CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS FROM *Mentha pulegium* L. AND *Mentha suaveolens* Ehrh. GROWING IN NORTH-EAST OF ALGERIA

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Abstract. *Mentha pulegium* L. and *M. suaveolens* Ehrh. are spontaneous plants growing in the wetlands of the Souarekh region (north-eastern Algeria). They are very well known and used by the natives of this region for different virtues. Moreover, these subspecies have been chosen for their great morphological variations. Hence, in this study, we aim at determining the chemical composition of the essential oils of both plants and evaluating their antibacterial activities.

The aerial parts of the plants were hydrodistilled using a Clevenger type apparatus and the essential oils were analyzed and identified using gas chromatography-mass spectrometry. Antibacterial screening of essential oils was carried out on the basis of the diameter of the inhibition zone using the disc diffusion method against two bacterial strains. The chemical analysis of the essential oils of both mints resulted in main compounds pulegone (61.24%), isomenthone (11.32%) and menthone (5.6%) for *M. pulegium*, and piperityone oxide (47.52%) for *M. suaveolens*. Accordingly, the study of the biological activity of essential oils manifested the antibacterial effect against the strains studied. The obtained results are therefore promising and thus pave the way for manufacturers to use the essential oils of the two plants in the pharmaceutical and food industries.

Keywords: Essential oils; *Mentha pulegium* L.; *Mentha suaveolens* Ehrh.; Chemical composition; Antibacterial activity; Algeria.

INTRODUCTION

In the recent years, the use of medicinal and aromatic plants in phytotherapy has been developed through exploiting several herbs such as the mint species, (family Lamiaceae) which includes nearly 20 species [52]. Several species are known as aromatic and medicinal plants in Asia Minor, the Middle East, Europe and North Africa (including Algeria) [7, 9, 22].

The study of the chemical composition of the essential oils showed variability of its constituents across the region [31, 48]. This variety is probably related to various vegetative phases of the plant, to the environmental conditions as well as to seasonal and geographical variations and to soil composition [35].

In Algeria, the use of spontaneous mints for food and phytotherapeutic is known in coastal regions such as the region of Souarekh (El Tarf, northeast - Algeria). This region is, also, famous for its richness in medicinal and aromatic plants, favored by its sub-humid climate and the important of the recovery of its meadows floodable with mints mainly pennyroyal (*M. pulegium*) and mint with round leaves (*M. suaveolens*).

In continuation of our research program on the valorisation of natural resources of Algeria, the objective of this work is to study the antibacterial activity of two medicinal palnts: *M. pulegium* and *M. suaveolens* from the Souarekh region which is frequently used for the treatment of various infectious diseases [15]. In addition, the chemical composition of the essentiel oils was investigated, to establish a potential link between antibacterial activity and specific compounds.

MATERIAL AND METHODS

Plant material

M. pulegium and *M. suaveolens* that are used in the study were identified at the Laboratory of Agriculture and Ecosystem Functioning following the procedure described by Dobignard and Chatelin (2010-2013) [25]. Aerial parts of these plants were collected from Souarekh region - northeast Algeria (Figure 1) in May 2018. Afterwards, leaves were separated and dried in open air for two weeks in darkness.

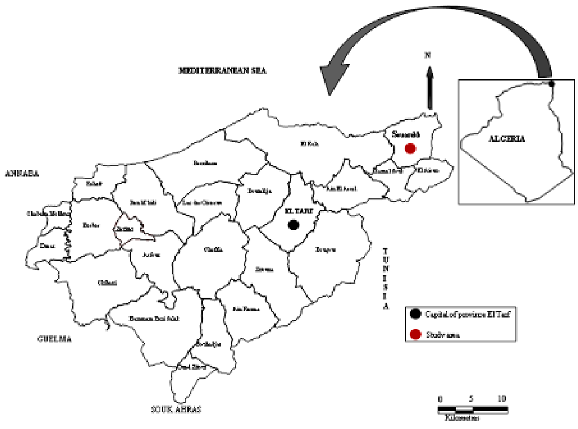

Figure 1. Geographic location of Study area (Souarekh)

Extraction of essential oils

After two weeks of drying, the essentiel oils were extracted from the dried plants (100 g) by hydrodistillation, at a temperature of 100 °C using a Clevenger-type apparatus and the recovery time was optimized to two hours. After extraction, the obtained essentiel oils were dried over anhydrous sodium sulphate and, kept in an amber color polyethylene vials and stored at 4 °C until tested.

Analysis of essential oils

The essential oils were analyzed using a Perkin Elmer Clarus 600 GC System, fitted with a Rtx-5 MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness; maximum temperature, 350 °C), coupled to a Perkin Elmer Clarus 600C MS. The carrier gas was helium at a flow rate of 1.5 mL/min. The injector, transfer line and ion source temperatures were 280, 270 and 260°C, respectively. The ionizing energy of MS was 70 eV. The injected sample volume was 0.3 mL with a split ratio of 200:1. The oven temperature program was 60 °C at a rate of 3 °C/mine 280 °C hold for 10 min. Components identification was achieved in comparing respective retention indices relative to C_8 C_{28} (n-alkanes); and respective mass spectra to those reported in the literature [1] and in (NIST 2011 v.2.3 and Wiley, 9th edition) mass spectral libraries.

Method for evaluating antibacterial activity

Choice of strains

The choice of strains is tested according to the use of traditional medicine of pennyroyal and round leaf mint; 4 strains were chosen: 2 of gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) and 2 of gram positive (*Staphylococcus aureus* and *Pseudomonas aeruginosa*).

Aromatogram method

The antibacterial activity is evaluated by the aromatogram method which makes it possible to determine the sensitivity of the various bacterial species with respect to the given essential oil. The aromatogram method consists of using Petri dishes containing a suitable agar medium, already solidified and inoculated with the microbial strain tested. Blotting paper discs 6 mm in diameter, previously impregnated with known quantities of essential oil (07µl), are then placed on the agar surface. Generally, microorganisms are classified as susceptible, intermediate or resistant, depending on the diameter of the inhibition zone [54].

Measurement of antimicrobial activity of essential oils

The aromatogram technique was applied to assess the antimicrobial activity of essential oils. This test is carried out by depositing a sterile disc 6 mm in diameter impregnated with an amount of essential oil on an agar medium previously seeded with a microbial culture. After incubation, the results are read by measuring the diameters of the zones of inhibition in millimeters [4, 20].

RESULTS

Yield of essential oils

The yields of essential oils of *M. pulegium* and *M. suaveolens* that are harvested from the Souarekh region are expressed in milliliters compared to 30 g of dry vegetable matter. These rates are 1.82% for *M. pulegium* and 1.03% for *M. suaveolens*.

Organoleptic characteristics

The organoleptic characteristics of the essential oils of *M. pulegium* and *M. suaveolens* obtained by hydrodistillation are presented in Table 1.

Organoleptic properties are a means of checking and controlling the quality of the essential oil. To this end, the results obtained are compatible those reported by AFNOR (1986) [2] having analyzed the essential oils of the two mints studied.

Chemical composition of essential oils from *M. pulegium* and *M. suaveolens* from the Souarekh region

The compositions of the extracted essentiel oils were identified on the basis of GC retention time (t_R) on fused silica capillary column and are shown in Table 2.

The GC/MS analyses showed the presence of twenty two chemical compounds for the *M. pulegium*, which represents about 97.9% of the total composition. Only Nineteen compounds were obtained for the *M. suaveolens*, representing over 75.2% of the total oil compositions.

The *M. pulegium* and *M. suaveolens* essentiel oils were composed of to 90.73% and 62.85% respectively of oxygenated monoterpenes. The high percentage component found in the *M. pulegium* essentiel oil was as pulegone (61.24%), isomenthone (11.32%), menthone (5.6%), neo-menthol (3.33%) and sabinene (2.09%). In the case of *M. suaveolens*, was as piperitenone oxide (47.52%), pulegone (8.44%) and limonene (2.81%). Other minor chemical components with different percentage obtained from the *M. pulegium* and *M. suaveolens* oils are also listed in Table 2.

Antibacterial activity of essential oils from *M. pulegium* and *M. suaveolens* from the Souarekh region

The aromatogram test of the essential oil of *M. pulegium* and *M. suaveolens* from the Souarekh region is shown in Table 3.

So, the results indicate that the essential oils of the mints studied have antimicrobial activity on all the bacteria tested. This sensitivity is different according to the strains and to the two species studied.

The *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains are very sensitive to the essential oils of both *M. pulegium* and *M. suaveolens*. While the *Escherichia coli* strain showed less sensitivity to essential oils of the same species.

The results obtained from Table 3, manifest that the bacteria examined are very sensitive to pure oil. On the other hand, these germs have a relative sensitivity towards ½ and / or ¼ dilutions of the previouslly stated mints essential oils of the two mints studied.

Table 1. Organoleptic characteristics of the essential oils from *M. pulegium* and *M. suaveolens*

Parametres	*M. pulegium*	*M. suaveolens*
Aspect	Clear, mobile liquid	Clear liquid
Odour	Very aromatic	Spicy, Penetrating
Color	Pale yellow	Yellow

Table 2. Chemical composition of the essential oils obtained from *M. pulegium* and *M. suaveolens* collected in the Souarekh region

N°	Compound name and class	t_R [min]	RI[a]	Content [%][b]	
				M. pulegium	*M. suaveolens*
1	α-pinene	3.00	934	0.73	0.45
2	camphene	4.31	945	0.02	-
3	sabinene	4.59	973	0.57	0.52
4	β-pinene	5.65	985	2.09	-
5	myrcene	6.40	993	0.28	0.67
6	limonene	6.52	1023	1.94	2.81
7	1,8-cineole	7.23	1036	0.62	0.19
8	linalool	8.43	1105	0.21	0.83
9	borneol	9.76	1146	-	0.92
10	menthone	10.03	1151	5.6	-
11	isomenthone	10.52	1159	11.32	1.64
12	menthofuran	11.47	1164	0.91	0.86
13	menthol	12.33	1168	0.99	-
14	neo-menthol	13.01	1172	3.33	-
15	*cis*-isopulegone	13.47	1174	1.83	-
16	α-terpineol	14.88	1193	0.45	0.22
17	bornyl acetate	15.77	1283	-	0.75
18	pulegone	16.45	1249	**61.24**	8.44
19	piperitone	17.20	1254	1.42	-
20	piperitenone	18.63	1332	0.89	1.28
21	piperitenone oxide	19.12	1343	1.92	**47.52**
22	(E)-β-caryophyllene	20.18	1409	0.64	1.39
23	δ-cadinene	21.30	1429	-	1.11
24	α-humulene	21.69	1445	0.38	-
25	α-gurjunene	22.38	1459	-	3.25
26	β-bourbonene	23.77	1469	-	0.14
27	germacrene D	29.45	1481	0.52	2.01
	Total identified			**97.9**	**75.2**
	Monoterpene hydrocarbons			5.63	4.45
	Oxygenated monoterpenes			90.73	62.85
	Sesquiterpene hydrocarbons			1.54	7.9

[a]RI : Retention index; [b] Content : expressed as percentage of the total oil composition in the *M. pulegium* and *M. suaveolens; –*: Not detected.

Table 3. Inhibition diameters (mm) of the different strains tested in the aromatogram in solid medium

Species	*M. pulegium*					*M. suaveolens*				
Dilution / Strains	Single disc	DMSO disk	Pure oil	Dilution 1/2	Dilution 1/4	Single disc	DMSO disk	Pure oil	Dilution 1/2	Dilution 1/4
Staphylococcus aureus ATCC 25923	6	6	17	10	8	6	6	18	11	9
Pseudomonas aeruginosa ATCC 27853	6	6	14	9	8	6	6	15	8	7
Escherichia coli ATCC 25922	6	6	9	7	6	6	6	10	8	8
Klebsiella pneumoniae ATCC 70603	6	6	15	8	6	6	6	16	10	8

DISCUSSION

As a matter of a fact, the study carried out in the commune of Souarekh of the wilaya of El Tarf (extreme northeast of Algeria) on the chemical composition of the essential oils of two known mints of the region was very interesting.

The results obtained indicate that the yield is higher in the species *M. pulegium* with 1.82% than in the species *M. suaveolens* with 1.03%. The same observation was recorded in Algeria: by Benabdallah (2017) [10] in other regions from the wilaya of El Tarf, by Brahmi *et al*. (2016) [19] in the Smaoun region from the wilaya of Bejaia and by Benazzouz & Hamdane (2012) [13] in the Ouagnoun region from the wilaya of Tizi-Ouzou; Besides in Tunisia by Soilhi *et al*. (2019) [48] in the Sedjnane region from the wilaya of Bizerte and Tabarka from the wilaya of Jandouba.

Additionally, for *M. pulegium*, the yield is lower than that of the species studied in Morocco by Cherrat *et al*. (2014) [21], Ait-Ouazzou *et al.* (2012) [3] and Hmiri *et al.* (2011) [33] with 1.9%, 2.7% and 3.30% respectively. However, it is higher than that obtained by: Mata *et al*. (2007) [43] in Portugal (0.7%); Sutour (2010) [49] in Corsica (0.62 to 0.84%); Kamkar *et al.* (2010) [36] in Iran (0.65%); Başer *et al*. (2012) [8] in Turkey (1.2%); Benabed *et al*. (2017) [11] at Djelfa in Algeria (1.47%) and Attou (2017) [5] at Ain Temouchent in Algeria (1.37%).

By the way, Brada *et al*. (2007) [18] having studied the phytochemistry of *M. suaveolens* in the regions of Chlef and Ain Defla (Algeria), indicate the recording of a yield in essential oils of between 0.7% and 0.9% during the end of flowering period. This yield corresponds to that obtained in this study and this for the early flowering phase. Still in the Ain Defla region but this time in the pre-flowering phase, a yield of 0.73% was noted by Sahnoune & Zebboudj (2019) [46].

Other studies carried out on this species in the Maghreb indicate that the yield is higher than that obtained in this work. It is 1.26% in Tunisia [44], 1.17%, 2.33% and 4.33% in Morocco [12, 24, 37]. The opposite would be for the yield recorded in Corsica since it is between 0.08% and 0.10% [49]. Variations in essential oil yield can be attributed to genetic, environmental and ontogenic factors, as well as to analytical methods [32, 40].

In the second part of our study, we are interested in studying the chemical composition of the essential oils of *M. pulegium* and *M. suaveolens*. We have found that the chemical composition of the essential oils of *M. pulegium* is, therefore, characterized by pulegone; the main constituent, as in many oils described in the literature [5, 6, 10, 19] in Algeria, [29, 47, 48] in Tunisia, [3, 14, 17, 21, 23] in Morocco, [27] in Egypt, [28] in Italy, [38] in Greece and [41] and in Uruguay.

Furthermore, it is important to mention that the association of pulegone (61.24%) with both isomenthone (11.32%) and menthone (5.6%) are no longer original from the essential oils of *M. pulegium* from Corsica in France as mentioned by Sutour (2010) [49] in his thesis because the present study indicates the existence of the aforementioned association. The same observation was made in Tunisia by Hadjlaoui *et al*. (2010) [29] and by Soilhi *et al*. (2019) [48]. The geographical location of the Souarekh region, on the one hand on the Tunisian border and on the other hand on the extreme eastern coast of Algeria (approximation compared to the island of Corsica), suggests that the unique outcome probably due to an ecotype of *M. pulegium*. The variations in the chemical composition of essential oils from one species, from one region to another and from one country to another are attributed to edaphoclimatic factors that can influence the regulation of the biosynthesis of essential oils [28, 35, 55].

In addition, the antibacterial activity of the essential oil was tested by the disc diffusion assay. Gram positive bacteria are known to be more susceptible to essential oils than Gram negative bacteria [45]. Gram-negative organisms are less susceptible because they possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering [53]. Gram positive bacteria with a simple membrane structure are less protected against the diffusion of fine essential oils particles [34]. However, the weak antibacterial activity of Gram negative bacteria observed in this investigation is in accord with other studies [5, 21, 50].

The essential oils of *M. pulegium* have shown a strong inhibitory effect against most of the bacteria tested and the results obtained are in agreement with those of pennyroyal from Tunisia [30, 42] and that from Portugal [51]. However, in Algeria, weak antibacterial activities against the same strains tested in this study were found for *M. pulegium* from the Sétif region [16] and that from the Béjaia region [19].

Also, it should be noted that the best bacteriostatic and bactericidal effects are found among the Moroccan medicinal and aromatic plants tested [3, 21].

The bactericidal effect of the essential oil of *M. pulegium* could be attributed to the presence of the pulegone in high quantity (61.24%) especially for the strain *Pseudomonas aeruginosa* known for its resistance to essential oils of Lamiaceae. This result consolidates that obtained by Taalbi (2016) [50] and Benabed *et al.* (2017) [11].

The essential oil of *M. suaveolens* has been shown to be more active than that of *M. pulegium* (Table 3). It has shown strong antimicrobial activities against certain microorganisms tested, particularly Gram-positive bacteria: *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The inhibition of growth was obtained by undiluted and diluted essential oils. The same result was obtained by Brahimi *et al.* (2016) [19]. In addition, a weak antibacterial activity against Gram negative bacteria has been noted mainly for *Escherichia coli*, this could be explained by the fact that it has an external lipopolysaccharide wall surrounding the cell wall of peptidoglycans, which limits the access of these compounds [44]. On the contrary, the essential oil of *M. suaveolens* collected in the south-east of Algeria, Tunisia and Morocco has shown an activity in particular against *Escherichia coli* [26, 39, 44]. The possible explanation for the differences in activity could be associated with the chemical compositions of the oil [19, 26]. Characterized by a high abundance of piperitenone oxide, the essential oil of *M. suaveolens* has good bactericidal activity for the strains tested. This result agrees with that of Ait-Ouazzou *et al.* (2012) [3] and Taalbi (2016) [50].

Due to their antimicrobial activity, the essential oils of *M. pulegium* and *M. suaveolens* can be used in the pharmaceutical industry for the production of new synthetic agents in the treatment of infectious diseases caused by the bacteria studied or can be suggested as natural agents food preservation as reported by some authors [3, 5, 50].

In conclusion we can say thatboth mint species studied are promising plants for further research in order to obtain a biopesticide allowing the fight against insect pests of stored products.

The essential oil of *M. pulegium* with a high abundance of isomenthone and menthone constitutes a good commercial alternative to that rich in pulegone. Like the essential oil of *M. suaveolens* rich in piperitenone oxide constitutes a strong economic potential because of the interest carried to this molecule by the pharmaceutical industry.

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