential oil

Regarding the volatile and semivolatile chemical composition referred to the essential oil, it has been extensively studied from many geographical origins (Lawrence, 2006; Sharopov *et al.*, 2013). These data show a great chemodiversity due to the two main sources of intraspecific variability: genotypic diversity and phenotypic plasticity. According to Viljoen *et al.* (2006), this chemodiversity cannot be related to geographical origin but, as pointed out by Lawrence (2006), can be explained by the characteristic high level of genetic polymorphism and tendency to hybridization of genus *Mentha*.

From the reported data in the above mentioned references and other more recent researches (Saeidi *et al.*, 2012; Segev *et al.*, 2012; Jamzad *et al.*, 2013; Moradalizadeh *et al.*, 2014) two main chemical profiles can be distinguished according to the oxygenated carbon (C2 or C3) in the menthane skeleton: (1) Menthane skeleton oxygenated in C2: Samples containing carvone and dihydrocarvone (*cis* and *trans* isomers) as major compounds have been reported in some studies (Mastelic *et al.*, 2002; Dzamic *et al.*, 2010; Bertoli *et al.*, 2011). (2) Menthane skeleton oxygenated in C3: Two typical profiles related to alternative metabolic pathways involving the double bond C4-C8 reduction can be distinguished: (a) piperitenone, piperitone and their epoxides (Saeidi *et al.*, 2012; Segev *et al.*, 2012; Jamzad *et al.*, 2013; Moradalizadeh *et al.*, 2014) and (b) pulegone, menthone or isomenthone,

menthofuran and other menthol derivatives as well (Hajlaoui *et al*, 2008; Mkaddem *et al*., 2009; Segev *et al*., 2012).

Furthermore, samples whose major components show other chemical structures have been also reported. Chemical profiles in which the main compound has menthane skeleton oxygenated in C8 (1,8-cineole) have been usually found together with the above two molecular structures as relative major component (Koliopoulos *et al*., 2010; Moradalizadeh *et al*.,2014) and it has been even reported as main compound by Fleisher and Fleisher (1998). It is closely related to α -terpineol as it derives from it by means of an ether bond between C1 and C8. On the other hand, α -terpinyl acetate was reported by Baser *et al*. (1999) as major component in a sample from Turkey.

With regard to menthane skeleton oxygenated in C4, terpinen-4-ol has been mentioned as major component in one sample from Turkey (Baser *et al*., 1999). Other profiles based on sabinene or bornane skeleton were also found in samples from Crete (Kokkini and Papageorgiou, 1988), Turkey (Baser *et al*., 1999) and Pakistan (Hussain *et al*., 2010), respectively. Likewise linalool rich samples (acyclic structure) have been also reported by Baser *et al*. (1999) from Turkey.

Likewise, despite oxygenated monoterpenes are generally the major compounds and they are considered for chemotype definition purposes, samples showing high amounts of sesquiterpenoids (α -caryophyllene and its oxide) have also been reported by Baser *et al*. (1999) in *longifolia* and *typhoides* subspecies.

Very scarce data are available concerning Spanish populations (Raya *et al*., 1990; Llorens-Molina *et al*., 2012) although *M. longifolia* is a widespread species in hygronitrophilous plant communities in Iberian Peninsula (Liendo *et al*., 2013).

Previous studies supporting the advisability of individual sampling

A previous study was conducted in the same populations involved in this work (Llorens-Molina *et al*., 2012) with samples coming from gathered and mixed individuals in order to analyze organ and diurnal variations. High intrapopulational variability affecting the main compounds was observed. It could be interpreted as consequence of the hypothetical co-occurrence of individuals belonging to different chemotypes in such a way that significant individual differences might have been hidden. This fact may be more probable when the concerned chemotype is defined by compounds belonging to different metabolic pathways. For example, samples rich in piperitone and piperitenone oxides have more probability to represent a well defined chemotype than those showing equilibrated amounts of (*Z*)-piperitone oxide, pulegone and piperitenone oxide (Gulluce *et al*., 2007). In the first case, both compounds are closely related from the metabolic point of view. In the 2nd case, the occurrence of pulegone as major compound leads to consider that the samples may come from individuals belonging both to the piperitone and piperitenone oxides and pulegone chemotypes. Thus, the individual sampling may be a more reliable way in order to improve the reliability of chemotypes identification.

Moreover, seasonal variations and environmental factors may affect each chemotype in a different way, and therefore its previous identification should be the starting point for this type of studies. Llorens-Molina *et al*. (2014) provided evidence for this possible specificity regarding diurnal variations in *M. longifolia* individuals belonging to two well-defined chemical profiles. In this study, appreciable diurnal changes were found in individuals rich in pulegone + isomenthone, whereas the composition of plants showing α-terpineol acetate + carvone acetate as major compounds remained almost constant. This way, if the influence of environmental factors affecting each chemotype is known, a higher control of essential oil quality can be achieved.

Material and methods

Plant material

The whole aerial parts of 47 individuals during their full blooming stage (August 2013) were collected during the sunniest time of the day from 5 populations located in streams and irrigation channel banks belonging to High Jiloca basin (Teruel, Spain) (Fig.1). These locations were selected in such a way that all of them had similar environmental features: altitude, orientation and habitat. In addition, no appreciable variations in climate conditions could be considered because their closeness. The collected individuals were more than 10 m apart, to avoid sampling from the same parent. The coordinates of sampling locations (Fig.1) were the following: (1) 40º 53' 50"N, 1º 18' 1" W; (2) 40º 55' 12" N, 1º 14' 25" W; (3) 40º 55' 58" N, 1º 19' 21" W; (4) 40º 56' 10" N, 1º 18' 6" W; (5) 40º 58' 12" N, 1º 18' 49" W.

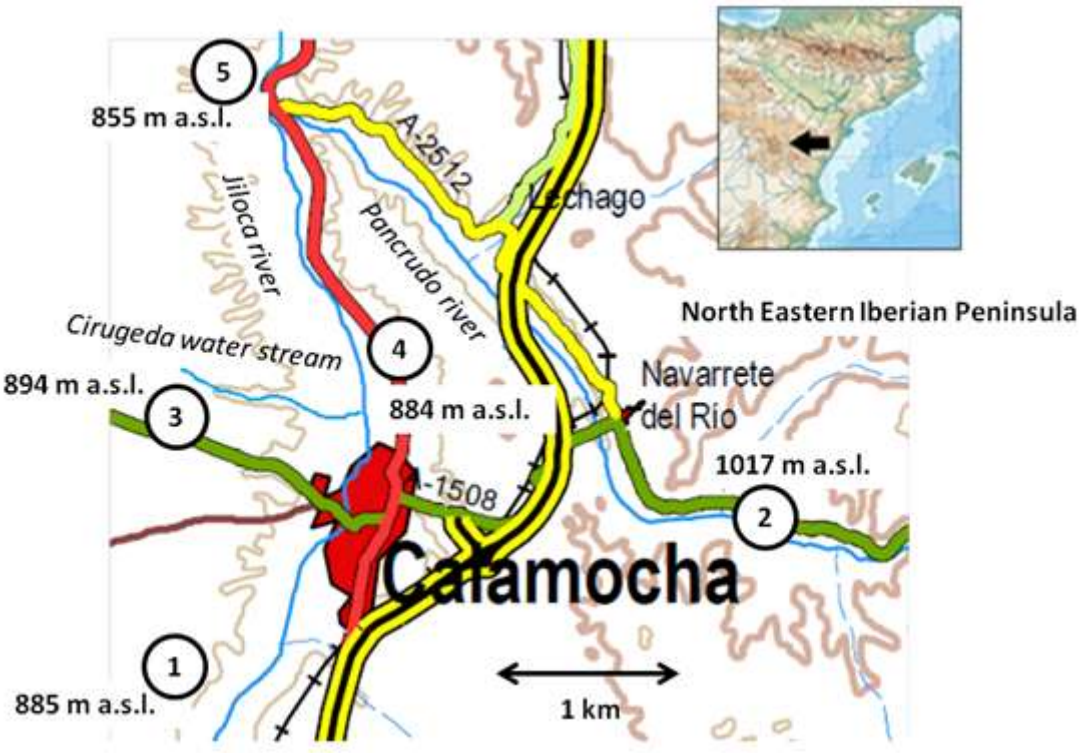

Fig. 1. Sampling locations.

After removing the woody parts from leaves, young branches and inflorescences, the fresh material (10-20 g) belonging to each individual was separately stored at -40 ºC until extraction for volatile and semivolatile fraction analysis. Voucher specimens from each population were deposited at the Herbarium of Mediterranean Agroforestry Institute, (Universitat Politècnica de València, Spain) VALA 9530, 9531, 9534, 9535, 9536.

Volatile and semivolatile compounds extraction

Simultaneous distillation-extraction (SDE) was performed for 2 h using a Likens and Nickerson apparatus with dichloromethane as solvent (Chaintreau, 2001). The extracts were dried with anhydrous sodium sulphate and concentrated up to 2 mL by N_2 stream.

GC analysis

Analysis of the SDE extracts was carried out using gas chromatography coupled to flame ionization detector (GC-FID) and mass spectrometry (GC-MS). A Clarus 500 GC (PERKIN ELMER INC., WELLESLEY, MA, USA) chromatograph equipped with a FID detector and a ZB-5 capillary column (30 m x 0.25 mm i.d. x 0.25 µm; PHENOMENEX INC., TORRANCE, CA, USA) was used for quantitative analyses (injection volume 1 µL). The GC oven temperature program was from 50 ºC to 250 ºC at a rate of 3 ºC min^{-1}. Helium was used as the carrier gas (1.2 mL min^{-1}), and the

injector and detector temperatures were set at 250 ºC. The percentage composition of the essential oil was computed from the GC peak areas (without correction factors) using Total Chrom 6.2 (PERKIN ELMER INC.) software.

Analysis by GC-MS was performed using a Clarus 500 GC-MS (PERKIN ELMER INC.) with the same capillary column, mobile phase and operating conditions as described above for the GC-FID analysis. The ionization source temperature was 200 ºC, and a 70 eV electron impact mode was used. MS spectra were obtained using the total ion scan (TIC) mode (mass range m/z 45-500 uma). The total ion chromatograms and mass spectra were processed using Turbomass 5.4 (PERKIN ELMER INC.) software.

The extracted components were identified based on their Kovats retention indexes relative to C7-C30 Saturated Alkanes (SIGMA-ALDRICH®), computer matching with the NIST MS Search 2.0 library, and comparison of their mass spectra with those of previously published data. Identification of some components was also confirmed by comparing their experimental retention times with those from co-injection of authentic standards (SIGMA-ALDRICH®): α-pinene, α-pinene, camphene, myrcene, camphor, terpinolene, borneol, terpinen-4-ol, and linalool. The percentage composition of the identified compounds was obtained from the GC peak areas without using response factors.

Statistical analysis

Cluster analysis was used to classify and group all the individuals according to their main components. Cluster analysis based on main components (Table 1) was calculated using the Euclidean distance measure. The development and data processing were performed using Statgraphics Centurion XVI (2011). Major chemical compounds from uniform chemical groups were submitted to analysis of variance. Percentage values were previously arcsin transformed. The means were compared by Fisher's least significant difference (LSD) test ($P<0.05$).

Results and discussion

The application of cluster analysis techniques to the results allowed us to establish four different chemotypes (Fig. 2). Mean values [percentage ± std. error] corresponding to the composition of each chemotype are listed in Table 1. The defined chemotypes are the following:

Chemotype I (α-terpineol acetate + carvone acetate)

Oxygenated monoterpenes compounds were the main fraction in this chemotype (18 individuals), accounting for 82.22% and characterized by high α-terpineol acetate and carvone acetate contents (39.81 % and 33.84 %, respectively). The amount of these compounds in this chemotype was significantly higher ($P \leq 0.05$; Table 1) than other chemotypes. The presence of pulegone, defining other chemotype, was also noticeable (6.03 %).

Carvone acetate has not been previously reported as major component in *M. longifolia*. It was identified in *Mentha haplocalyx* Briq. (De-sheng and Han-dong, 1983) emphasizing its insect repellent activity.

Chemotype II (piperitone oxide + piperitenone oxide)

Oxygenated monoterpenes were again the main fraction in this chemotype, comprised by 16 individuals. The major component in this chemotype was piperitenone oxide (50.32%), followed by piperitone oxide (15.53%), with significant differences with the other chemotypes, and to a lesser extent pulegone (7.12%) and α-terpineol acetate (5.70%) compounds.

Chemotype III (pulegone + isomenthone)

This chemotype was characterized by the high content of pulegone ranging from 50.06 to 84.90% in the 9 individuals belonging to it with a mean of 63.94%. Isomenthone, accounting for 16.67% of the essential oil, was the second compound quantitatively. Other compounds as α -terpineol acetate and carvone acetate represented less than 4.00%.

With regard to the major components in the last two chemotypes, they are characterized by its insecticidal

activity. As reported by Tripathi *et al.* (2004), piperitenone oxide shows strong toxicity against the malarial vector *Anopheles stephensi*. The same way, pulegone and mint oils containing it were found highly active against *Drosophila melanogaster* (Franzios *et al.*, 1997).

Chemotype IV ((Z)-sabinenhydrate + 1-terpinen-4-ol)

The fourth chemotype showed a quite different composition with respect to the other ones. It was comprised by 4 individuals. Monoterpene alcohols were the main fraction, with (*Z*)-sabinenhydrate as the major component (41.15%), followed by terpinen-4-ol and α-terpinene (13.99 and 6.04 %, respectively). The same way as above mentioned, some compounds defining other chemotypes accounted for no negligible amounts: piperitenone oxide (8.40 %) and α-terpineol acetate (2.08 %).

Table 1. Chemical composition [%] of essential oils of *Mentha longifolia* L. individuals (average values for each chemotype).

			Chemotypes			
			I α-terpineol acetate + carvone acetate	II piperitone oxide + piperitenone oxide	III pulegone + isomenthone	IV (*Z*)-sabinen hydrate + 1-terpinen-4-ol
Compounds	KIE[a]	KIL[b]				
2-hexenal	866	855	tr	tr	tr	tr
α-thujene	931	930	tr	-	-	0.68 ± 0.23
α-pinene	939	939	tr	0.43 ± 0.04	0.42 ± 0.05	0.55 ± 0.03
sabinene	977	975	1.53 ± 0.15	0.79 ± 0.14	0.38 ± 0.05	3.28 ± 0.15
β-pinene	982	979	0.19 ± 0.04	0.68 ± 0.04	0.54 ± 0.05	0.43 ± 0.05
β-myrcene	992	991	0.89 ± 0.10	0.69 ± 0.06	0.51 ± 0.06	1.12 ± 0.02
(*Z*)-3-hexenyl acetate	1004	1005	-	0.14 ± 0.03	0.19 ± 0.04	0.10 ± 0.03
3-octen-1-ol acetate	1107	1113	0.21 ± 0.03	tr	tr	tr
hexyl acetate	1010	1009	-	-	-	tr
α-terpinene	1019	1017	tr	-	-	3.37 ± 0.20
p-cymene	1026	1025	-	-	-	0.13 ± 0.06
limonene	1033	1029	0.74 ± 0.20	2.21 ± 0.19	1.63 ± 0.21	0.92 ± 0.16
1,8-cineole	1037	1031	2.21 ± 0.41	3.03 ± 0.36	1.16 ± 0.33	2.81 ± 0.39
(*Z*)-β-ocymene	1042	1037	0.14 ± 0.03	0.91 ± 0.09	0.30 ± 0.08	0.59 ± 0.13
(*E*)-β-ocymene	1053	1050	tr	tr	tr	tr
α -terpinene	1065	1060	tr	tr	-	6.04 ± 0.36 a
(*Z*)-sabinen hydrate	1080	1070	0.27 ± 0.20 b	0.13 ± 0.05 b	-	41.15 ± 3.83 a
terpinolene	1090	1089	tr	tr	-	-
linalool	1106	1097	tr	-	-	1.38 ± 0.07
(*E*)-sabinen hydrate	1108	1098	tr	-	-	2.01 ± 0.19
menthatriene <1,3,8-para>	1114	1110	0.26 ± 0.05	tr	-	-
(*E*)-*p*-menth-2,8-dien-1-ol	1125	1123	0.19 ± 0.04	tr	-	0.92 ± 0.07
3-octanol acetate	1126	1123	0.49 ± 0.06	tr	tr	tr
(*Z*)-menth-2,8-dien-1-ol	1139	1138	0.15 ± 0.03	tr	-	0.27 ± 0.02
menthone	1155	1153	tr	tr	tr	-
isomenthone	1164	1163	0.44 ± 0.20 b	0.43 ± 0.21 b	16.67 ± 4.31 a	tr b
(*Z*)-isopulegone	1172	1173[c]	tr	tr	0.45 ± 0.12	-
menthol	1177	1172	tr	0.15 ± 0.08	0.21 ± 0.21	-
1-terpinen-4-ol	1183	1177	tr	tr	0.78 ± 0.22 b	13.99 ± 0.95 a
(*E*)-isopulegone	1183	1177[d]	tr	0.28 ± 0.15	0.23 ± 0.12	-
4,8-dimethyl-1,7-nonadien-4-ol	1187	1191[e]	0.15 ± 0.07	tr	-	-
α-terpineol	1202	1189	2.04 ± 0.24	0.56 ± 0.09	0.39 ± 0.07	1.47 ± 0.05
(*E*)-dihydrocarvone	1208	1201	tr	tr	-	-
(*E*)-carveol	1219	1217	-	-	tr	-
(*Z*)-sabinen hydrate acetate	1220	1221	-	-	-	0.62 ± 0.46
pulegone	1238	1237	6.03 ± 2.26 b	7.12 ± 3.71 b	63.94 ± 4.53 a	0.12 ± 0.03 b
carvone	1244	1243	0.18 ± 0.08	-	-	-
piperitone	1255	1253	1.06 ± 0.32	0.12 ± 0.09	0.20 ± 0.11	tr
piperitone oxide	1264	1254	tr b	15.53 ± 2.31 a	0.47 ± 0.36 b	1.93 ± 0.77 b
phenethyl acetate	1277	1258[f]	-	tr	tr	tr
(*E*)-pinocarvyl acetate	1297	1298	tr	tr	tr	-
(*Z*)-pinocarvyl acetate	1313	1312	tr	tr	tr	-
dihydrocarveol acetate <iso>	1325	1329	0.11 ± 0.04	tr	tr	-
piperitenone	1332	1248	0.39 ± 0.04	tr	tr	-
α-terpineol acetate	1353	1349	39.81 ± 2.63 a	5.70 ± 3.00 b	3.31 ± 1.34 b	2.08 ± 0.90 b
(*Z*)-carvyl acetate	1372	1368	0.14 ± 0.09	-	-	-

piperitenone oxide	1378	1369	tr	50.32 ± 4.08 a	1.12 ± 1.12 b	8.40 ± 3.35 b
β-bourbonene	1385	1388	0.79 ± 0.05	tr	tr	tr
(*Z*)-jasmone	1405	1393	tr	tr	tr	tr
β-cariophyllene	1421	1419	3.09 ± 0.28	4.83 ± 0.57	1.78 ± 0.24	2.22 ± 0.19
β-farnesene	1455	1457	tr	tr	tr	-
α-humulene	1458	1455	0.15 ± 0.05	0.13 ± 0.02	tr	tr
allo-aromadendrene	1464	1460	tr	tr	tr	-
germacrene-D	1483	1485	1.67 ± 0.17	3.11 ± 0.19	1.42 ± 0.14	2.01 ± 0.25
byciclogermacrene	1497	1500	0.14 ± 0.04	0.40 ± 0.10	tr	tr
α -cadinene	1519	1523	tr	tr	-	-
carvone acetate	1575	1566	33.84 ± 4.13 a	0.47 ± 0.36 b	2.65 ± 1.51 b	0.60 ± 0.36 b
cariophyllene oxide	1582	1583	1.03 ± 1.00	0.11 ± 0.04	-	tr
Monoterpene hydrocarbons			3.95 ± 0.33	5.82 ± 0.40	3.80 ± 0.37	17.13 ± 0.63
Monoterpene alcohols			4.98 ± 0.62	3.82 ± 0.46	2.34 ± 0.50	64.63 ± 5.08
Other oxygenated monoterpenes			82.22 ± 1.47	80.25 ± 1.35	89.42 ± 0.91	13.18 ± 5.03
Sesquiterpene hydrocarbons			5.95 ± 0.42	8.60 ± 0.69	3.41 ± 0.37	4.37 ± 0.5
Oxygenated sesquiterpenes			1.03 ± 1.00	0.11 ± 0.04	-	tr
Others compounds			0.88 ± 0.13	0.35 ± 0.08	0.34 ± 0.09	0.26 ± 0.06
Total			99.00 ± 0.23	98.95 ± 0.13	99.30 ± 0.22	99.60 ± 0.17

Notes:

The bold typed compounds are those define the proposed chemotypes. (Values within a row for each compound having different letters are significantly different from each other according Tukey's HSD test (P<0.05)).

tr: traces (<0.1%).

[a] KIE: Kovats retention index, experimentally determined, relative to C7-C24 n-alkanes on the ZB-5 column.

[b] KIL: Kovats retention index from literature (Adams, 2007), except for those expressly referred).

[c]Slavkovska *et al.* (2005).

[d]Benzo *et al.* (2007).

[e]Mondello *et al.* (2012).

[f]Campo *et al.*(2005).

This chemotype has not been reported either in the literature so far. Only a sample reported by Baser *et al.* (1999) exhibited a close profile: 1-terpinen-4-ol (39 %) and (*E*)-sabinen hydrate (15 %). (*Z*)-sabinen hydrate is a valuable fragrance ingredient extensively used in toiletries, household cleaners and detergents (Bhatia *et al.*, 2008).

According to the results obtained, no relation could be noted between locations and chemotypes, given that all of them were present in each one of the sampling zones. Chemotypes II and III (both C3 oxygenated) are extensively referred in the literature. Samples in which the major compounds are piperitenone or piperitone oxides are reported by Maffei (1988), Baser *et al.* (1999), Singh *et al.* (2008),

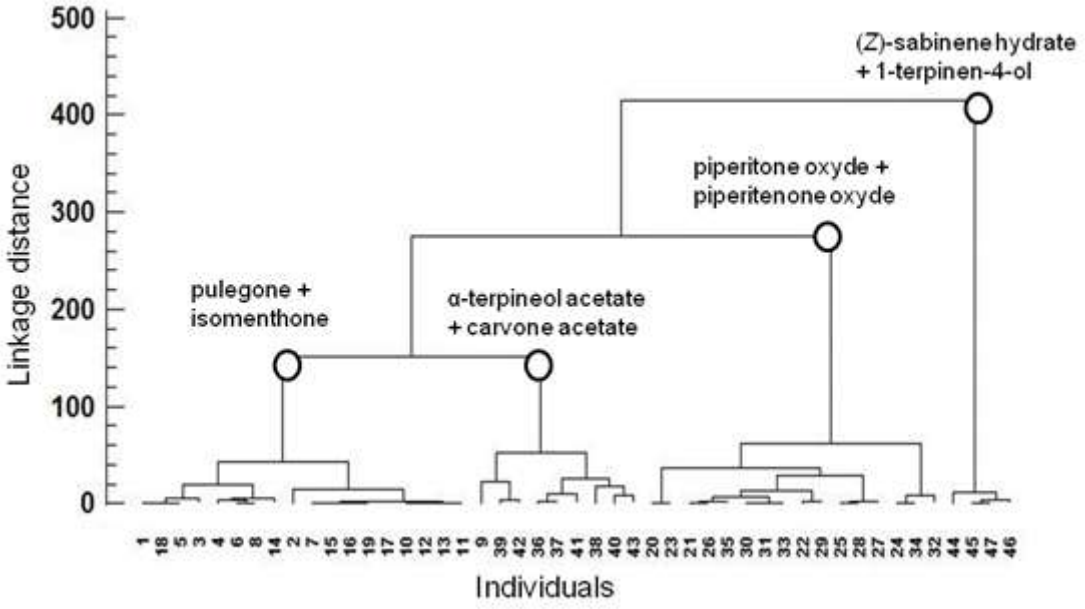

Fig. 2. Dendrogram of clusters resulting from chemical diversity showing the grouping of the studied individuals of *M. longifolia* L.

Segev *et al.* (2012), Sharopov *et al.* (2012), Jamzad *et al.* (2013). The same way, chemotypes with pulegone and menthone (or isomenthone) as the major components are reported by Fleisher and Fleisher (1991), Mimica-Dukic *et al.* (2003), Sharopov *et al.* (2012); Asekun *et al.* (2007), Mkaddem *et al.* (2009), Segev *et al.* (2012). Nevertheless, the most abundant chemotype turned out to be the one defined by equilibrated rates of α-terpineol acetate (C8 oxygenated) and carvone acetate (C2 oxygenated) (chemotype I), which has not been reported so far. This last compound marks the difference with regard to chemical profiles from literature. However, as reported by Sharopov *et al.* (2012), carvone was found as a main compound by several researches (e. g. Monfared *et al.*, 2002). Bearing in mind the relative amount of α-terpineol (0.39-2.04 %) and carvone (0-0.18 %) with respect to α-terpineol acetate and carvone acetate, the high level of acetylation can be highlighted as an important feature about which it may be worth to go deeply into. A similar comment can be done regarding to epoxidation if the relationship between piperitone (tr-1.06 %) and piperitenone (tr-0.33 %) and their epoxides is taken into account.

Some of the most extended chemical profiles reported in the literature have been found in a reduced area (less than 50 km^2). Furthermore, as the five sampling zones included in this work were selected in such a way that the climate and soil conditions were similar, the chemodiversity found does not seem to be related neither to geographical origin nor environmental factors, but to genetic ones. On the other hand, the fact that these chemotypes were found together in the different sampling zones reinforces the advisability of individual sampling.

Two new well defined chemotypes have been identified in this study: (*Z*)-sabinenhydrate + 1-terpinen-4-ol, and α-terpineol acetate + carvone acetate (the most numerous). Thus, the selection and propagation of these chemotypes rich in active compounds could be of undoubted practical usefulness. Moreover, from a wider point of view, the possibility of growing pure chemotypes could contribute to conduct more accurate studies on seasonal and environmental factors affecting essential oil composition. It allows, in turn, obtaining suitable chemical profiles according to each biological activity related to practical purposes.

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