ANTIRADICAL, ANTIMIC ROBIAL EFFECT AND CHEMICAL COMPOSITION OF ESSENTIAL OIL OF MENTHA ROTUNDIFOLIA. L FROM MOUNTAINS EL HAMDANIA, NORTH OF ALGERIA

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Abstract : This work is part of the valuation of essential oil of *Mentha Rotundifolia* harvested in the mountains of El Hamdania, in the Atlas Blideen (northern Algeria). The essential oil of *Mentha rotundifolia* extracted by hydro distillation have been analysed by means of Gas Chromatography GC-FID and GC-FID coupled to a Mass Spectrometer GC-MS in combination with retention indices. 34 constituents were identified accounting for 92 % of the essential oil. The main components were piperitenone oxide (30.37%) and piperitone oxide (23.78%). The antimicrobial activity evaluated on seven microbial strains revealed sensitivity for the majority of the bacteria except *pseudomonas aeruginosa*. 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 21.8% comparing with Quercetin (98.2%) of these oils can be attributed to the absence of some phenolic components, which may play an important role. The inhibitory effect of this essential oil on the development of bacteria and more particularly its effect on *staphylococcus aureus* sees glimpses of applications in the pharmaceutical industry as a natural antibiotic, and for other applications in the food and cosmetic industries.

Keywords: Antimicrobial effect, Chemotype, Essential oil, *Mentha rotundifolia* L, piperitone oxide, piperitenone oxide.

INTRODUCTION

Medicinal plants have been the sole source of remedies, defined by the European Pharmacopoeia as herbal drug or natural raw material with medicinal properties, used in the

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manufacture of medicines. These plants are known for their soothing and analgesic virtues, and all aim to overcome suffering and improve the health of humans (Wicht and Anton 2001). It is estimated that at least 25% of all modern medicines derive directly and indirectly from medicinal plants related mainly to the application of modern technologies to traditional knowledge (Robinson and Zhang 2011).

Algeria has a very rich plant heritage with 3000 species with several botanical families that remain little studied and little exploited pharmacologically.

The valorisation and the study of the chemistry of these plants is always topical. This is mainly due to the fact that the vegetable kingdom represents an important source of bioactive molecules.

Mentha rotundifolia. L. Huds, is synonymous with *Mentha suaveolens* ssp suaveolens L Huds, it is a perennial belonging to the family of lamiaceae frequently found in the edges of the ways and the wet places (Kokkini and Papageorgiou 1988). *Mentha suaveolens* has two subspecies, subspecies suaveolens, present in Europe, North Africa, America and Japan and subspecies insularis absent in Algeria, endemic to Corsica and Sardinia (Mailhebiau 1994) The differentiation of subspecies can be done by taking into consideration the different morphological parameters (colours, shape of the leaves and flowers, absence or presence of the petioles).

In Algeria, *Mentha rotundifolia* is widely used in traditional medicine for its multiple therapeutic virtues: Cardiovascular, hypotensive activities, vasodilators and bradycardias, antibacterial and antifungal properties. Acts as agents delaying the reproduction of the malaria vector (Page and Stearn 1990)

The leaves and stems of this plant are generally consumed an oral decoction against digestive disorders and colic, against vertigo and cooling. The dried leaves are used as a laxative (Brada et al 2006).

Research conducted on *Mentha rotundifolia* in Algeria and in the world has focused mainly on their chemical composition, who has demonstrated a great chemical diversity due to environmental factors and geographical location (Brada et al 2006, 2007; Sutour et al 2008; Beghidja et al 2007; Hussein et al 2010).

The purpose of our work is the determination of chemical composition followed by evaluation of the antimicrobial effect in vitro on some microbial strains (Sonago et al 2006) and antioxidative of essential oil of the *Mentha rotundifolia*. L. huds Algerian medicinal plant

harvested in the mountains of El Hamdania, at an altitude of 400 m , in North of Atlas Blideen.

Material and methods

Plant material

The aerial parts of *Mentha rotundifolia L.* Huds were harvested in March to April 2018 at the flowering stage from the mountains of El Hamdania, in the Atlas Blideen (northern Algeria). The identification of the plant was done in three laboratories: The Herbarium of the Vegetal Biology and the Botany Laboratory of Agronomy, University of Blida 1 and the Botany Laboratory of National School of Agronomy, El Harrach. The aerial part of the plant (leaves) was air dried and protected from light and moisture for a period of (10 days) it was then crushed and immediately subjected to isolation from essential oils.

Extraction of essential oil

The sample treated above (100gr), was submitted to hydro-distillation using a Clevenger-type apparatus containing 500mL of distilled water for 3h. The essential oil obtained were collected in compact flasks and stored in the refrigerator at 4 ° C. until used.

Gas chromatography analysis

Each oil was analysed by GC on a HP 6890 standard model, using the following conditions : two fused silica capillary columns were respectively used with an apolar stationary phase, HP5-MS (30m x 0.25mm x 0.25µm) and polar stationary phase, HP wax (60m x 0.32mm x 0.25µm); column temperature, 60°C for 8min, rising to 250°C at 2°C/min, then held for 30min at 250°C; detector FID; injector and detector temperatures, 250°C and 320°C, respectively; injected volume 0.2µL; split less mode; carrier gas, N_2, flow rate, 0.4mL/min and 0.9mL/min respectively.

The homologous series of C_7-C_{28} n-alkanes, injected into the GC under the same conditions as the oils, were used for calculating retention indices. Peak area percentages were calculated from the non-polar capillary column HP5-MS, by using the normalization method, in which the response factor for each component was assumed equal to 1.

Gas chromatography/mass spectrometry analysis

The essential oils were analysed by GC/MS using a Hewlett-Packard Computerized System Comprising a 6890 gas chromatograph coupled to a 5973A mass spectrometer, equipped with a fused-silica capillary column with an apolar stationary phase HP5-MS (30m x 0.25mm x 0.25µm). GC/MS spectra were obtained using the following conditions.

Carrier gas He, flow rate 0.5mL/min; split 1:20; injection volume 0.1µL, injection temperature 250°C; oven temperature progress from 60 to 250°C at 2°C/min; the ionization mode used was electronic impact at 70eV.

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) and with previously published spectra, and verified by comparison of linear retention indices of the identified compounds with published index data (Adams 2007; Shibamoto et al 1987). On apolar and polar columns. Relative percentage amounts of the separated compounds were calculated from FID chromatograms using the HP-5 capillary column. For each analysis, each compound was expressed as peak area by integration from the total ion current.

Experimental results were the means ± standard deviation of three values by using Microsoft Excel statistical analysis programme (n=3).

Scavenging of DPPH radical activity

The antioxidant activity of the different essential oil was determined according to the ability of the tested samples to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) (Brand-Williams *et al* 1995) by off-line spectrophotometric measurements. Methanolic solutions (2 mL) of $DPPH^{\cdot}$ (40g/L) with an absorbance at 515nm of 0.800 ± 0.030AU (Absorbance unit) were mixed with methanol solutions (1.2 mL) samples at different concentrations (300-1000) µg/mL (Leitao 2002; Chen et al 2004). Standard solutions (Quercetin and Rutin) are prepared following the same steps and the same experimental conditions. Triplicate samples were shaken and allowed to stand for 30 min in the dark at room temperature, and the decrease of absorbance at 515nm 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:

$$\text{DPPH scavenging ability (\%)} = \left[\left(A_A - A_B\right)/ A_A\right]\times 100,$$

Where A_A is the absorbance of the blank sample (t =0) methanol (containing all reagents except the sample); A_B is the absorbance of the tested sample after 30 min.

Afterwards, a curve of % DPPH scavenging capacity versus concentration was plotted and IC_{50} values were calculated. IC_{50} denotes the concentration of sample required to scavenge 50 % of DPPH free radicals.

Antimicrobial activity

The aromatogram, also called the agar medium diffusion method, consists in measuring in vitro the antibacterial effect of essential oils, this method is the equivalent of an antibiogram, and in this case the antibiotic is replaced by the essential oils

In our study, we used the agar diffusion technique (Benayad 2008; haddache et al 2017; Ouzmil et al 2002). It consists of depositing Wattman paper disks (9mm in diameter) soaked with essential oils on an agar-seeded culture to study. This substance diffuses on the surface from the disc and a decreasing gradient is established around the disc.

The microbial strains used are referenced ATCC (American type culture collection), come from the bacteriology laboratory of the Pasteur Institute: *Staphylococcus aureus* ($Gram^+$), *Pseudomonas aeruginosa* ($Gram^-$), *Salmonella typhimurium* ($Gram^-$), *Escherichia coli* ($Gram^-$), *Citrobacter freundii* ($Gram^-$), *Klebsiélla pneumonea* and a fungal strain *Candida albicans*. The antibiotic discs used are Pipemidic acid, Chloramphenicol and Fucidic acid.

RESULTS

Chemical composition

The average yield of the essential oil of *Mentha rotundifolia* was calculated from 100g of dry vegetable matter (leaves), evaluated at 0.69 ± 0.02%.

The qualitative and semi quantitative analysis of the essential oil of *Mentha rotundifolia* from the El Hamdania are listed in order of their elution on the HP-5MS non-polar column in table 1. Thirty-four compounds representing 92 % to the total composition were identified in the *Mentha rotundifolia* essential oil. The mainly components have piperitenone oxide (30.3%), piperitone oxide (23.8%) and cinerolone (21.3%). Others main components in oil were carvone (3.7%), borneol (2.6%), γ-terpinene (3.6%) and terpine-4-ol (1.3%). The rest of the compounds are in the form of traces (Figure 1)

The percentage composition of the main family classes of the essential oil of *Mentha rotundifolia* L. reveals that it is rich in ethers oxides (55.1%) followed by ketones (25.3%) and then alcohols (5.5%) and finally the monoterpenes (3.9%). the esters do not exceed 1%.

The chemical composition of the essential oil of *Mentha rotundifolia* L. is highly variable. Many compounds and their associations are at the origin of a great diversity of chemical compositions.

Table 1: Chemical composition of the essential oil of *Mentha rotundifolia* L.

№	Compounds	R I[1]	R I[2]	%	Identification
1	tricyclene	925	1012	tr	RI, MS
2	α-thujene	929	1029	0,1± 0.01	RI, MS, co-GC
3	α-pinene	937	1025	0.1± 0.01	RI, MS, co-GC
4	Camphene	952	1065	tr	RI, MS, co-GC
5	Sabinene	969	1119	0.1± 0.02	RI, MS, co-GC
6	β-pinene	974	1103	0.2± 0.04	RI, MS, co-GC
7	Octan-3-ol	975	1478	0.3± 0.03	RI, MS
8	β-myrcene	992	1165	0.1± 0.01	RI, MS
9	α-terpinene	1017	1184	0.2± 0.04	RI, MS, co-GC
10	limonene	1028	1197	tr	RI, MS, co-GC
11	β-ocimene	1039	1217	0.3± 0.04	RI, MS
12	γ-terpinene	1058	1242	2.7± 0.11	RI, MS, co-GC
13	cis-sabinene hydrate	1066	1476	0.1± 0.01	RI, MS
14	1-octen-3-yl acetate	1117	-	0.3± 0.04	RI, MS
15	borneole	1166	1677	2.6± 0.11	RI, MS
16	terpinene-4-ol	1179	1592	1.3± 0.08	RI, MS
17	myrtenal	1191	1618	tr	RI, MS
18	carvone	1244	1723	3.7± 0.13	RI, MS, co-GC
19	piperitone	1254	1713	0.2± 0.02	RI, MS
20	piperitone oxide	1274	-	23.8± 0.41	RI, MS
21	thymol	1299	-	0.9± 0.04	RI, MS, co-GC
22	dihydrocarvyl acetate	1328	-	0.2± 0.02	RI, MS
23	piperitenone oxide	1374	-	30.4± 0.43	RI, MS
24	cis-jasmone	1389	1935	0.1±0.01	RI, MS
25	cis-cinerolone	1392	-	21.3± 0.31	RI, MS
26	trans-caryophllene	1419	2038	1.1± 0.01	RI, MS, co-GC
27	β-copaene	1429	-	tr	RI, MS
28	α-cubebene	1441	-	0.1±0.02	RI, MS
29	α-humulene	1453	-	0.3± 0.04	RI, MS, co-GC
30	D germacrene	1471	-	0.1± 0.01	RI, MS
31	γ-Muurolene	1480	1694	0.1± 0.02	RI, MS, co-GC
32	α-muurolene	1500	1708	tr	RI, MS
33	Caryophyllene oxide	1583	1980	0.9± 0.05	RI, MS, co-GC
34	Viridiflorol	1591	2078	0.4± 0.03	RI, MS
Identified components (%)				92	

Monoterpene hydrocarbons	3.30
Sesquiterpene hydrocarbons	1.60
Ketones	25.30
Alcohols	5.50
Ether oxides	55.10
Ester	0.60

Components are listed according to their elution from the HP 5MS column; tr : trace < 0.05%; RI^1 and RI^2: temperature programmed indices referred to n-alkanes C_7-C_{28}, determined respectively on HP5-MS and HP-Wax capillary columns according retention to Van Den Dool and Kratz; Values represent the average of three measurements; peak area of a constituent ± SD); RI, identification based on comparison of retention index with those of published data (Adams, 2011); MS, tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N, 11N and Nist 2007 libraries and published data (Adams, 2011); co-GC, co-injection with authentic compound. * Yield expressed as in grams of oil per 100g of plant material;

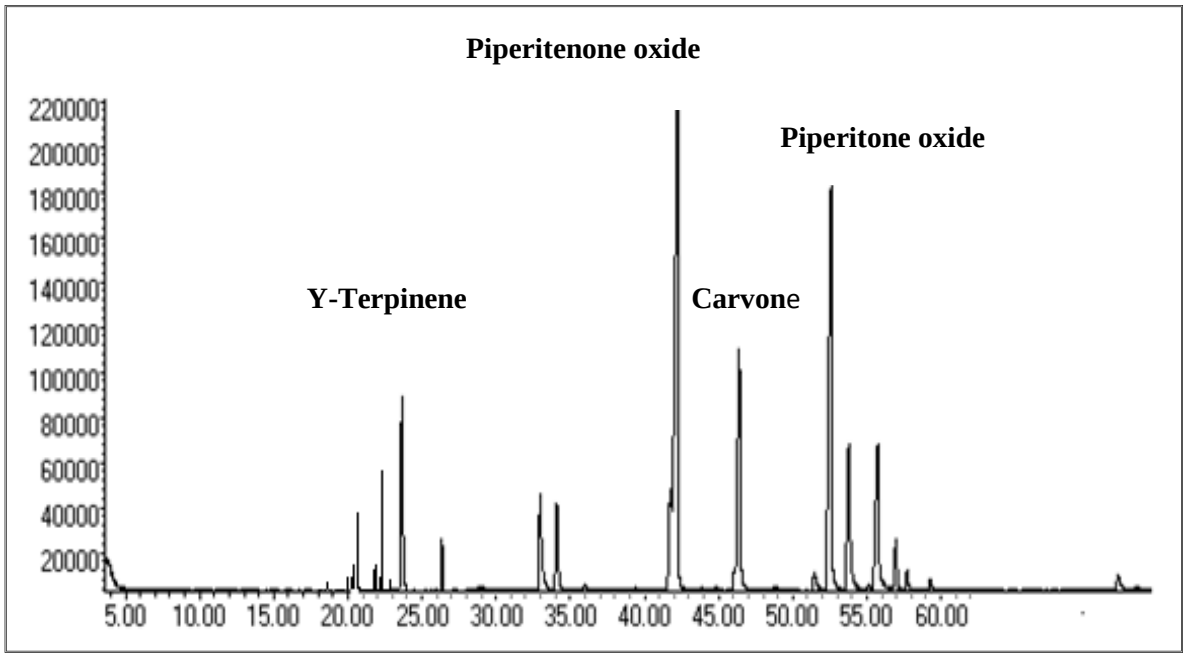

Figure 1: Chromatogram of essential oils of *Mentha rotundifolia* L. from the mountains of El Hamdania (Atlas Blideen).

Scavenging of DPPH radical activity

The antioxidant activity of essential oil of *M. rotundifolia* was examined by comparing it to the activity of known antioxidant such as Quercetin and Rutin by the in vitro assay of inhibition of DPPH radical. The results showed IC_{50} that *M. Rotundifolia* essential oil was found to be less active (1420 ± 0.04µg/mL) than Quercetin (3.60 ± 0.04µg/mL) and Rutin (160± 0.01µg/mL). The scavenging ability of essential oils and positive control is presented in table 2.

Table 2. Radical scavenging activity of the essential oil against DPPH radical

Sample	Radical scavenging DPPH		
	IC_{50} (µg /ml)	**ARP (1/ IC_{50})**	**DPPH scavenging ability %**
Essentials oil	1420 ± 0.04	0.70	21.7
Quercetin	3.90 ± 0.04	185.87	98.2
Rutin	160 ± 0.01	6.21	64.8

Antimicrobial activity

The antibacterial activity of gasoline aromatic of *Mentha Rotundifolia* performed by aromatogram, was performed on seven microbial strains. Analysis of variance, showed a significant difference, by comparison of means by pair of diameters of Inhibition Zones (DZI).

The results obtained (table 3) show that the essential oil of *Mentha rotundifolia* L. is active on the majority of bacteria tested and weakly active on *candida albicans*. Depending on the diameters of inhibitions, the essential oil is more active on *staphylococcus aureus* (26.26 mm) and *citrobacter freundii* (18 mm), moderately active on *salmonella typhimurium* (14.16 mm), *escherichia coli* (14.23 mm), and *klebssiella pneumonia*. The pseudomonas aeruginosa strain is particularly resistant to the essential oil.

Table 3. Averages of inhibition zone diameters of essential oil on strains tested.

Microorganism		Gram	DZI (mm ± sd)	
	ATTC		EO	DMSO (NC)
Staphylococcus aureus	6538	+	26.26 ± 0.25	-
Salmonella typhimurium		+	14.16 ± 0.15	-
Escherichia coli	4157	-	14.23 ± 0.18	-
Pseudomonas aeruginosa	9027	-	0	-
citrobacter freundii		-	18.23 ± 0.20	-
klebssiella pneumonia	4352	-	14.33 ± 0.28	-
Candida albicans	24433	Fungi	12.4 ± 0.13	-

ATCC: American Type Culture Collection; DZI: diameters of the inhibition zones; NC: negative control; the sensitivity of different strains to essential oil is classified according to their diameter and according to the following criteria:

- Not sensitive (-) for a diameter less than 9 mm.
- Sensitive (+) for a diameter of between 9-14 mm.
- Very sensitive (++) for diameters between 15-19 mm
- Extremely sensitive (+++) for diameters greater than 20mm. [25].

DISCUSSION

The yield of essential oil of *Mentha rotundifolia* (0.69 ± 0.02%) is similar to that obtained for from southern Algeria (0.70% and 0.90%) (Beghidja et al 2007) and (0.73%) (Haddache et al 2017). The highest yield (4.33%) is that of *Mentha rotundifolia* from Morocco (Derwich et al 2010); nevertheless, low levels were reported on *Mentha rotundifolia* from Naciria (60 km in the east of Algiers) obtained by hydrodistillation and microwave, (0.22 and 0.13% respectively) (Haddache et al 2017). The very low yields were report on *Mentha rotundifolia* of Corsica (0.08% - 0.10%) (Sutour et al 2008). The low value obtained can be attributed to several intrinsic parameters (growth stages and the age of the plant material) and extrinsic the plant as the period and middle of harvest, cultural practices, and all soil and climate conditions, drying and extraction methods) (Benayad 2008). Factors abiotic or physicochemical characteristics have an effect on the essential oil yield, humidity, temperature, sunshine time

The chemotype of the essential oil analysed is piperitenone oxide (30.3%), piperitone oxide (23.8%) which are ether oxides and cinerolone (21.3%) which is a ketone. GC and GC / MS analysis of the essential oil of *Mentha rotundifolia* from northern, northern east and east Algeria (Beghidja et al 2007; Chen et al 2004; Galombosi et al 1998) revealed the existence of two chemotypes characterized by different levels of piperitone oxide and piperitenone oxide.

The piperitenone oxide has been report as the major constituent of the essential oils of *Mentha rotundifolia* (87.3% and 80.8%, respectively) in Japan and Uruguay, piperitone oxide has been report of the essential oils from Greece, Morocco and Italy (40.5%) (Kokkini and Papageorgiou 1988; Ouzmil et al 2002; Lawrence 2007; Lorenzo et al 2002). Some research was shows that the most important constituents are oxygenated monoterpenes (Mailhebiau 1994), such as: pulegone (50%) in Morocco, carvone (62.3% and 40.30%, respectively) in Finland and Argentina (Derwich et al 2010; Handa et al 1964; Galombosi et al 1998).

In conclusion, our results corroborate with those obtained in essential oil of Naciria in Northern east of Algeria (Haddache et al 2017) which show richness in piperitone oxide (25.06%) and piperitenone oxide (8.92%).

The difference in the composition of essential oils depend on several biotic and abiotic factors, such as the climate specific to regions of origin of plants, the geographical factors (altitude, the nature of the soil and period of harvest) and the extraction techniques.

Our results DPPH radical scavenging activity of the oil (IC50 = 1420 µg/ml) are significantly lower compared with those obtained, on *Mentha rotudifolia* from Iran, (IC50 21.7 µg/ml) (Nickavar et al 2008), on *Mentha rotudifolia* from two regions in Tunisia (Bizerte, Bejaia), with an IC50 = 26.11µg/ml, IC50 = 29.52 µg/ml respectively (Riahi et al 2013).

The weak DPPH radical scavenging activity of the oil could be attributed to the absence of some phenolic components, which may play an important role. It is interesting that Quercetin and Rutin is a pure chemical substance, while the essential oil of *Mentha Rotundifolia* used consists of several natural active substances or a few of them must have this antioxidant capacity. However, it has been shown that the antioxidant activities of the major compounds of essential oils tested separately, often give inferior results compared to the total activity of the essential oil (Safaei-Ghomi, 2009). In general, the synergy of the different constituents of an essential oil is at the origin of the antioxidant power. In addition, certain constituents of essential oils such as the hydrocarbon constituents also have a strong antioxidant activity (Vardar-Ünlü, 2003).

This difference in the IC50 values can be attributed on the one hand to the difference in the concentration of DPPH• used in the test and the incubation time (Sharma et al, 2009) and to the influence of other intrinsic factors and extrinsic which can affect the chemical composition of plants. On the other hand, it should also be noted that this strong activity in reducing free radicals is due in large part to the chemical composition of the essential oil.

The results of the antimicrobial activity reveal that the essential oil of *Mentha Rotundifolia* inhibited the growth of all the bacteria and yeasts tested, with the exception of the pseudomonas aeruginosa strains. Staphylococcus aureus Gram + bacteria, was strongly inhibited by the essential oil with (26.26 ± 0.25 mm). This result seems interesting especially that this bacterium is pathogenic for humans and that it is the source of dangerous food poisoning. It is interesting to note that the essential oil of *Mentha rotundifolia* L. is sensitive to *salmonella typhimurium* (14.16 ± 0.15), knowing that this microbial species has been shown to be very resistant to antimicrobial agents (Sivropoulou et al 1995).

Among the Gram – bacteria, *citrobacter freundii* is strongly inhibited (18.23 ± 0.20 mm) by essential oil followed by *klebsiella pneumoniae* (which is also pathogenic bacteria for humans causing many serious infections) and *escherichia coli* with a similar inhibition zone respectively (14.33 ± 0.28 mm), and (14.23 ± 0.18 mm). This result is not surprising because these bacteria possess an intrinsic resistance to biocidal agents, this is in relation with the nature of the outer membrane, which is composed of lipopolysaccharides, is a layer impervious to hydrophobic compounds (Ponse et al 2003). The *pseudomonas aeruginosa* has been shown to be resistant compared to the microorganisms studied, as this bacterium has been cited by several authors as being very resistant to all antimicrobial agents and antibiotics (Hussein et al 2010; Mimica-Dukié et al 2003). According to these results, the resistance of this strain is probably not linked to the chemical composition of the essential oil but it may be due to the structure and the composition of the wall of this strain; responsible for this resistance.

Regarding *candida albicans*, the essential oil is weakly active on this strain (11.25 mm). Work on *Mentha rotundifolia* shows that the essential oils of this species exert a strong activity on *staphylococcus aureus* and a moderately strong effect on *escherichia coli*. Fungi are resistant and less vulnerable than bacteria. This high activity has been attributed to the high pulegone content (Arras and Usai 2001). The same result was obtained for our species but for an essential oil rich in piperenone oxide (30.32%).

The biological activity of an essential oil is linked to its chemical composition, to the functional groups of the main compounds and to the possible synergistic effects of these constituents (Chebli et al 2003).

Durraffourd et al 2002, affirm that it is probable that the minority compounds act in synergy; in this way, the value of the essential oil is due to all of these components.

CONCLUSION

This work carried out on the study of the chemical composition and the antimicrobial activity of the essential oils of *Mentha rotundifolia* made it possible to obtain a chromatographic profile consisting of 34 compounds representing 92% of the total composition of the oil. Majority compound is the piperitenone oxide, this result allowed to deduce that our studied plant is chemotyped: essential oil piperitenone oxide-piperitone oxide.

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 21.8% comparing with Quercetin (98.2%) of these oils can be attributed to the absence of some phenolic components, which may play an important role.

The essential oil of this plant inhibits the bacterial growth of all bacteria with the exception of *pseudomonas aeriginosa* which is particularly resistant. Its activity is very weak on the *candida albicans* fungal strain.

The effect of essential oil is very marked on *staphylococcus aureus*. These results can contribute to the valorisation of this plant by the local production of this essential oil. The inhibitory effect of this essential oil on the development of bacteria and more particularly its effect on *staphylococcus aureus* sees glimpses of applications in the pharmaceutical industry as a natural antibiotic, and for other applications in the food and cosmetic industries.

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