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Study on essential oil of *Mentha aquatica* L. from Vietnam++++++

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Abstract
The characterization of essential oils of *Mentha aquatica* L grown in two Provinces of Vietnam was performed by means of gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The major constituents of the essential oils were *epi*-bicyclosesquiphellandrene (58.9% and 52.4% respectively) and limonene (21.1% and 31.4% respectively) for Thanh Hóa and Nghệ an Provinces, Vietnam. This compositional pattern may represent a new chemotype of the essential oils of *M. aquatica.*

Keywords: *Mentha aquatica*, essential oil composition, chemotype, *epi*-bicyclosesquiphellandrene, limonene

1. Introduction

Mentha aquatica L., (Lamiaceae) is a perennial plant in the genus *Mentha* native throughout Europe except for the extreme north, and also northwest Africa and southwest Asia. It is an herbaceous rhizomatous perennial plant growing to 90 cm tall. The stems are square in cross section, green or purple, and variably hairy to almost hairless. The rhizomes are wide-spreading, fleshy, and bear fibrous roots. The leaves are ovate to ovate-lanceolate, 2 to 6 cm long and 1 to 4 cm broad, green (sometimes purplish), opposite, toothed, and vary from hairy to nearly hairless. The flowers are tiny, densely crowded, purple, tubular, pinkish to lilac in colour; flowering is from mid to late summer [1]. Naringenin, a component of *M. aquatica* has anxiolytic effects [2]. The essential oils of *M. aquatica* inhibit acetycholinestrase [3] and possessed both antibacterial [4, 5] and antioxidant [5] activities. Viridiflorol from the essential oil and (*S*)-naringenin from an ethanolic extract show strong affinity to the GABA-benzodiazepine receptor [6]. Extracts of the plants have displayed antihemolytic and antioxidant activities [7]. Phenolic compounds with antibacterial and antiradical activities were present in *M. aquatica* [8]. *M. aquatica* synthesizes and emits (+)-menthofuran, which acts as a deterrent to *Chrysolina herbacea* [9]. Furthermore, *M. aquatica* contains psychoactive compounds that display both monoamine oxidase-inhibitory activity and mitochondrial respiration uncoupling [10, 11]. Literature information has shown that some reports are available on compositions of essential oils from *M. aquatica*. It appears that *M. aquatica* essential oil exhibits chemical variability which varies depending on the part of the plant being studied and the country of origin. The major chemical compounds so far identified in *M. aquatica* among the monoterpenes includes α-pinene, limonene, *trans*-β-ocimene, α-terpinene, linalool, linalyl acetate, 1,8-cineole, pulegone, menthofuran, menthone, menthol, isopinocamphone, piperitenone oxide and dihydrocarveyl acetate. On the other hand, the common sesquiterpene compounds were β-caryophyllene, germacrene D, elemol, viridiflorol, caryophyllene oxide [1, 4, 5, 9, 12-35].

The objective of the present study is to report the volatile compounds identified in the leaf of *M. aquatica* grown in Vietnam. The chemical compositions of essential oils of some plants cultivated in Vietnam were recently published [36-38].

2. Materials and methods

2.1 Collection of plant samples

Aerial parts of *M. aquatica* were collected from Thanh Hóa and Nghệ an Provinces, Vietnam, in July 2012. A voucher specimen, DND 108, was deposited at the Herbarium of the Institute of Ecology and Biological Resources, National Centre for Science and Technology, Ha Noi, Vietnam.

2.2 Extraction of essential oils

Sample was shredded, dried and the oil was obtained by steam distillation for 3h at normal pressure, according to the Vietnamese Pharmacopoeia [39]. The yields of oils was 0.42% and 0.34% (v/w), calculated on a dry weight basis for Thanh Hóa and Nghệ an Provinces respectively.

2.2 Chromatographic analysis of the essential oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technology). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 min hold) to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 µL. Inlet pressure was 6.1 kPa.

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s. Compounds identification was performed on the basis of comparison of retention indices (RI) and MS data with reference to literature data [40, 41].

2.3 Numerical cluster analysis

A cluster analysis was performed to determine the chemical relationships between the studied *M. aquatica* oil from Vietnam and the oils of this species reported in the literature from several other locations around the world. The 44 *M. aquatica* samples were treated as operational taxonomic units (OTUs). The percentage composition of twenty-four main essential oil components were used to determine the chemical relationships between the different *M. aquatica* oil samples by cluster analysis using the NTSYSpc software, version 2.2 [42]. Correlation was selected as a measure of similarity, and the unweighed pair-group method with arithmetic average (UPGMA) was used for cluster definition.

3. Results & Discussion

The main compounds of both oil samples were *epi*-bicyclosesquiphellandrene (58.9% and 52.4% respectively) and limonene (21.1% and 31.4% respectively) from Thanh Hóa and Nghệ an Provinces (Table 1). However, bicyclogermacrene (3.0%), dihydrocarvyl acetate (2.4%) and *trans*-carveol (2.1%) were the other notable compounds of Thanh Hóa oil which were present in lower amounts in Nghệ a sample. However, other known compounds of previously investigated samples from other parts of the world were not detected in the present oils. These are menthofuran, pulegone, menthyl acetate, linalyl acetate, caryophyllene oxide, viridiflorol and elemol.

Previous analyses of *M. aquatica* essential oils from various parts of the world have reported variation in its chemical constituents (Table 2). This has led to the delineation of various chemotypes such as menthofuran/1,8cineole/β-caryophyllene [1, 12, 13], menthofuran [4, 9, 14-22], pulegone/menthone [23, 24], elemol/ menthofuran [14], menthofuran/1,8-cineole [18, 25], viridiflorol/β-caryophyllene/caryophyllene oxide [14], β-caryophyllene/viridiflorol [14], menthofuran/viridiflorol [14], viridiflorol [26], linalyl acetate [27], α-terpinene/piperitenone oxide/1,8-cineole [25], menthofuran/limonene/*trans*-β-ocimene [28], β-caryophyllene/ viridiflorol/1,8-cineole [29], piperitenone oxide/β-caryophyllene/1,8-cineole [29], 1,8-cineole [5, 30], isopinocamphon [16], linalool/linalyl acetate [16], menthol/menthyl acetate/menthofuran [17], 1,8-cineole/limonene [21], limonene/caryophyllene/germacrene D [30], limonene/1,8-cineole dihydrocarveyl acetate [32], menthols/menthyl esters/menthones [33], α-terpinene/piperitenone oxide/1,8-cineole [25], linalyl acetate/α-pinene/linalool [34] and menthofuran/limonene [35]. Accordingly menthofuran appears to be the main constituent of *M. aquatica* oils despite a few exceptions.

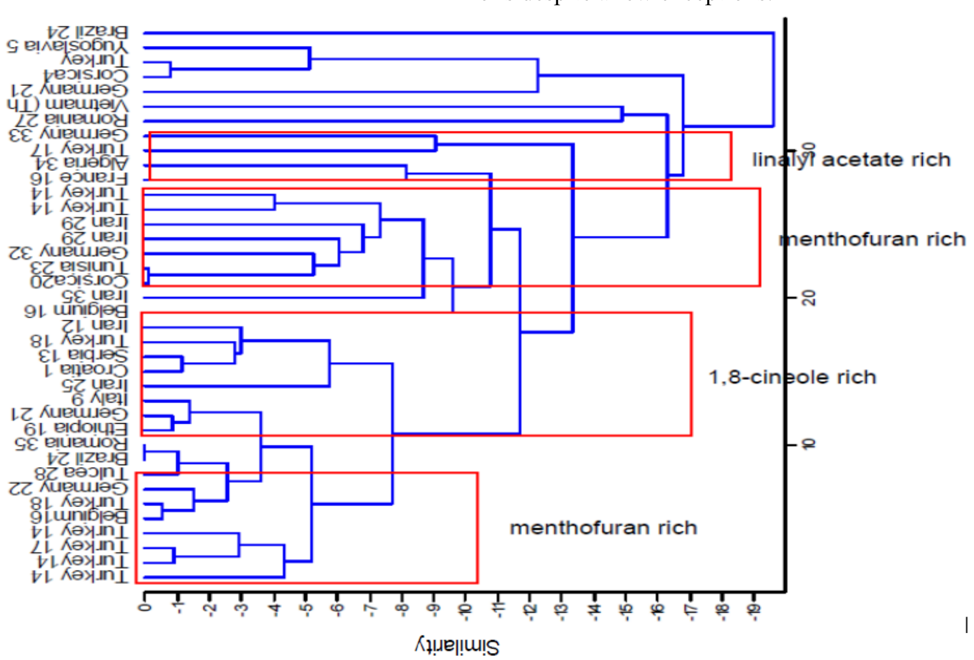

Fig. 1: Cluster analysis of the essential oil of M. aquatic.

The cluster analyses of the principal components are depicted in Fig 1. There are some apparent clusters: menthofuran-rich, linalyl acetate-rich and 1,8-cineole-rich. However, Table 2 provides a more comprehensive delineation of the chemotypic forms of essential oils of *M. aquatica.* The analyses of this study indicate a number of differences from previous studies. It is known that the environmental factors represented by altitude, temperature, luminosity, soil, animal-plant interactions, besides anthropic factors, may result in variations in the plant metabolic pathways so that one often finds a variation of the chemical compounds present in the essential oils extracted from the plants of different origins. It should be noted that the chemical combination of *epi*-bicyclosesquiphellandrene/limonene in the oils under study may be described as a new chemotype of the essential oil of *M. aquatica*.

3.1 Tables

Table 1: Percentage composition of the distilled aerial parts oil of *Mentha aquatica* L. from Vietnam

Compounds [a]	RI [B]	RI [c]	Thanh Hóa	Nghệ An
α-Pinene	939	932	0.8	0.8
Sabinene	976	969	0.4	0.4
β-Pinene	980	976	0.9	0.9
β-Myrcene	990	988	1.2	0.8
3-Octanol	994		Tr	0.2
α-Phellandrene	1006	1002	Tr	0.2
Limonene	1032	1020	21.1	31.4
1,8-Cineole	1034	1032	Tr	Tr
(*Z*)-β-Ocimene	1042	1034	Tr	0.1
(*E*)-β-Ocimene	1053	1044	Tr	0.1
Linalool	1100	1095	0.2	0.1
3-Octanol acetate	1120	1120	0.1	0.2
trans-Limonene oxide	1137	1137	Tr	0.1
Menthone	1142	1148	Tr	0.5
iso-Menthone	1150	1158	0.2	0.1
Menthofuran	1157	1159	0.1	0.1
cis-Dihydrocarvone	1190	1191	1.6	0.2
Methyl chavicol	1203	1195	0.3	0.1
trans-Carveol	1217	1215	2.1	1.9
Dihydrocarvyl acetate	1304	1306	2.4	0.5
β-Bourbonene	1388	1387	0.1	Tr
β-Caryophyllene	1419	1417	0.4	0.6
α-Humulene	1454	1452	1.6	0.3
(*Z*)-β-Farnesene	1450	1440	Tr	0.1
Germacrene D	1480	1484	0.1	0.2
epi-Bicyclosesquiphellandrene	1482	1488	58.9	52.4
Bicyclogermacrene	1500	1500	3.0	0.8
cis-Calamenene	1530	1528	0.1	0.1
TOTAL			95.4	93.2

RI: Retention indices on HP-5MS column; Tr. Trace amount, < 0.1%

Table 2: Major components and chemotypic forms of essential oils of *M. aquatica* from literature

Source/Chemotypic forms	Part	Major constituents	Reference
Chemotype 1			
Iran	Aerial	1,8-cineole (27.2%), menthofuran (23.2%) and β-caryophyllene (12.8%)	[12]
Croatia	-	menthofuran, 1,8-cineole and β-caryophyllene[a]	[1]
Serbia	Vegetative phases	menthofuran (19.0–24.5%), 1,8-cineole (11.8–20.2%) and β-caryophyllene (6.7–16.6%)	[13]
Chemotype 2			
Turkey	-	menthofuran (21-48%)	[14]
-	-	menthofuran [a]	[15]
Belgium	Seeds	menthofuran (49%)	[16]
Turkey	-	menthofuran (35%)	[17]
Turkey	-	menthofuran (35% and 58%)	[18]
Ethiopia	Leaf	menthofuran (70.5%)	[19]
Corsica	-	menthofuran (50%)	[20]
Germany	-	menthofuran (66·4%)	[21]
Germany	-	menthofuran (54.95%)	[22]
Corsica	-	menthofuran (51.7%)	[4]
Italy	Stolons	menthofuran	[9]
Chemotype 3			
Tunisia	Leaf	pulegone (39.36 %) and menthone (27.69 %)	[23]
Brazil	-	methone (77.9%) and pulegone (14.39%)	[24]
Chemotype 4			
Turkey	-	elemol (24%) and menthofuran (11% and 17%)	[14]
Turkey	-	menthofuran (34%) and elemol (l2%)	[14]
Chemotype 5			
Turkey	-	menthofuran/1,8-cineole (14–30%/15–27%)	[18]
Iran	Flowering phase	menthofuran (45.1%) and 1,8-cineole (26.6%)	[25]
Iran	Flowering phase	menthofuran (45.1%) and 1,8-cineole (26.6%)	[25]
Chemotype 6			

Turkey	-	viridiflorol (27%), β-caryophyllene (15%) and caryophyllene oxide (15%)	[14]
Cheemotype 7			
Turkey	-	β-caryophyllene (19%) and viridiflorol (16%)	[14]
Chemotype 8			
Turkey	-	(a) menthofuran (33%) and viridiflorol (17%) (b) menthofuran (41%) and viridiflorol (10%)	[14]
Chemytpe 9			
	-	viridiflorol [a]	[26]
Chemotype 10			
Romania	-	linalyl acetate [a]	[27]
Chemotype 11			
	Vegetative stage	α-terpinene (25.2%), piperitenone oxide (19.2%), 1,8-cineole (15.1%)	[25]
Chemotype 12			
Tulcea	Vegetative phases	menthofuran (58.59 to 51.26%) and limonene (12.06 to 5.94%)	[28]
Brazil	Aerial parts	menthofuran (51.27%) and limonene (12.06%)	[24]
Chemotype 13			
Iran	Stem	β-caryophyllene (22.4%), viridiflorol (11.3%) and 1,8-cineole (10.9%)	[29]
Chemotype 14			
Iran	Leaf	piperitenone oxide (25.7%), β-caryophyllene (12.0%) and 1,8-cineole (10.3%)	[29]
Chemotype 15			
Turkey	Aerial	1,8-cineole (55.3%)	[30]
Yugoslavia	-	1,8-cineole [a]	[5]
Chemotype 16			
Belgium	Seeds	(-)-isopinocamphone [a]	[16]
Italy, Portugal, Belgium	Seeds	isopinocarphone (49%)	[16]
Chemotype 17			
France	Seeds	linalool (43.1%) and linalyl acetate (22.1%)	[16]
Chemotype 18			
Turkey	-	menthol (37%), menthyl acetate (33%) and menthofuran (17%)	[17]
Chemotype 19[b]			
Germany	-	1,8-cineole (78·7%) and limonene (53·8%)	[21]
Chemotype 20[b]			
-	-	limonene, caryophyllene and germacrene-D [a]	[31]
Chemotype 21[b]			
Germany	-	limonene (19·0%), 1,8-cineole (11·8%) and dihydrocarveyl acetate (11·0%)	[32]
Chemotype 22[b]			
Germany	-	menthols (47·8%), menthyl esters (14·3%) and menthones (10·5%)	[33]
Chemotype 23			
Iran	Before flowering	α-terpinene (25.2%), piperitenone oxide (19.2%) and 1,8-cineole (15.1%)	[35]
Chemotype 24			
Algeria	Aerial parts	linalyl acetate (26.1 %), α-pinene (22.7 %) and linalol (13.8 %)	[34]
Chemotype 25			
Romania	Aerial parts	menthofuran (51.27%) and limonene (12.06%)	[35]

a Quantitative data not available; [b] produced by gene substitution into *M. aquatica*; - not known

4. Conclusions
The study on the chemical composition of essential oils of *M. aquatica* grown in Vietnam revealed a new chemotype hitherto unknown. This may be due to the ecological and climatic condition between Vietnam and the rest of the world.

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