Original article

Chemical Characterization, Evaluation of the Antibacterial and Antioxidant Activities of the Essential Oil of Algerian (*Myrtus communis* L).

Benhadid Rym[1], *Hadef Youcef*[2], *Djahoudi Abdelghani*[3], *Hosni Karim*[4]

[1]Departement of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria;[2]Laboratory of Analytical Chemistry, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar University, Annaba, Algeria; [3]Laboratory of Microbiology, Department of Pharmacy Faculty of Medicine - Badji Mokhtar University Annaba, Algeria; [4]Laboratory of Natural Substances, National Institute of Research and Physical-Chemical Analysis, INRAP Sidi Thabet, Tunisia

Abstract. The essential oil extracted from Algerian Myrtle were analysed by gas chromatography/mass spectrometry (GC/MS), it revealed 62 components accounting for 98.62 % of the oil. The major components were α-pinene (24.83 %), 1,8-cineole (10.8 %), limonene (6.07 %), α-Terpineol (4.14%). Methyl eugenol (3.48%), The in vitro antibacterial activity was performed by agar disc diffusion and (MIC). The oil was tested against *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus (MRSA), Escherichia coli ATCC 25922, ciprofloxacin resistant Escherichia coli* (*E. coli* cip R). *Pseudomonas aeruginosa* VIM 2, imipenem- resistant *Pseudomonas aéruginosa* (*Pseudomonas aeruginosa* IMP), *Pseudomonas aéruginosa* ATCC 27853, *Enterobacter cloacea*, carbapenemase-negative *Klebsiella pneumonia* (KPC -), carbapenemase-positive *Klebsiella pneumoniae* (KPC+), *Klebsiella pneumoniae*, *Acenitobacter baumannii*. The antibacterial test revealed that myrtle oil showed a good antibacterial effect except against pseudomonas. The antioxidant activity of essential oil was evaluated by DPPH assay. The results showed the highest free radical scavenging activity with dpph (93.39 %) at the concentration of EO 4 mg/ml.

Key words: *Myrtus communis* L, essential oil, chemical composition, Pathogenic Bacteria, antibacterial activity, antioxidant activity

Introduction

Essential oils are volatile organic compounds found in various plant tissues such as fruits, leaves, flowers, bark, stem, seeds, wood and roots (1). Essential oils as natural products, are complex chemical mixtures that consist of several types of molecules. Most of the volatile constituents of essential oils are terpenoid derivatives (2). A wide spectrum of biological activities have been reported for essential oil including antioxidant, antibacterial, antiviral, antifungal, anticancer, anticonvulsant, spasmolytic, expectorant, immunomodulatory and antidiabetic activities (3).

Myrtus communis L. (Myrtle) (Myrtaceae) is an evergreen shrub which grows mainly in Mediterranean climates and has long been used by locals for its culinary and medicinal properties (4).

Algeria is the largest country in the Mediterranean region. It is recognized by its varietal diversity in medicinal and aromatic plants, and in particular the myrtle which grows spontaneously in the coastal region, in the internal hills, and in the north of the forest

areas. Its aromatic properties have long been known. The benefits of Myrtle are diverse. The different parts of the plant are used in cosmetology and medicine. Leaves, fruits, flowers and roots are indicated as a treatment for several diseases (5, 6).

In Algeria, infusions or decoctions of the plant are used for the treatment of hypotension, diabetes, rheumatism, diarrhea, gastrointestinal system diseases and anxiety (7, 8). In Morocco, the plant is used to treat fatigue (9) and dermatologic affections (10).

According to an ethnobotanical study in the region of Kahramanmaras in Turkey *M. communis* leaves and fruits are used in the treatment of skin disorders, wounds, eczema, heart diseases (11) and constipation (12).

M. communis contains essential oils in its leaves, flower and fruit glands. The essential oil obtained from Myrtle has a variable composition. The main constituents are normally α-pinene, 1,8-cineole and myrtenyl acetate (13). Even today, more than 50 active ingredients of M. communis oil have been identified and the major components were determined by gas chromatography-mass spectrometry are α-pinene, limonene, 1, 8- cineole, 4- terpineol, α-terpineol, linalool, geranyl acetate, methyl eugenol, phenolic and acetate compounds (14).

According to the previously published papers on this particular topic, myrtle essential oil possesses strong antimicrobial and antioxidant activity that makes it a valuable raw material for the cosmetic, pharmaceutical and food stuff industries. (15) (16).

Therefore, the present work attempts to determine the chemical composition of the leaf essential oil of Algerian *M. communis* and to evaluate its antibacterial and antioxidant activities.

Materials and methods

Plant Material

Fresh leaves of *M. communis L.* were collected from Seraidi region (North-East of Algeria) in august 2016. Samples were air-dried in shade.

Extraction of essential oil

The essential oils were extracted by hydro-distillation using a Clevenger-type apparatus. The oil was stored at 4°C in the dark until analysis. The yield was expressed in percentage.

Analysis of the essential oil

The gas chromatography–mass spectrometry (GC–MS) analyses were performed on a gas chromatograph HP 6890N interfaced with an HP 5975 mass spectrometer (Agilent Technologies, Palo Alto, Ca, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (60 m × 0.25 mm, 0.25 mm film thickness) was used for the separation of volatile compounds. 1 µL of Diluted oil samples in hexane (2%) was injected with a split ratio of 1:60. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 mL/ min. Scan time and mass range were 1 s and 50–550 m/z, respectively. The volatile compounds were identified by comparison of retention indices relative to C_7-C_{24} n-alkanes with those of literature and/or with those of authentic compounds available in our laboratory, and by matching their mass spectral fragmentation patterns with corresponding data (Wiley 275.L and NIST 05 libraries). Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak areas without use of the correction factor.

Evaluation of antibacterial activity
Bacterial strains

The bacterial strains tested were provided by the Laboratory of Medical Microbiology, Faculty of Medicine Annaba. the bacterial species and strains used in this study were:

Gram-positive

Staphylococcus aureus, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* (MRSA).

Gram-negative

Escherichia coli ATCC 25922, ciprofloxacin resistant *Escherichia coli* (*E.coli* cip R). *Pseudomonas aeruginosa* VIM 2, imipenem- resistant *Pseudomonas aéruginosa* (*Pseudomonas aeruginosa* IMP), *Pseudomonas aéruginosa* ATCC 27853, *Enterobacter cloacea*, carbapenemase-negative *Klebsiella pneumonia* (KPC-), carbapenemase-positive *Klebsiella pneumoniae* (KPC+), *Klebsiella pneumoniae, Acenitobacter baumannii*. The strains were revived from frozen (–70°C) stocks and subcultured for purity. Bacteria were grown on nutrient agar and incubated at 37°C for 24 hours.

Antibacterial tests

The antimicrobial activity of oils was determined through the aromatogram (agar disc diffusion) and minimum inhibitory concentration (MIC)

The aromatogram is a qualitative method to test the antimicrobial activity of a substance against a particular microorganism. This method has been prepared using essential oils of *M. communis.* Essential oil was used at different concentrations: pure oil, diluted oil in Dimethyl sulfoxide (DMSO) to ratio 1/2, 1/4 and 1/8. Little sterilized disks of blotting paper saturated with 10 **µ**L of essential oil were placed on the surface of a Müeller Hinton plate count agar previously spread with bacterial inoculum.. After a latency period at 37°C±1 for 24 h, the diameter of the inhibition halo of was measured with a caliber measured and expressed in mm (including the diameter of the disc of 6 mm). (17)

Determination of minimum inhibitory concentration (MIC):

The MICs of the HE were determined by incorporation method in an agar medium (18, 19). Serial dilutions of essential oils were performed in dimethylsulfoxide (DMSO). The dilutions obtained are added MH agar, melted in a water bath and cooled to 45 °C, in order to obtain the concentrations of HE per milliliter of culture medium: 1, 0.5, 0.25, 0.125, 0.075 and 0.05. Witness discs containing culture medium and only DMSO were also prepared. Seeding was done as a deposit of bacterial suspension. After incubation at 37°C for 24 hour, the growth was compared to the control. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms.

Evaluation of antioxidant activity with 2,2;- di-phenyl-1-picrylhydrazyl (DPPH) method

The DPPH solution was prepared by solubilizing 2.4 mg of DPPH in 100 ml of methanol.100 **µ**l of the essential oil at different concentrations was added to 2 mL of DPPH solution. The mixture was shaken vigorously for 1 min and left to stand for 30 min in the dark at room temperature. Absorbance was measured at 517 nm against a blank (DPPH / methanol).

Vitamin C (ascorbic acid) was used for comparison as standard antioxidants. Inhibition of free radical DPPH as percentage [I (%)] was calculated as follows.

I (%) = (Ablank -A sample) / Ablank ×100.

where I (%) is the total antioxidant activity, A blank is the absorbance of the control and A sample is the absorbance of the test com- pound. IC50 value (**µ**g /mL) is the concentration at which DPPH radicals are scavenged by 50 %. This was obtained by interpolation and using linear regression analysis.

The results are expressed as mean values ± standard deviation (SD).

Results and discussion:

Essential oil yield

The essential oil yields obtained of *M. communis* leave was 0.62%, These results are in agreement with those obtained by (20) who found a yield of 0.6% for the essential oil of myrtle in the region of Chlef Algeria.

According to (21), the average yield of myrtle essential oil in Morocco is of the order of 0.3 to 0.4%, which is lower than that obtained in our

work. Values of 0.6 and 0.4% were obtained for two varieties of myrtle namely (beatica and italica) in Tunisia (22).

The oil yield of *M. communis* aerial parts in Italy and Turkey would have been 0.33% and 0.38% (23).

Chemical composition of essential oil

Chromatographic analysis of essential oils resulted in the detection of 62 compounds that are showed in the Table1. The monoterpenes hydrocarbons were predominant chemical group (38.4%) of *M. communis*, followed by oxygenated monoterpenes (30.46), while sesquiterpenes hydrocarbons (11.82%) and oxygenated sesquiterpenes (10.6%) were low. Major oil components were α-pinene (24.83 %), 1,8-cineole (10.8 %), limonene (6.07 %), α-terpineol (4.14%) methyleugenol (3.48%). The chemical composition of myrtle essential oil was previously investigated (21,24,25).

For example, it has been shown that the myrtle essential oil collected from Morocco is composed of; α -pinene (10%), I,8-cineole (43%) and myrtenyl acetate (25%)(21). Ben Ghnaya et al. (1) compared the essential oil of myrtle from Algeria and Tunisia and found that the highest percentages of α-Pinene (45.4%) and 1.8 cineole (35.7%) were observed in the Algerian population.

In other study from Northeastern Algeria, a rather low content of α-pinene (39.3%) and 1.8-cineole (33.3%) has been observed in the essential oil of myrtle collected in the region of Gouraya (24). Similar results have also been reported from Northern Algeria (25).

In contrast, Hennia et al. (20) reported a quite different composition of myrtle samples collected in the region of Chlef (northern Algeria) with the main compound being limonene (23.4%), linalool (15.4%), Geranyl acetate (10.9%), α-pinene (10.7%), linalyl acetate (8.2%) and 1,8-cineole (6.6%).

Antibacterial activity assays:

The antibacterial activity of the essential oil was evaluated using disc diffusion and MIC method. The disc diameters of zone of inhibition (DD), minimum inhibitory concentrations (MIC) of essential oils for the microorganisms tested (Table 2 and 3).

Variables zones of microbial growth inhibited by various dilutions of *Myrtus communis* essential oil were noted. The highest activity of pure oil was observed against: *Enterobacter cloacae* with strongest inhibition zones (23.14 mm), *E coli* ATCC 25922 (20.66 mm), *Staphylococcus aureus* ATCC25923 (19.32 mm),

Klebsiella pneumonia (16.33 mm). The dilution 1/2 showed a good antibacterial activity against: *Enterobacter cloacae* (19.20 mm), *Staphylococcus aureus* ATCC25923 (15.2 mm), *Acenitobacter baumannii* (14.17 mm). The dilution 1/4 have a moderate activity against *Staphylococcus aureus* ATCC25923, *Acenitobacter baumannii* and MRSA with inhibition zones of 12 mm.The dilution 1/8 of essential oil seems to be no active against the majority of bacteria strains . The essential oil of *M. communis* has an inhibitory effect on the growth of all pathogenic bacteria tested except *P. aeruginosa*.It has an intrinsic resistance to biocidal agents, in relation to the nature of its external membrane. The latter is composed of lipopolysaccharides which form a barrier impermeable to hydrophobic compounds. In the presence of permeabilizing agents of the membrane, inactive substances against *P. aeruginosa* become active (19). It appears that this strain is resistant to a very large number of essential oils (26, 27).

The MIC values were 0.62 mg/ml in *E .coli* ATCC 25922 and *Enterobacter cloacae*,1.04 mg/ml in *staphylococcus aureus* and *s. aureus* (ATCC 25923) 2.08 mg/ml. In *E.coli* cip R, *Klebsiella pneumonia, Acenitobacter baumannii* and *S. aureus* (ATCC 29213) and 4.16 mg/ml was in KPC +,KPC –and MRSA.

Our results are in agreement with those of Rasooli et al. (28) who showed that myrtle essential oil was lethal to *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13183).

Ben Ghnaya et al. (1) showed that the essential oil of Algerian myrtle have significantly inhibited the growth of *E. coli, Staphylococcus aureus, Bacillus subtilis, Salmonella sp.*, and *Listeria sp*.

The essential oil showed an inhibitory effect on the growth of all pathogenic bacteria tested except for *P. aeruginosa*, wich appeared to be resistant to myrtle essential oil.

Table 1. Composition of the essential oil (% peak area) of *M. communis* leaves

N^0	components	RI	Content%
1	Isobutyl isobutyrate	909	0.86
2	*α*-Pinene 939	939	24.83
3	Camphene	953	1.2
4	Sabinene	976	0.05
5	*β*-Pinene	981	1.5
6	*α*-phellandrene	998	0.77
7	Limonene	1031	6.07
8	1.8 cinéole	1033	10.8
9	Cis-*β*-Ocimene	1040	0.43
10	*γ*-Terpinene	1059	1.64
11	2-Nonanone	1095	2.05
12	Linalool	1101	1.98
13	*α*-Fenchol	1112	0.44
14	*trans- allo*-Ocimene	1141	1.91
15	Camphor	1145	0.87
16	Borneol	1165	0.28
17	Terpinen-4-ol	1178	0.78
18	*α*-Terpineol	1189	4.14
19	Estragole	1199	1.04
20	*trans*-Verbenone	1205	0.09
21	Fenchyl acetate	1226	0.18
22	*Cis*-Carveol	1229	0.17
23	Cuminal	1236	0.15
24	Linalyl acetate	1247	0.62
25	Geraniol	1254	2.04
26	Phellandral	1276	0.23
27	Bornyl acetate	1285	0.13
28	Myrtenyl acetate	1326	0.28
29	Terpinyl acetate	1354	0.1
30	Carvyl acetate	1368	1.3
31	*β*-damascenone	1371	0.37
32	Geranyl acetate	1385	2.95
33	Methyleugenol	1405	3.48
34	*β*-Caryophyllene	1418	2.4
35	*γ*-Elemene	1440	0.23
37	Geranyl acetone	1455	0.14
38	*α*-Humulene	1456	1.75
39	*allo*-Aromadendrene	1458	0.23
40	Bicyclosesquiphellandrene	1463	0.17
41	*β*-cubebene	1478	0.48
42	*β*-Ionone	1482	0.25
43	Germacrene D	1503	0.39
44	Isoledene	1508	0.25
45	Geranyl isobutyrate	1514	0.74
46	*δ*-ledene	1534	3.52
47	*δ*-cadinene	1541	1.71
48	Germacrene *B*	1558	0.69
49	Spathulenol	1576	2.83
50	Caryophyllene oxide	1583	2.09
51	*α*-Cedrol	1608	0.43
52	*α*-Humulene epoxide	1611	1.46
53	T-cadinol	1635	1.68
54	*α*-muurolol	1643	0.48
55	*α*-cadinol	1663	0.85
56	Isoaromadendrene epoxide	1681	0.75
57	9-Pentadecenol	1727	0.78
58	*trans*-Farnesol	1775	0.06
59	Hexahydrofarnesyl acetone	1845	0.15
60	Isopimaradiene	1996	0.07
61	Abitatriene	2054	0.1
62	Heneicosane	2100	0.39
Total identified			98.62
Monoterpene hydrocarbons			38.4
Oxygenated monoterpenes			30.46
Sesquiterpene hydrocarbons			11.82
Oxygenated sesquiterpenes			10.6
Others			7.34

RI: Retention index on a HP-5MS column relative to C7-C24 n-alkanes

(29) have tested the essential oil of Irakian myrtle against *E.coli*, *proteus mirabilis, Staphylococcus aureus,Pseudomonas aeruginosa, Acinetobacter sp* and *Salmonella typhi.*

They found that the fraction of the essential oil containing eugenol acetate was the most active against the bacteria tested. The results of the disk diffusion method revealed that the inhibition zone was 36.5 mm against *S. aureus.* followed by *E. coli* 25.5 mm while the minimum zone of inhibition was found 13.5 mm in diameter against *Acinetobacter sp.* While

Table 2. Diameter of inhibition of essential oils against the bacterial strains (mm).

Bacteria	**Oil dilutions and corresponding bacterial Inhibition zone (mm)**			
Gram negative	**B**	**½**	**¼**	**1/8**
E. coli ATCC 25922	20.66±1.67	11.17±0.75	08.74±0.26	8.30±1.33
E. coli cip R	13.33±2.94	12.11±0.91	09.27±1.23	/
Pseudomonas aeruginosa VIM 2	/	/	/	/
P. aeruginosa ATCC 27853	/	/	/	/
P. aeruginoa IMP	/	/	/	/
Enterobacter cloacae	23.14±0.72	19.20±1.43	11.25±0.69	10.14±2.12
KPC –	11.22±0.72	10.13±0.66	09.6±1.82	07.26±0.35
KPC +	12.9±1.76	11.8±0.46	8.15±0.38	/
Klebsiella pneumoniae	16.33±0.61	11.11±0.78	10±1.36	/
Acenitobacter baumannii	14.62±1.28	14.17±0.72	12.28±1.23	12.21±0.28
Gram positive				
Staphylococcus aureus	11.13±1.17	09.26±1.09	/	/
S. aureus ATCC 29213	14.16±0.88	12.16±1.91	07.10±0.30	07.09±0.11
S. aureus ATCC25923	1 9.32±1.33	15±0.64	12.32 ±0.65	11.14±0.82
MRSA	13.18±0.72	11.26±2.56	12.25±0.68	12.21±0.63

Table 3. (MIC) of essential oil of *Myrtus communis* L.

	Concentration of essential oil (%)					
	1%	**0.5 %**	**0.25 %**	**0.125 %**	**0.075 %**	**0.05 %**
MIC (mg/ml)	8.32	4.16	2.08	1.04	0.62	0.41
Bacteria strains						
E. coli ATCC 25922	-	-	-	-	-	+
E. coli cip R	-	-	-	+	+	+
Enterobacter cloacae	-	+	+	+		
KPC –	-	-	+	+	+	+
KPC+	-	-	+	+	+	+
Klebsiella pneumoniae	-	-	-	+	+	+
Acenitobacter baumannii	-	-	-	+	+	+
Staphylococcus aureus	-	-	-	-	+	+
S. aureus ATCC 29213	-	-	-	+	+	+
S. aureus ATCC25923	-	-	-	-	+	+
MRSA	-	-	+	+	+	+

- No culture; + presence of culture

P. aeruginosa was resistant, the MIC was between 25.0 and 100 µg/ml. The antibacterial activity of essential oil of *M. communis* may be attributed to the high level of monoterpene hydrocarbons such as :α-pinene and limonene (30).

Regarding the mechanism of action of 1,8-cineole; once the phenolic compound has passed through the membrane of the microbial cell, interactions with membrane enzymes and proteins would cause an inverse flow of protons, affecting cellular activity (31).

Table 4. antioxidant activity of *Myrtus communis* L essential oil and ascorbic acid using DPPH radical.

Antioxidant activity of EO		Antioxidant activity of AsA	
Conc. of EO (mg.mL–1)	**P. inhibition (%)**	**Conc. of AsA (µg.mL–1)**	**P. inhibition (%)**
0.100	7.61%±0.73	1	10.84%±4.84
0.200	12.65%± 2.07	2	6.94%±3.04
0.400	25.62%±0.69	4	27.60%±2.43
0.800	51.20%±0.84	6	39.28%±1.38
1.200	76.22%±4.50	8	49.26%±1.26
2	89.93%±4.64	12	65.83%±1.53
2.400	91.59%±4.00	24	84.76%±0.73
4	93.39%±2.01	36	88.82%±1.51

It can therefore be concluded that the antibacterial activity of myrtle can be the result of a synergistic effect between several compounds of the essential oil.

Antioxydant activity of essential oil of Myrtus communis L

The antioxidant activity of the different EO concentrations (0.1 - 4 $mg.mL^{-1}$) varied between 7.61% to 93.39%, with IC_{50} **values lower than 0.8 $mg.mL^{-1}$**, which is below the IC 50 of the standard ascorbic acid (12 µg/ml).

Respectively Hateet et al. (29) showed that eugenol acetate (1.95 - 1000µg /ml) isolated from the essential oil of *M. communi*s had a strong antioxidant activity (10-80%).

It seems to be a general trend that EOs which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes; have greater antioxidative properties.

These activities may be attributed to the synergistic effects of two or more compounds present in the oil.

Boukhris et al. (32) mentioned that most natural antioxidative compounds often work synergistically with each other to produce a wide spectrum of antioxidative properties that create an effective defense system against free radicals.

Table 5. Values of Ic 50

Antioxydant	**IC 50 µg/ml**
Essential oil	794.75±2.87
Ascorbic acid	8.09±0.26

Conclusion

The aim of this study was to analyze of chemical composition of essential oil of Algerian Myrtus communis L. and to evaluate its antibacterial and antioxidant activities. The main constituents of EO are α-pinene, 1,8-cineole and Limonene. The essential oil has a good antibacterial and antioxidant activity,so it could be considered as a potential source of natural compounds that can be used for food cosmetic and pharmaceutical applications.

Acknowledgement: This study was supported by the Laboratory of Analytical Chemistry, Department of pharmacy, Faculty of Medicine - Badji Mokhtar Annaba .

References

1. Ben Ghnaya A, Chograni H, Messoud C, Boussaid M. Comparative Chemical - Composition and Antibacterial Activities of Myrtus communis L. Essential Oils Isolated from Tunisian and Algerian Population. Journal of Plant Pathology and microbiology. 2013; 4:7.
2. Emami SA, Asgary S, Naderi GA, Shams MR, Kasher T. Antioxidant activities of Juniperus foetidissima essential oils against several oxidative systems. Revista Brasileira de Farmacogno sia 2011 ;21:627-634.
3. lsmaili R, Fechtal M, Zine El Abidine A, Hachimi M, Sesbou A. Effet de Ia transplantation sur le rendement et Ia composition chimique du Myrte (Myrtus communis L.) Annales de la recherche forestière du Maroc. 2001; 34: 87-93.

4. Atzei AD. Le piante nella tradizione popolare della Sardegna. Sassari, Italy: CarloDelfino Editore 2003; 319–23.
5. Bellakhdar J, Miki w, Honda G. Herboristes et pharmacopée à Marrakech et Salé (Maroc) AI Biruniya, Revue de pharamcie Maroc 1994; 10 (1): 26-27.
6. Sijelmassi A. Les plantes medicinales du Maroc. Ed. Le Fennec, Casablanca 1991. 137.
7. Bouasla A, Bouasla I. Ethnobotanical survey of medicinal plants in northeastern of Algeria Phytomedicine. 2017 ;36:68-81.
8. Senouci F, Abadou A, Chouieb M. Ethnobotanical survey of medicinal plants used in the southern Mediterranean. Case study: The region of Bissa (Northastern Dahra mountains Algeria. Pharmacognosy journal 2019;11 (4):647-659.
9. Slimani I, Najem M, Belaidi R, et al. Ethnobotanical survey of medicinal plants used in Zerhoun region-Morocco. International journal of innovation and applied studies .2016;846-863.
10. El Hilah F, Ben Akka F, Bengueddour R Rochdi A, Zidane L. Etude ethnobotanique des plantes médicinales utilisées dans le traitement des affections dermatologiques dans le plateau central marocain.Journal of Applied Biosciences 2016 ;98 :9252-9260.
11. Uzun S P, Koca C. Ethnobotanical survey of medicinal plants traded in herbal markets of Kahramanmaras. Plant divers.2020;42 (6):443-454.
12. Emre G, Dogan A,Haznedaroglu M Z, et al. An Ethnobotanical study of medicinal plants in Mersin (Turkey). Frontiers in pharmacology.2021;12:664500.
13. Lawrence B,M. Progress in essential oils. Myrtle oil. Perfumer and Flavorist 1996; 21: 57-58.
14. Chryssavgi G, Vassiliki P, Athanasios M, Kibouris T, Michael K. Essential oil compo-sition of Pistacia lentiscus L. and Myrtus communis L.: evaluation of antioxidant capacity of methanolic extracts. Food Chemistry 2008; 107:1120–30
15. Bouzouita N, Kachouri F, Hamdi M, Chaabouni M . Antimicrobial activity of essential oils from Tunisian aromatic plants. Flavour Fragrance journal. 2003; 18: 380-383.
16. Yadegarinia D, Gachkar L, Rezaei M B, Taghizadeh M, Shakiba A A, Rasooli I, Biochemical activities of Iranian Mentha piperita L. and Myrtus communis L. essential oils. Phytochemistry 2006; 67, 1249–1255.
17. Tajkarimi M M, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control 2010 ;21:1199-1218.
18. Billerbeck VG, Roques C, Vaniére P, Marquier P . Activité antibactérienne et antifongique des produits à base d'huiles essentielles. Hygiénes X-n°3.2002 ;248-251
19. Mann C.M, Cox S.D, Markham J.L. The outer membrane of Pseudomonas aeruginosa NCTC 6749 contributs to its tolerance to the essential oil of Melaleuca alternifolia (Tea tree oil). Letters in Applied Microbiology 2000; 30: 294-297.
20. Hennia A, Brada M, Nemmiche S, Fauconnier M L, Lognay G. Chemical composition and antibacterial activitiy of the essential oils of Algerian Myrtus communis L . Journal of Essential Oil Research 2015; 27 (4): 324-328
21. Satrani B, Farah A, Talbi M. Effect of fractional distillation on chemical composition and antimicrobial activity essential oils of Myrtle (Myrtus communis L.) from Morocco. Acta Botanica Gallica 2006; 153:2, 235-242.
22. Wannes W A, Mhamdi B, Marzouk B .Variations in essential oil and fatty acid composition during Myrtus communis var. Italic fruit maturation. Food Chemistry 2006; 112(3): 621-626.
23. Senatore F, Formisano C, Napolitano F, Rigano D, Ozcan M. Chemical composition and antibacterial activity of essential oil of Myrtus communis L. growing wild in Italy and Turkey. Journal of Essential Oil Bearing Plants 2006; 9(2): 162-169.
24. Foudil-Cherif Y, Boutarene N, Yassaa N. Chemical composition of essential oils of Algerian Myrtus communis and chiral analysis of their leave volatiles. Journal of Essential Oil Research 2013;25:5, 402-408.
25. Bouzabata A, Cabral C, Goncalves M J et al., "Myrtus communis L. as source of a bioactive and safe essential oil,"Food and Chemical Toxicology,vol.75.2015;166–172.
26. Hammer K A, Carson C F, Riley T V.in vitro activity of essential oils in particular Melaleuca alternifolia oil and tea tree oil products against candida Spp. Journal of antimicrobial chemotherapy. 1998;42:591-595.
27. Deans S G, Ritchie G. Antibacterial properties of plant essential oils. International Journal of Food Microbiology1987; 5: 165-180.
28. Rasooli I, Moosavi M L, Rezaee M B, Jaimand K. Susceptibility of microorganisms to Myrtus communis L. essential oil and its chemical composition. Journal of agricultural and food chemistry. 2002; 4, 127–133 .
29. Hateet R, Khadhim A, Shawi H H . Biological Activity of Eugenol Acetate as Antibacterial and Antioxidant Agent, Isolation from Myrtus communis L. Essential Oil. International Journal of Bioengineering & Biotechnology 2016; 1(2): 6-11.
30. Marino M, Bersani C, Comi G . Impedence measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositeae. International journal of food microbiology. 2001; 67:187-195
31. Stojkovic D, Sokovic M D,Glamoclija J, et al. Chemical composition and antimicrobial activity of vitex agnus castus L, fruits and leaves essential oils, food chemistry. 2011; 128 :1017-1022.
32. Boukhris M, Regane G, Yangui T, Sayadi S, Bouaziz M. Chemical composition and biological potential of essential oil from Tunisian *Cupressus sempervirens* L. Journal of Arid Land Studies 2012;22:329-332.

Correspondence
Benhadid Rym
Departement of Biology, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria
E-mail: rym.biologie@yahoo.fr