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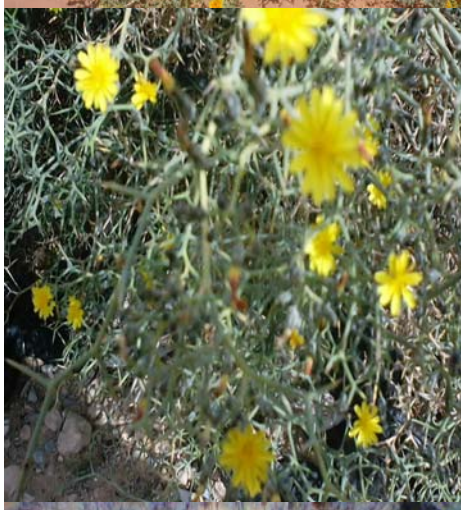

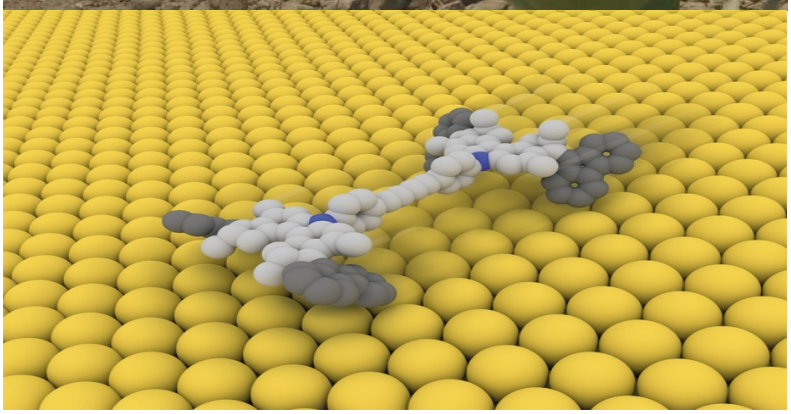

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Comparative Analysis of Chemical Composition, Antioxidant and Antibacterial Activities of *Mentha rotundifolia* Essential oils from Algeria extracted by microwave and hydrodistillation

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Abstract. *The essential oil of Mentha rotundifolia (L.) Huds growing wild from East Algeria (Naciria at 60Km in East of Algiers) obtained by hydrodistillation (HD° and a microwave distillation process (MD) have been analysed by means of GC-FID and GC/MS in combination with retention indices. In total, 54 constituents were identified (accounting for 96.7 and 95.6% in HD and MD oils, respectively).*
The main components were piperitone oxide (25.1 and 29.1% in HD and MD oils, respectively), piperitenone oxide (8.9 – 11.8%), terpinen-4-ol (9.3 – 3.4%), β–caryophyllene (5.4 – 7.3%), allo-aromadendrene (5.3 - 6.4%) and D-germacrène (5.4 – 7.1%). In comparison with HD, MD allows to obtain oil in a very short time, with the reduction of solvent used similar yields, comparable qualities and substantial savings of energy.
The antioxidant activity was determined according to the ability of the tested samples to scavenge the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH). The essential oil were slightly active (32.6 and 21.8% in HD and MD oils, respectively) comparing with BHT (64.7%). The antibacterial activities of the essential oils indicated that Staphylococcus aureus was the more sensitive strain tested to the oils of Mentha rotundifolia with the strongest inhibition zone 28.3 for HD and 26.5 mm for MO.*

***Key Words*:** *Mentha rotundifolia (L.) Huds, essential oil composition, piperitenone oxide, piperitone oxide, piperitenone, Chemotype, Antimicrobial activity*

1. Introduction

The genus Mentha (Lamiaceae) includes aromatic Herbs of difficult taxonomic classification due to great variability in their morphological characters and frequent hybridisation. Previous investigation of their essential oils have revealed the existance of an important chemical polymorphism [1] and several varieties and chemotypes have been described for *M. specata.*

Mentha rotundifolia is an hybrid between *Mentha longifolia* and *Mentha suaveolens* Ehrh., whose essential oil has been the object of several studies [2, 3, 4] and different chemotypes have been characterised. Some authors have considered *Mentha rotundifolia* as a synonym of *Mentha suaveolens* [5].
Algeria is blessed with a rich source of aromatic plants, many of which have not been previously investigated for their chemical constituents and biological potentials. *Mentha rotundifolia* grows in Algeria region and is a potential source of essential oils. It is very widely distributed a round the Mediterranean basin, in American and in occidental Asia [6, 7]. She has been used for their flavours in cooking, in folk medicine as antiseptic and as antimicrobial agents [8].
In Algeria and northern Africa, this aromatic plant is well known such as "timarssad" or " timaja" Multiple studies have been reported on the chemical composition of the essential oils of *Mentha rotundifolia* belonging to different regions in the world [9, 10, 11] have been reported and related chemotypes have been defined. One of them is particularly rich in piperitenone oxide, an oxygenated monoterpene whose biological effects (cardiovascular effects, activity antibacterial and antifungal, toxic, repellent and reproduction retardant toward malarial vector *Anopheles stephensi*) have been investiguated [12, 13].
In this work, we studied the chemical composition of the leaves essential oils of *Mentha rotundifolia*, plants collected in Atlas mean, a mountainous region from Algeria where people frequently use this plant in traditional medicine

2. Material and Methods

2.1. *Plant material:* *The* aerial parts of *Mentha rotundifolia* were collected at Naciria near Boumerdes (60 km in the east of Algiers). Voucher specimens of the different samples were stored in the Herbarium of the Vegetal Biology, University of Sciences and Technology Houari Boumediene, Bab Ezzouar, Algiers. Plant samples were air-dried (3 – 6 days), minced, and subjected immediately to oil isolation.

Microwave Hydrodistillation: Microwave hydrodistillation (MD) was performed at atmospheric pressure in a microwave laboratory oven, as described previously [14, 15]. 100 g of fresh plant material were heated using a fixed power density of 1000 W for 15 min with 30 ml distilled water. The direct interaction of microwaves with biological water the release of essential oils trapped inside the cells of plant tissues. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4 °C until used. Extractions were performed at least three times and the mean values are reported.

Hydrodistillation: One hundred grams of each aromatic aerial parts plant were submitted to hydrodistillation using a Clevenger-type apparatus according to the European pharmacopoeia and extracted with 3L of water for 2.5h.
The essential oil was collected, dried under anhydrous sodium sulphate, and stored at 4°C until used. Extractions were performed at least three times and the mean values are reported.

***2.2. Gas chromatography analysis*:** GC analysis was carried out using a Hewlett-Packard 6890N gas chromatograph equipped with a flame ionisation detector (FID), under the following operation condition: vector gas, N_2; injector and detector temperatures, 250 °C and 320 °C, respectively; injected volume 0.2 µl; split-less mode; HP5MS (30 m x 0.25 mm LD., film thickness 0.25 µm; constant gas flow 0.3 mL/min) and HP wax (60 m x 0.32 mm LD., film thickness 0.25 µm; constant flow 0.9 ml/min); the oven temperature program was 60 °C for 8 min, rising to 250 °C at 2 °C/min, then held for 30 min at 250 °C; Retention indices were determined with C_5-C_{28} alkanes standards as

reference. Relative amounts of individual components are based on peak areas obtained without FID response factor correction.

***2.3. Gas chromatography/mass spectrometry analysis*:** GC/MS analysis was carried out using an Agilent 6890N coupled to an Agilent 5973A mass spectrometer. Samples were analysed on a fused-silica capillary column HP5MS (30 m x 0.25 mm LD., film thickness 0.25 µm) and HP wax (60 m x 0.32 mm LD., film thickness 0.25 µ m). Carrier gas He, injector and detector temperatures, 250 °C flow rate 0.5mL/min; split 1:20; injection volume 0.1µl; oven temperature progress from 60 to 250°C at 2°C/min; ionisation mode used was electronic impact at 70eV; electron ionisation mass spectra were acquired over the mass range 35-400 µ.

***2.4. Identification components*:** Component identification was confirmed by comparison of mass spectral fragmentation patterns with those stored in the MS data bank (NIST 2002, Wiley 7), laboratory mass spectra libraries built up from pure substances, and with previously published spectra, and verified by comparison of linear retention indices of the identified compounds with published index data [26, 27, 28] on apolar and polar columns.

2.5. Antioxidant activity: The antioxidant activity of the different essentials oils were determined according to the ability of the tested samples to scavenge the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH*) [29, 30] by off-line spectrophotometric measurements. Methanolic solutions (2.4 ml) of DPPH* (10^{-5}M) with an absorbance at 515 nm of 0.800± 0.030AU were mixed with methanolic solutions (1.2 ml) of samples at different concentrations: 1000 µg/ml. Triplicate samples were shaken and allowed to stand for 15 min in the dark at room temperature, and the decrease of absorbance at 515 nm was measured using a Perkin-Elmer Instruments, Nor-walk, CT, USA). The radical scavenging activity of the tested samples, expressed as DPPH* scavenging percentage, was calculated by the following formula:

$$RC\ \% = \left[\left(A_A - A_B\right)/ A_B\right] \times 100$$

A_B is the absorbance of the blank sample (t =0); A_A is the absorbance of the tested sample after 15min; *RC is the* radical scavenging (%)

***2.6. Antimicrobial activity*:** The essential oils were individually tested against Gram positive bacteria and Gram negative bacteria. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli, Streptococcus pyogenes* and *Candida albicans*.
The agar diffusion method was employed to determine the antimicrobial activity of the essential oils.of *M. Rotundifolia* essential oil was assessed using the paper disk agar diffusion method. [31] with some modifications. A 16-h culture was diluted with sterile physiological saline solution with reference to the MC Farland 0.5 standard to achieve an inoculum of approximately 10^6 CFU/ ml. A suspension was swabbed in three directions on 4 mm thick Mueller Hinton agar (MHA). Absorbent disk (Whatman disk No. 4 of 6 mm diameter) containing 15 µl of filter sterilized test essential oil were applied on the surface of the plate (90 mm) inoculated with different microbial strains. The plates were then incubated for 24 h at 37°C. Negative control was prepared using a disk impregnated with sterile water. Finally, antimicrobial activity was evaluated by measuring the diameter (mm) of the growth inhibition zones including the 6 mm disk. The measurements of inhibition zones were carried out for three samples.

3. Results and Discussion

The yields, isolation times and energy consumption of HD and MD oils were listed in table I. MD was clearly quicker than conventional HD. The isolation took 15 min, whilst 2.5 h were required by hydrodistillation. For HD or MD, the isolation temperature was equal to boiling temperature of water at atmospheric pressure (100°C). A prolonged heating and stirring in a greater quantity of boiling water (3 L for HD vs. 30 mL for MD) could involve a possible degradation of oil components by hydrolysis, trans-esterification and oxidation as noted previously [14,15,16].
The yield of oil obtained from *Mentha rotundifolia was* 1.22 ± 0.02% by HD and 1.13 ± 0.02% by MD. These results mean a substantial savings of time, quantity of water and energy. Contrary to the *Mentha rotundifolia* essential oils yield of Morocco which is very high level (4.33%) [17]. In this study the yield is low to those of essential oils analyzed in Morocco [18], which the yield was 1.86%.
The compounds of *mentha rotundifolia* leaf oil from Algeria are listed in order of their elution on the HP-5MS non polar column. Fifty four constituents were identified by GC and GC/MS in *mentha rotundifolia* oils isolated by HD and MD (Table I), which made up (95.9 and 96.7% in HD and MD oils, respectively) of the total essential oil.
A comparison between the HD and MD oils shows that the first sample has slightly higher concentrations of the family classes than the second one. These results are different than those obtained from HD and MD oils of orange peel [19], where as significant variations were reported, mainly related to the nature of the oil organs (sacs,glands) located at different depths of each plant.
The major component were piperitone oxide (25.1 and 29.1% in HD and MD oils, respectively), piperitenone oxide (8.9 – 11.8%), terpinen-4-ol (9.3 – 3.4%), trans-caryophyllene (5.4 – 7.3%), allo-aromadendrene (5.3 - 6.4%) and D-germacrène (5.4 – 7.1%).
This investigation has shown that the oils were rich in ether oxides (41.2% - 34.2%) and sesquiterpene were highest (30.1 HD v.s 24.9% MD). The Algerian *Mentha rotundifolia* oil from the Nasiria region, is characterized in this work by the chemotype piperitone oxide - piperitenone oxide, where as different Algerian chemotypes, such as pipéritone oxide (Miliana : 31,4 % ; Rouina : 19,7 %)- piperiténone oxide (Miliana : 27,8 % ; Rouina : 29,4 %) for the first chemotype and pipériténone (54,9 %) - pipériténone oxide (17,6 %) second chémotype [20].
The essential oils composition showed a different pattern to those published for other geographical regions: menthol, menthyl acetate and menthone were reported as the major component in the essential oil from Serbia [21] and Marroco [22].
The total essential oil in this study is different to those found in *Mentha rotundifolia* oil in Uruguay [4] which is of piperitenone oxide (80.8%) and in Tunisie the major component is pulegone (47.15%) [23]. Intense study on the essential oil of *Mentha rotundifolia* revealed the existence of different principal constituent: piperitone oxide [3, 10], methyl acetate [10], dihydrocarvone [3], carvone [21] and piperitenone [17].
The antioxidant activity of essential oil of *M. Rotundifolia* was examined by comparing it to the activity of known antioxidants such as BHT by the following in vitro assay; inhibition of DPPH radical. These results showed that *M. Rotundifolia* essential oil was found to be less active than BHA since their CR % values were found to be higher. But with careful and cautious consideration, it is interesting that BHT is a pure chemical substance, while the essential oil of *M. Rotundifolia* used consists of several natural active substances or a few of them must have this antioxidant capacity.
The scavenging ability of essential oils and positive control BHT is presented in table 2. The weak DPPH radical scavenging activity of these oils could be attributed to the absence of some phenolic components, which may play an important role.

The antibacterial activities of the essential oïl are shown on table 3. The data indicated that *Staphylococcus aureus* was the more sensitive strain tested to the oils of *Mentha rotundifolia* with the strongest inhibition zone 28.3 for HD and 26.5 mm for MO. *Escherichia col*, was less sensitive with 15.3 and 14.2 mm for HD and MO respectively. Modest activities were observed against *Candida albicans*. On the other hand the essential oil of *Mentha rotundifolia* show no activity against *Streptococcus pyogenes* and *Pseudomonas aeruginos*a. The antimicrobial activities were mainly explained by C_{10} and C_{15} terpenes with aromatic rings and phenolic hydroxyl groups capable of forming hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, Aldehydes and esters may contribute to the overall antimicrobial effect of essential oils [24, 31, 32], On the other hand, the enantiomers of α-pinene, β-pinene, limonene and linalol have a strong antibacterial activity [25].

4. Conclusion

This study completes our chemical composition on *M. rotundifolia* essential oils from Algeria. Among the 54 constituents isolated by HD and MD and identified by GC and GC/MS, a great number has not been reported previously, to our knowledge.
In comparison with HD, microwave extraction can produce essential oil in concentrated form, free from any residual solvents, contaminants or artefacts. The new systems developed to date indicate that microwave extraction offers net advantages in terms of selectivity, with shorter extraction times and better essential oil compositions, and is environment friendly. In this article we have discussed how microwave extraction highly accelerated the extraction process, without causing considerable changes in the volatile oil composition and properties, phenomena which were previously described. The MD offers important advantages over traditional alternatives: shorter isolation time, same yields, reduced cost; less energy consuming; cleaner features, and a better possibility of the natural aroma reproduction of the essential oil compared to HD. The oïl was found to have significant antibacterial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious

Table 1: Chemical composition (%) of *Mentha Rotundifolia* oils isolated by HD and MD

N°	Compounds	RI [1]	HD	MD
1	2-hexanal	865	0.2	0.2
2	tricyclene	925	tr	tr
3	α-thujene	929	0.2	0.1
4	α-pinene	935	0.6	0.5
5	camphene	950	0,3	0.1
6	sabinene	978	0,4	0.3
7	β-pinene	979	1.1	0.4
8	1-octen-3-ol	988	0.4	0.2
9	β-myrcene	997	2.1	1.2
10	α-terpinene	1021	1.1	0.3
11	o-cymene	1029	tr	tr
12	limonene	1034	1.8	1.0
13	1,8-cineol	1035	tr	tr
14	trans- β-ocimene	1047	3.5	2.1
15	cis- β-ocimene	1055	0.5	0.5
16	γ-terpinene	1063	2.1	0.5
17	cis-sabinene hydrate	1072	2.5	3.8
18	α-terpinolene	1065	1.4	1.3

N°	Compounds	RI [1]	HD	MD
19	trans-sabinene hydrate	1096	0.7	0.6
20	linalool	1100	tr	tr
21	1-octen-3-yl-acetate	1115	1.6	1.2
22	camphre	1127	0.7	0.5
23	Borneol	1165	0.3	0.3
24	Terpinene-4-ol	1177	**9.3**	**3.4**
25	trans-piperitone oxide	1193	**26.1**	**29.1**
26	bornyl acetate	1285	0,2	0.3
27	thymol	1298	2.0	1.5
28	α-copaene	1392	0.7	0.4
N°	Compounds	RI [1]	HD	MD
29	β-bourbonene	1383	tr	tr
30	oxyde de pipéritenone	1386	**8.9**	**11.8**
31	trans-caryophyllene	1389	**5.4**	**7.3**
32	cis-jasmone	1393	0.6	0.8
33	α-humulene	1452	tr	tr
34	trans- β-farnesene	1458	0,2	0.2
35	allo-aromadendrene	1462	**5.3**	**6.4**
36	bicyclo sesquiphellandrene		3.0	2.4
37	γ-muurolene	1478	1.9	2.2
38	D-germacrene	1482	**5.4**	**7.1**
39	valencene	1488	tr	0.1
40	bicyclogermacrene	1493	0.9	0.6
41	A-germacrene		tr	0.7
42	γ-cadinene	1512	0.4	0.6
43	cis-calaménene	1521	1.4	1.8
44	δ-cadinene	1529	tr	tr
47	cadina-1,4-diène	1533	0.1	tr
46	α-cadinene	1537	0.4	0.6
47	cis-muurol-5-en-4-α-ol	1546	tr	0.1
48	4-ol-germacrene D	1574	0.1	0.2
49	oxyde de caryophyllene	1581	0.2	0.3
50	viridiflorol	1590	0.3	0.2
51	1,10-di-epi-cubenol	1615	1.8	1.4
52	α-epi-cadinol	1639	0.7	0.6
53	α-epi-muurolol	1653	0.2	0.9
Isolation time (min)			150	15
Energy consumption (Kwh)			1.75	0.21
Yield %*			0.22	0.13
Identified components (%)			95.9	96.7
Monoterpene hydrocarbons			8.1	15.1
Sesquiterpene hydrocarbons			30.1	24.9
Ketones			1.4	1.3
Alcohols			7.3	14.0
Ether oxides			41.2	34.2
Esters			5.6	5.0
Others			2.3	2.2

tr : trace < 0.05%; RI[1]: temperature programmed indices referred to n-alkanes C_7-C_{28}, determined respectively on HP5-MS capillary columns according retention to Van Den Dool and Kratz
HD: hydrodistillation; MD: Microwave distillation; * Yield expressed as in grams of oil per 100g of plant material

Table 2: Radical scavenging (%) activity of the essential oils of *M. rotundifolia* against DPPH radical

Sample	Radical scavenging (%)	
	HD [b]	**MD** [c]
M. rotundifolia	32.62	21.80
BHT	64.72	

DPPH• scavenging percentage values are means of three replicates and the RSD is less than 1%; HD: hydrodistillation; MD: Microwave distillation ; BHT : 2,6-di-*tert*-Butyl-4-methylphenol (used as reference compound).

Table 3: Antimicrobial activity of the essential oils of *M. Rotundifolia* as determined by diffusion technique

Microorganism	Essential oil zone inhibition (mm)	
	HD	MO
Staphylococcus aureus CIP7625	28.3 ± 0.28	26.5 ± 0.24
Streptococcus pyogenes CIPA22	-	-
Escherichia coli CIP54.8	15.3 ± 0.20	14.2 ± 0.21
Pseudomonas aeruginosa CIPA22	-	-
Candida albicans CLM	13.4 ± 0.40	12.5 ± 0.39

CIP: collection of Institut Pasteur of Paris, French; CLM: collection to Laboratory of Microbiological of ENS, Kouba, Algiers. ; HD: hydrodistillation; MD: Microwave distillation.

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