Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(4); 537-545

ISSN: 0975-4873

Research Article

Comparison of the Chemical Composition and the Bioactivity of the Essential Oils of Three Medicinal and Aromatic Plants from Jacky Garden of Morocco

Moutaouafiq Salma[1*], Farah Abdellah[1], Alilou El Houssine[2], Benbrahim Kawtar[3], Bousta Dalila[1]

[1]*Laboratory of Medicinal, Aromatic Plants and Natural Substances, National Institute of Medicinal and Aromatic Plants Sidi Mohamed Ben Abdellah University, Fez, Morocco.*
[2]*Laboratory of Bioactive Molecules, Structure and Functions, Faculty of Science and Technology Saïss. Sidi Mohamed Ben Abdellah University, Fez, Morocco.*
[3]*Laboratory of Microbial Biotechnology, Faculty of Science and Technology Saïss. Sidi Mohamed Ben Abdellah*

Available Online:13th March, 2016

ABSTRACT
The purpose of this work is the comparative study of the bioactivity of three aromatic plants: Geranium (*Pelagronium graveolens*), bitter orange (*Citrus aurantium*) and lemon grass (*Cymbopogon citrates*) used by the local population for their medicinal virtues. Our samples came from an orchard in Agadir, Morocco. We examined their chemical components and their antibacterial activities against seven pathogens (*Enterobacter aerogenes*, *Salmonella typhi*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis and Staphylococcus aureus*). The extraction was carried out by hydrodistillation. The analysis of the essential oil was done through GC/MS. The antimicrobial activity was examined using the disc diffusion method. *C.citratus* showed the highest yield 0.992% vs. 0.763% for *P.graveolens* and 0.313% for *C. aurantium*. The chemical analysis of *C. citratus* revealed the presence of three major constituents namely Geranial, Neral, and Geraniol. The essential oil extracted from *C. aurantium* is essentially composed of Limonene, Linalool, γ-elemene and α- terpineol. On the other hand, the essential oil of *P. graveolens* is mainly composed of alcohol Geraniol, Citronellol, Nerol, and Linalool. The biological tests have shown that all the essential oils are active against all the tested bacteria.

Keywords: Antibacterial activity- Aromatic Medicinal Plant- Essential oil - *Pelargonium graveolens*– *Cymbopogon citratus*– *Citrus aurantium* – MIC.

INTRODUCTION

The Mediterranean coastal climate of Morocco is very good and favorable for the healthy and diverse growth of great vegetation. The coastal vegetation of a city named Agadir is prominently a large exotic collection of Eucalyptus, Citrus, Argan trees, Acacia and many others. These medicinal and aromatic plants are important sources for therapeutic drugs and play an important role in the survival of the tribal and ethnic communities. Today, despite the many advances in modern medicine, there is a marked revival of interest with respect to medicine and traditional pharmacopoeia. In the rural areas of developing countries, herbal medicine serves the health needs of about 80% of the world's population according to World Health Organization (WHO). This is due to the therapeutically and organoleptical virtues of the aromatic and medicinal plants as well as their aroma. This shift towards nature has led scientists to be interested in studying the active components of medicinal and aromatic plants. In this study, we are interested in the essential oil (EO) portion of these plants and their bioactivity. The EO has the ability to fight many epidemic and infectious diseases, caused by antibiotic resistant germs[1]. In our study, we picked three different aromatic species from an orchard called "Jacky Garden" in Agadir Morocco:

Geranium or *Pelargonium graveolens* is an uncommon species in the *Pelargonium* genus within the plant family Geraniaceae. It has great importance in the perfume industry. "Geranium oil", are sold for aromatherapy and massage therapy applications. It has since become indispensable aromatherapy oil. Other uses of geranium essential oil include the treatment of dysentery, hemorrhoids, inflammation, heavy menstrual flows, and even cancer. The French community is currently treating diabetes, diarrhea, gallbladder problems, gastric ulcers, jaundice, liver problems, sterility and urinary stones with this oil[2].

Cymbopogon citratus or Lemongrass oil of the Family Poaceae, has great benefits as a muscle and skin toner and revitalizes the body and mind, helps with infections and keeps the family pet flea and tick free and smelling nice. It is useful with respiratory infections such as sore throats, laryngitis and fever and helps prevent spreading of

**Author for Correspondence*

Table 1: Dilution for MIC

Dilutions	Concentration	EO (µl)	agar solution 0,2% (µl)
1	1/25	40	960
2	1/50	20	980
3	1/100	10	990
4	1/200	5	995
5	1/250	4	996
6	1/400	2,5	997,3
7	1/500	2	996
Negative control	0	0	1000

Table 2: Organoleptic characteristics

plant	color	Smell of the flower
Pelargonium. graveolens	Dark greenish-yellow	Strong smell
Cymbopogon. citratus	Dark orange	Lemon and verbena scent
Citrus. aurantium	*orange-yellow*	Very strong smell and powerful

MATERIALS AND METHODS

Material

Plant materials

The samples of aerial parts (stems, leaves and flowers) of *Cymbopogon citratus* (lemongrass), *Citrus aurantium* (Bitter Orange) flowers and *Pelargonium graveolens* (geranium) were collected in April 2013 from Jacky Garden in a region called Aït Melloul in Agadir, Morocco. These plants in this garden are from organic cultivation. The plantations were watered by natural water from a 230 meter deep well. The flowers and the leaves were then dried for one month. The dried samples are taken to the Green Extraction Laboratory of National Institute of infectious diseases. It is helpful with colitis, indigestion and gastro-enteritis[3].

Bitter orange or *Citrus aurantium* belongs to Rutacea Family. The bitter orange flower and bitter orange oil are used for gastrointestinal (GI) disorders including ulcers in the intestine, constipation, and diarrhea. *Citrus* genus is useful in diminishing the symptoms of anxiety or insomnia, and *C. aurantium* has more recently been proposed as an adjuvant for antidepressants[4]. Studies concerning these plants are still very few and this work is a contribution to value them. We aim to do the extraction of the essential oil and the chemical analysis of these three medicinal plants: *Pelargonium graveolens*, *Cymbopogon citratus* and *Citrus aurantium*. The antibacterial activity of their essential oils is also reported.

Aromatic and Medicinal Plants (NIAMP), Taounate, Morocco for extraction.

Microbial strains

The antimicrobial activity of our samples was evaluated on seven isolated strains. The bacteria used in this work are: Gram (+): *Bacillus subtilis* ATCC6633, *Micrococcus luteus* ATCC14452, *Staphylococcus aureus* ATCC29213. Gram (–): *Klebsiella pneumoniae*, *Escherichia coli* ATCC25922, *Enterobacter aerogenes*, *Salmonella typhi*. These pathogenic bacteria are chosen for their high antibiotic resistance and toxicity in humans.

Methods

Extraction

The extraction of essential oils was performed by hydrodistillation in a Clevenger type apparatus[5]. Each plant undergoes a number of distillations by boiling 100g of a plant material for three hours in 500 ml of water in a 1l round bottom flask surmounted by a column of 25 ml connected to a condenser. The Yield of the essential oils is determined from dried plants estimated from three samples dried for 24 hours in an oven at 100°C (humidity calculation)[6]. The yield is represented in ml/100g of dry matter. The essential oils samples are then stored in opaque sealed bottles under low temperature (4 to 5°C).

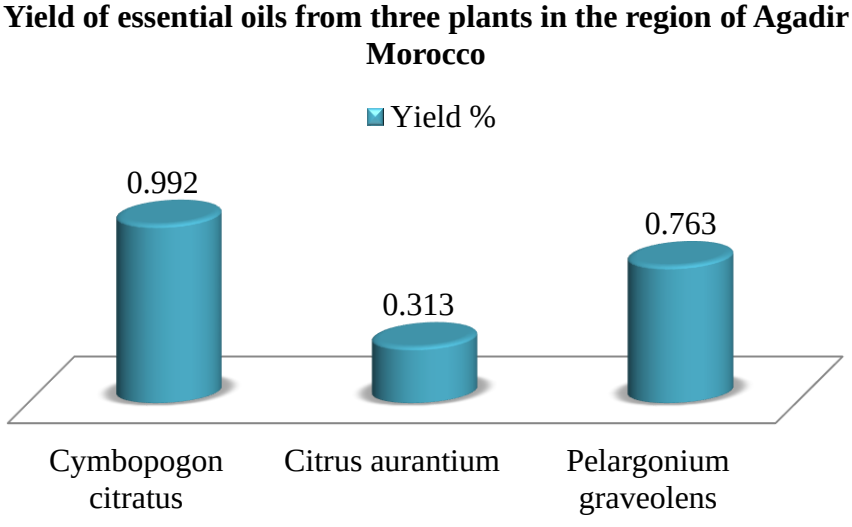

Figure 1: The yield of essential oils from 3 plants in the region of Agadir Morocco:

Table 3: Chemical composition of *Cymbopogon citratus*, *Pelargonium graveolens* and *Citrus aurantium* collected in the region of Agadir Morocco

S No.	Compounds	IK	Composition %		
			P. graveolens	*C. citratus*	*C. aurantium*
1	α-pinene	937	t	-	-
2	β- pinene	980	-	-	1.86
3	α-phellandrene	1008	-	-	1.95
4	Limonene	1030	-	-	40.81
5	cis-linalool oxide	1067	-	-	0.90
6	Trans-linalool oxide	1084	-	-	0.38
7	Linalool	1100	5.37	-	26.66
8	p-menth-2-en-1-ol	1118	0.30	-	-
9	p-Menthone	1158	0.54	-	-
10	α-Terpineol	1189	0.08	-	4.97
11	Nerol	1230	13.65	-	-
12	Citronellol	1235	25.59	-	-
13	Neral	1247	-	36.50	-
14	Geraniol	1258	29.98	3.36	-
15	Linalyl acetate	1260	-	-	1.84
16	Geranial	1277	3.38	55.30	-
17	α-terpinyl acetate	1350	-	-	2.07
18	β-Caryophyllene	1419	-	-	1.13
19	γ-elemene	1436	-	-	7.97
20	α- Bourbonene	1444	1.48	-	-
21	Germacrene-D	1480	2.47	-	-
22	Ledene	1493	2.62	-	-
23	ε-cadinene	1538	2.40	-	-
24	Spathulenol	1578	1.16	-	4.02
25	Caryophyllene oxide	1581	-	-	2.41
26	Veridiflorol	1590	0.97	-	-
27	α-Guaiene	1651	1.98	-	-
28	γ-costol	1745	1.34	-	-
29	unidentified	-	-	-	1.66
	TOTAL		93.31%	95.16%	98.63%
Chemical Classification			Monoterpenes %		
	Hydrocarbon		-	-	44,62
	Alcohol		74,97	3,36	31,63
	Aldehyde		3,38	91,8	-
	Ketone		0,54	-	-
	Oxide		-	-	1,28
	Ester		-	-	3,91
			Sesquiterpenes %		
	Hydrocarbon		10,95	-	9,1
	Alcohol		3,47	-	4,02
	Oxide		-	-	2,41

Gas chromatography-mass spectrometry analysis (GC/MS)

The chemical analysis of the essential oils was carried out with a Gas Chromatograph (GC) coupled to a Mass Spectrometer (MS). The coupling of GC and MS was done by using a chromatographic apparatus (Trace GC Ultra) and a mass spectrometer (Polaris Q) (El 70 eV) equipped with a fused silica capillary column 30 m x 0.25 mm x 0.25 µm of VB-5 (5% phenyl, 95 % methylpolysiloxane). This was operated under helium pressure with a flow of 1.4 ml/min, and accompanied with a quadrupolar selective detector, where the ionization potential is fixed at 40 to 200°C, with a rise of 4°C/ min and S/Sl injection. The injection mode is split and the temperature of injection is 200°C. The components were identified on the basis of their retention time and their mass spectral fragmentation compared to those reported in the database "NIST MS Search".

Microbiological assays

Aromatogram or disc diffusion method (screening)

The Aromatogram is a qualitative technique that determines the sensitivity of the microorganisms toward an antimicrobial substance. National Committee standardizes this method for Clinical Laboratory Standards (NCCLS) [7, 8]. In our case, we studied the sensitivity of seven strains (*Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Micrococcus luteus, Enterococcus aureus*) against the

Table 4: Inhibition Zone diameter (mm) of the essential oils (EO) of three Moroccan species on seven selected bacterial strains

EO	Strain	*Ec*	*B*	*Sa*	*Kl*	*S*	*Ml*	*Ea*
Cymbopogon citratus	P	25.5	30	17.3	14	18.5	39.5	15.5
	1	10	25	13	13.5	14	13.5	14
	2	9	24	12	10	11.75	10.5	11.75
	3	8	12	11.5	10	10	9	10.5
	4	7	11	11	12	10	7.5	8.75
	5	7	10	10	11.5	8.25	7	7.75
Citrus aurantium	P	13	37	15.5	11	28.5	18	13
	1	13	34	10	10.5	21.5	9.5	12.5
	2	11.5	33	9.75	9.75	14.75	8.5	10
	3	10	15	9	9	13.5	8	8.75
	4	11.75	12	9	9	10.25	9	8.5
	5	9.5	11	8	8	9	8.5	8.25
Pelargonium graveolens	P	13	18.5	14	13	24	12	14.5
	1	10	18	11	12	15.25	12	13
	2	9	12.5	9.5	11	10	9	10
	3	8.5	11	9	8.5	9.5	8	9.5
	4	8	9.5	9	10.5	9	11	9.5
	5	7	8	9	8	9	10	9.5

**The disc diameter (6 mm) is included in all tests.* Concentration of essential oils (P=1; 1= 1/2; 2= 1/5; 3=1/10; 4=1/20; 5=1/100)*, , Ec=Escherichia coli, B=Bacillus subtilis, Sa=Staphylococcus aureus , Kl=Klebsiella pneumoniae, S=Salmonella typhi, Ml=Micrococcus luteus, Ea=Enterobacter aerogenes.*

essential oils of the following plants: *P. graveolens, C. citratus* and *C. aurantium* flowers. This test is performed in the same way as an antibiogram where the antibiotics are substituted by the substances to be tested in this study. The method depends on the migratory effect of these substances on a solid agar medium (Mueller-Hinton agar). Due to the immiscibility of essential oils in water and therefore in the medium culture, the emulsification is carried out with a solution of 0.2% agar to promote contact germ/compound[9]. This technique consists of inoculating a pre-culture of the bacterial strains with an autoclaved liquid of the Luria broth (LB), and incubating the inoculums at 37°C with agitation. When 0,7< DO_{660}nm <0,8 spreading in Petri plate (9 cm in diameter), containing the Mueller Hinton agar through bacterial suspensions of 10^8 CFU (the culture is done by spreading out 2ml of this suspension in each plate), then they are incubated at37°C for 30 minutes. Wattman paper discs (6 mm in diameter) are sterilized and put on the dried agar already inoculated with bacterial suspension. Then, these discs are impregnated with increasing dilutions of each essential oil (1, 1/2, 1/5, 1/10, 1/20 and 1/100) with a micropipette. The Petri dishes are then stored at 4°C for one hour before incubating them at 37°C for 24 hours[10] in inverted position to avoid the condensed water of the plate's cover to drop into our culture. After incubation, we measure the diameters of the inhibition zones in millimeters for our results.

Determination of Minimum Inhibitory Concentrations (MIC)

This technique consists of incubating a range of decreasing concentrations of essential oils. The MIC is determined as the lowest concentration of oil able to inhibit the visible growth of each microorganism on the agar plate[11,12].

We determined the MIC with the dilution technique in a solid medium[13,9], which consists of a 1000 ml of a solid nutrient medium TSA (Tryptic Soy Agar) is prepared, autoclaved and poured into the test tubes. Then, 1 ml of different dilutions of the essential oil in the agar-agar solution (0.2%) was added in all the tubes with 9 ml of TSA (Table 1). Each tube is vortexed to make the mixture homogenous then the content is put in a Petri dish of 9cm, we let it cool down to solidify.

The minimum inhibitory concentration of the essential oil is defined via the first agar plate of the range devoid of the bacterial grow

Negative controls containing the culture medium and the agar solution at 0.2% only, were also prepared.

RESULTS AND DISCUSSION

The extraction and characterization of essential oils

The yield of essential oils

Average yields of the essential oils were calculated based on the dry plant material from the aerial part of the plant. The essential oil yield (w/w %) of our samples changes from one plant to another.The *Cymbopogon citratus* from Agadir provided a yield of almost 1%, whereas *Pelargonium graveolens* reached a yield of 0.76% and a yield of 0.31% for the *Citrus aurantium.*

The yield of *C. citratus* essential oil is relatively high (0.99%) in comparison to that obtained from a sample in Ivory Coast (0.7%) and from Cameron (0.67%)[14].

The essential oil of the moroccan *P. graveolens* with a yield of 0.763% is clearly superior than this reported in literature 0.22%[15]. The yield ranges from 0.2 to 0.25% in South Africa[16]; 0.08 to 0.16% in Reunion[17] and 0.2% in Algeria[18] according to AFNOR[19].

As for *C.aurantium*, the yield is 0.3%, much higher than that of previous studies 0.12% in Tunisia[20].

Organoleptic characteristics

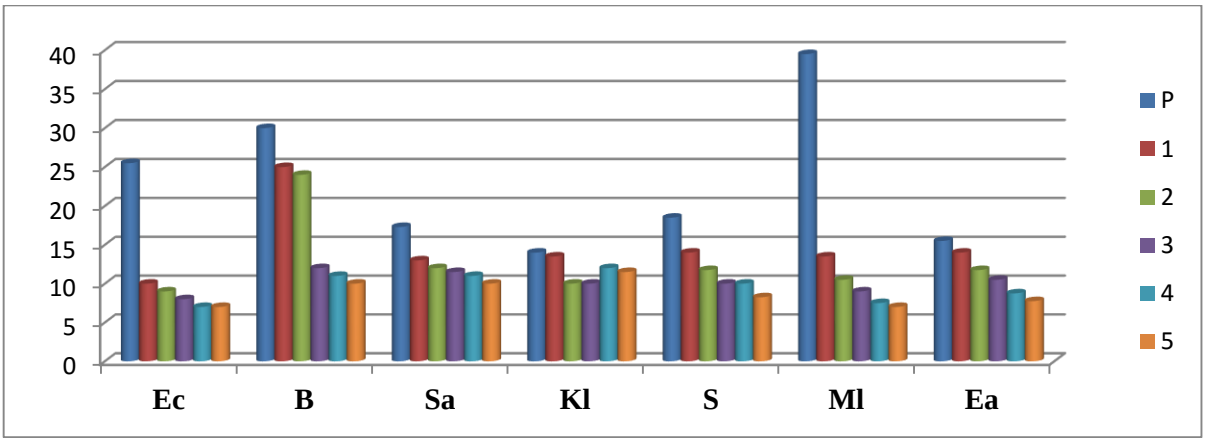

Figure 2: Zone of inhibition of *Cymbopogon citratus* Essential Oil on 7 bacterial strains
Concentration of essential oils (P=1; 1= 1/2; 2= 1/5; 3=1/10; 4=1/20; 5=1/100), , Ec=Escherichia coli, B=Bacillus subtilis, Sa=Staphylococcus aureus , Kl=Klebsiella pneumoniae, S=Salmonella typhi, Ml=Micrococcus luteus, Ea=Enterobacter aerogenes.*

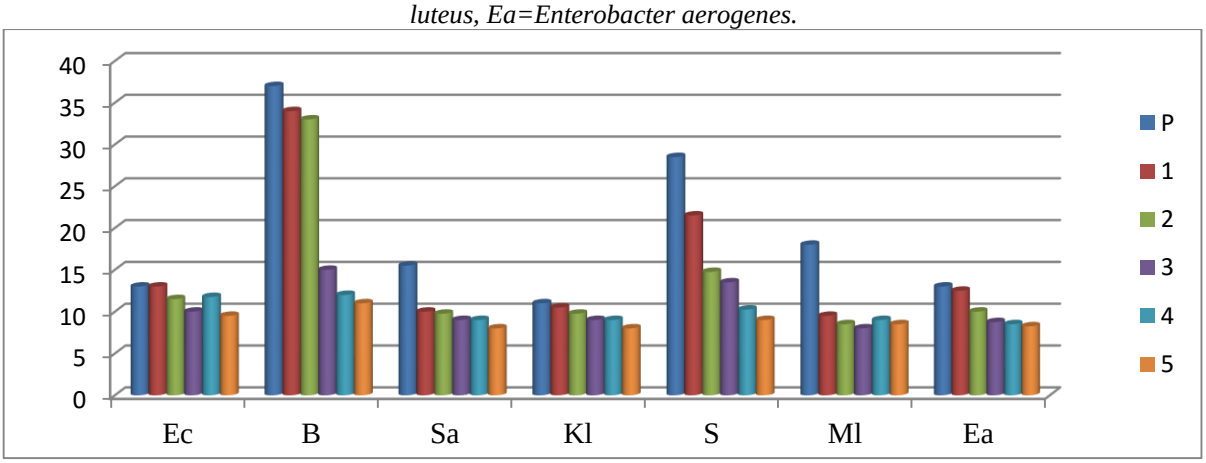

Figure 3: Zone of inhibition of *Citrus aurantium* Essential Oil on 7 bacterial strains
Concentration of essential oils (P=1; 1= 1/2; 2= 1/5; 3=1/10; 4=1/20; 5=1/100) , Ec=Escherichia coli, B=Bacillus subtilis, Sa=Staphylococcus aureus , Kl=Klebsiella pneumoniae, S=Salmonella typhi, Ml=Micrococcus luteus, Ea=Enterobacter aerogenes*

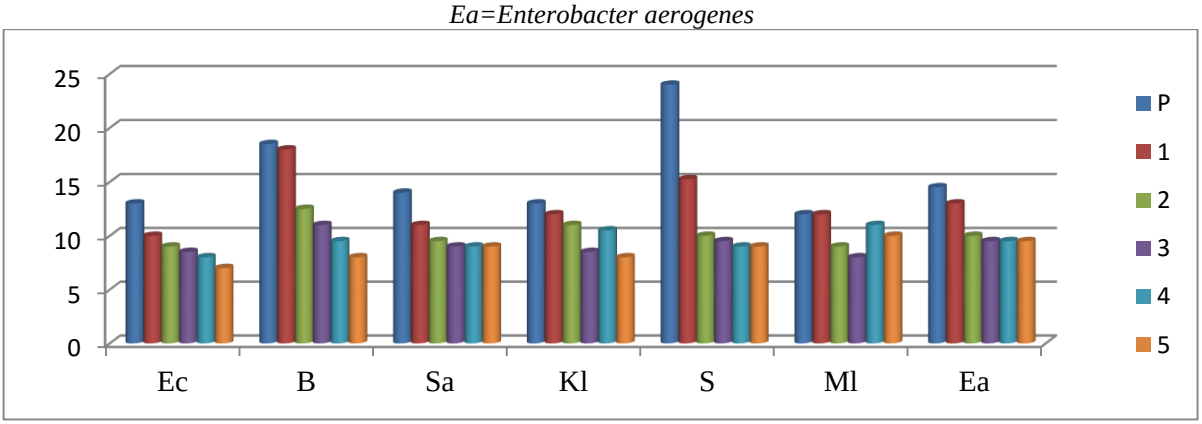

Figure 4: Zone of inhibition of *Pelargonium graveolens* Essential Oil on 7 bacterial strains
Concentration of essential oils (P=1; 1= 1/2; 2= 1/5; 3=1/10; 4=1/20; 5=1/100), , Ec=Escherichia coli, B=Bacillus subtilis, Sa=Staphylococcus aureus , Kl=Klebsiella pneumoniae, S=Salmonella typhi, Ml=Micrococcus luteus, Ea=Enterobacter aerogenes.*

The distillation of essential oils showed that *P. graveolens* EO and the *C. aurantium* EO have a viscous nature, which indicates the presence of easily polymerizing components.

Chemical composition

The volatile components percentages of the EO analyzed by GC and GC/MS of *Cymbopogon citratus*, *Pelargonium graveolens* and *Citrus aurantium* collected in the region of Agadir Morocco are reported in Table 3.

The essential oil of *P.graveolens* analyzed in this study was dominated by Geraniol (29.98%), Citronellol (25.59%), Nerol (13.65%), Linalool (5.37%), α-Terpineol (0.08 %), p-menth-2-en-1-ol (0.30 %).Same goes for the

Table 5: Antibacterial activity of essential oils of *Cymbopogon citratus*, *Pelargonium graveolens* and *Citrus aurantium* collected in the region of Agadir Morocco

	B			*Ml*			*Kl*			*S*			*Sa*			*Ec*			*Ea*		
	Cc	*Ca*	*Pg*	*Cc*	*Ca*	*Pg*	*Cc*	*Ca*	*Pg*	*Cc*	*Ca*	*Pg*	*Cc*	*Ca*	*Pg*	*Cc*	*Ca*	*Pg*	*Cc*	*Ca*	*Pg*
1/25	-	+	+ -	-	-	-	-	-	-	-	+ -	-	-	-	-	-	+	+ -	-	+	+ -
1/50	-	+	+ -	-	-	-	-	+	+	-	+ -	-	-	-	-	-	+	+ -	-	+	+ -
1/100	-	+	+	-	-	-	+	+	+	-	+ -	+	-	+ -	-	+	+	+	+	+	+
1/200	-	+	+	-	-	+	+	+	+	+	+ -	+	-	+ -	+	+	+	+	+	+	+
1/250	+	+	+	-	+	+	+	+	+	+	+	+	+	+ -	+	+	+	+	+	+	+
1/400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1/500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Cc: Cymbopogon citratus; Ca: Citrus aurantium; Pg: Pelargonium graveolens * Ec=Escherichia coli, B=Bacillus subtilis, Sa=Staphylococcus aureus , Kl=Klebsiella pneumoniae, S=Salmonella typhi, Ml=Micrococcus luteus, Ea=Enterobacter aerogenes*;* -: inhibition, +: growth

geranium from India[21], the main constituents of the *P.graveolens* essential oil from Comoros[22] are geraniol (14.12%), citronellol (29.98%), and linalool (5.97%). The *P. graveolens* EO is also composed of Geranial (3.38%), p-Menthone (0.54%) and sesquiterpenes hydrocarbons (10.95%). However, the EO from Algeria contains terpenes alcohols (41%), (Menthone + Isomenthone) (6%), geranial (0.679%) and sesquiterpenes alcohols (0.77%)[18].

The chemical composition of C. citratus essential oil revealed three compounds representing 95.16% of the total essential oil. The major fractions were Geranial (55.30%), Neral (36.50%)[23] and Geraniol (3.36%). These results are almost similar to those shown by other researchers who reported that the major compounds of the *C. citratus* EO are Neral (33.31%), Geranial (39.53%) and Geraniol (3.05%)[24]. As to the *Citrus aurantium* EO, 14 compounds were identified, representing 98.63% of the total oil. Limonene (40.81%) was the main component followed by Linalool (26.66%), γ-elemene (7.97%), α-terpineol (4.97%) and α-terpinyl acetate (2.07%). These components are clearly different compared to EO of Tunisia that has only 27.5% of Limonene as major component followed by E-nerolidol (17.5%), α-terpineol (14%), α-terpinyl acetate (11.7%) and E-farnesol (8%)[20].

Antibacterial activity of the essential oils

Disc diffusion method

The antibacterial activity was tested by the disc diffusion method in a solid nutritive media on Gram-positive bacteria *(Micrococcus luteus, Staphylococcus aureus, and Bacillus subtilis) and* Gram-negative bacteria *(Escherichia coli, Salmonella typhi, klebsiella pneumonia, Enterobacter aerogenes).* The results of the aromatogram that shows the antibacterial activity of the EOs of *Cymbopogon citratus*, *Pelargonium graveolens* and *Citrus aurantium* are summarized in the table 4 and the figures (2, 3 and 4)

The table 4 shows the measurements of the inhibition zone diameters of the discs impregnated with 5 µl of the decreasing concentration of each essential oils (1, 1/2, 1/5, 1/10, 1/20, 1/100). The values are the average of tree repetitions.

This measure has allowed us to classify the bacterial strains according to their degree of sensitivity to the concentration of each tested EO. To interpret our results, we followed the spectrum showed below of the bacterial strains sensitivity depending on the inhibition diameter (mm)[25, 26]:

Resistant bacteria: < 8 mm

Sensitive: 9 – 14 mm

Very sensitive: 15- 19 mm

Extra sensitive: > 20 mm

The inhibition zone diameter of the *Cymbopogon citratus* EO varies from 7 to 39.5 mm. *Escherichia coli* has proved to be more resistant than the rest of the bacteria Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*) and Gram-negative (*Klebsiella pneumoniae*, *Salmonella typhi*, *Enterobacter aerogenes*). It is resistant at the concentration of 1/10. The *Cymbopogon citratus* EO has displayed an important inhibitory activity against Gram-positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis)* with 10 to 17.3mm and from 10 to 30 mm of diameter. This antibacterial activity increases with the concentration of the essential oil. On the other hand, *klebsiella pneumoniae* is the most sensitive towards the *Cymbopogon citratus* EO with a diameter between 10 and 14 mm.

As far as the *Citrus aurantium* EO is concerned, the inhibitory diameters vary from 8 to 37mm. This oil has displayed a significant inhibitory activity toward *Bacillus Subtilis* with diameters ranging from 11 to 37 mm for the different concentrations.

Escherichia coli has proved to be more sensitive than the other bacteria except for *Bacillus subtilis* starting from a concentration of 1/100, with the diameters of 9.5 to 13 mm. Then comes *Salmonella typhi* which is sensitive to extremely sensitive starting from the concentration of 1/20 with the diameters from 10.25 to 28.5 mm. *Klebsiella pneumoniae* and *Staphylococcus aureus* are sensitive toward the EO of *Citrus aurantium* starting from a concentration of 1/20 with diameters ranging between 9 and 11 mm.

Micrococcus luteus and *Enterobacter aerogenes* are the most resistant strains to the EO of *Citrus aurantium.* However, they are sensitive to important concentrations of 1/2 and 1/5 with diameters of 9.5 and 10 mm respectively.

The inhibition zone diameters of the *Pelargonium graveolens* essential oil vary from 7 to 24 mm. *Salmonella typhi* is sensitive to extremely sensitive to the EO from a concentration of 1/100 with diameters from 9 to 24 mm. This EO showed important antibacterial activity

against *Bacillus subtilis* with inhibition zone diameters of 9.5 to 18.5 mm starting from the dilution of 1/20, *Enterobacter aerogenes* is sensitive with diameters ranging from 9.5 to 14.5 mm. *Escherichia coli* has proved to be less sensitive up to concentration of 1/5, with diameters of 9 to13 mm. It is resistant at a concentration of 1/100. The susceptibility of the bacteria changed with the dilution of the essential oil.

The Minimal Inhibitory Concentration (MIC)

The results of a Minimal Inhibitory Concentration of the essential oils studied are shown in Table 5.

Cymbopogon citratus EO has exhibited a total inhibition on all types of bacteria. Hence, it presents a significant antibacterial activity. *Micrococcus luteus* displays the most important inhibitory effect with *Cymbopogon citratus* EO at a concentration of 1/250, followed by *Bacillus subtilis* and *Staphylococcus aureus* which are sensitive at a concentration of 1/200. Whereas *Salmonella typhi* is inhibited at 1/100 as a minimal concentration. On the other hand, *Escherichia coli, Klebsiella pneumoniae* and *Enterococcus aureus* are sensitive at a weak concentration of 1/20. As for the concentrations 1/25 and 1/50 of EO, no bacterial colony has developed for all the strains. The agar plate remained entirely translucent, even after several days. We may deduce, then, that the area is sterilized and that the effect for *Cymbopogon citratus* EO is bactericidal against different bacteria ranging between the concentration 1/25 and 1/50. The EO extracted from *Citrus aurantium* inhibits the growth of *Micrococcus luteus* starting from 1/200, followed by *Staphylococcus aureus* at a concentration of 1/50, while *Klebsiella pneumoniae* is inhibited at 1/25. *Bacillus Subtilis*, *Escherichia coli* and *Enterobacter aerogenes* are resistant at a concentration of 1/25. *Pelargonium graveolens* EO inhibits the growth of *Micrococcus luteus* and *Staphylococcus aureus* at a concentration of 1/100 followed by *Salmonella typhi* which is inhibited at 1/50, whereas *Klebsiella pneumoniae* has proved to be less sensitive compared to *Micrococcus luteus*, *Staphylococcus aureus* and *Salmonella typhi*). The results showed that the pure essential oils of *C.citratus*, *P.graveolens* and *C.aurantium* represent the most extensive inhibition zones. Bacillus strains are resistant to all of the essential oils with a concentration of 1/25 except with the *C.citratus* EO which displays an inhibition of this bacterium at 1/200. The strong antibacterial activity is generally due to the presence of some components having antibacterial effect. The antimicrobial activity of some terpene compounds is in the following increasing order: Phenol› aldehydes> alcohols> ketones> hydrocarbons[27]. This strong activity of *C.citratus* is due to the presence of 91.8% of monoterpene aldehydes: citral as major components[23] with the presence of alcohol. Previous works attributed the antimicrobial property of the *C. citratus* EO to the presence of Geranial[28] and Neral[23]. These components possess an interesting antibacterial activity against Gram + and Gram – bacterial strains[29] after the terpenic phenols[30,31]. The results of previous works showed that the oxygenated monoterpenes exhibited a variable degree of antibacterial activities by producing a weak zone of inhibition from 7 to 11 mm in diameter[32]. The essential oil of *P.graveolens* showed strong antibacterial activity related to the presence of oxygenated monoterpenes which accounted for 78.89%. Another study reported that major components of geranium especially Geraniol, Citronellol and α-Terpineol alone or combined elicit antibacterial action against gram positive and gram negative bacteria[33]. It has been also reported that antimicrobial effects of Linalool, Geraniol and Geranial against Escherichia coli have been evaluated, they found, that Geraniol and Geranial were more potent than linalool against E. coli.[34], linalool and α-terpineol were less effective against Salmonella typhi and E. coli.[35,36]. The *P. graveolens* EO is also composed of ketone p-Menthone and sesquiterpenes hydrocarbons (10.95%). As to the *Citrus aurantium* EO, the limonene, β- pinene and α-phellandrene are Monoterpene Hydrocarbons that have a moderate antibacterial activity on the majority of the bacteria such as *Escherichia coli* and *Staphylococcus aureus*; they are not active on *Bacillus*[29,30]. This EO contains also monoterpene alcohols Linalool[31], α-terpineol[32] and sesquiterpene alcohol, which have a strong antibacterial activity[33].

The yields, chemical compositions and antibacterial activities of the essential oils vary according to different factors: provenance, soil type and climate, the part of the plant distilled the extraction technique, etc[34,35].

CONCLUSION

In this work, we studied the chemical composition of three essential oils from *Pelagronium graveolens, Citrus aurantium and Cymbopogon citratus* flowers collected in an orchard in Agadir, Morocco. Additionally, we contributed to study their antibacterial activities against seven bacterial strains: *Enterobacter aerogenes, Salmonella typhi, Micrococcus luteus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis and Staphylococcus aureus)*, The yields of the essential oils of the examined plants (*Pelagronium graveolens, Citrus aurantium and Cymbopogon citratus*) are acceptable and may be rewarding at an industrial level. Moreover, the major components of these EOs showed an interesting antibacterial activity. However, it is essential to state that the biological activity of a given EO is not only due to its major components, but to the mixture of the components contained in this oil. The chemical analysis of the essential oil of *C. citratus* showed the presence of three major constituents Geranial, Neral and Geraniol. The EO of *C. aurantium* is mainly composed of Limonene, Linalol, γ-elemene and α- terpineol. While *P.graveolens* EO is mainly composed of alcohol: Geraniol**,** Citronellol**,** Nerol and Linalol.

ACKNOWLEDGEMENTS

We are grateful to Professor Jean-Jacques Descamps and Lhadj Larbi Rada (Jacky Garden in Agadir) for their collaboration in the achievement of this work.This work was supported by the MAP2ERA project (7PCRD).

REFERENCES

1. Bouhdid S, Idaomar M, Zhiri A, Baudoux D, Skali NS, Abrini J. Thymus essential oils: chemical composition and in vitro antioxidant and antibacterial activities. Biochimie substances naturelles et environnement. International Congress of Biochemistry Agadir Morocco. 2006; 324-327.
2. Peterson A, Goto M, Machmuah SB, Roy BC, Sasaki M, Hirose T. Extraction of essential oil from geranium (*Pelargonium graveolens*) with supercritical carbon dioxide. J. Chem. Technol. Biotechnol. 2005; 81: 167-172.
3. Rauber CS, Guterres S, Schapoval EE. LC determination of citral in Cymbopogon citratus volatile oil. J. Pharm. Biochem.Analysis 2005; 37: 597-601.
4. Celso AR, Thaís CC, Bruna OC, Regina KT, Jorge CF, Mirtes C. Citrus aurantium L. essential oil exhibits anxiolytic-like activity mediated by 5-HT_{1A}-receptors and reduces cholesterol after repeated oral treatment .BMC Complementary and Alternative Medicine (ISCMR). 2013; 13:42.
5. Clevenger J, Apparatus for the determination of volatile oil. Journal of the American Pharmacists Association. 1928; 17:345–349.
6. Simard S, Hachey JM, Colin GJ. The variation of the essential oil composition with the extraction process, the case of Thuya occidentalis L and Abies balsamea L. J. Mill Wood Techn. 1988; 8:561-573.
7. NCCLS. Performance standards for antimicrobial susceptibility testing. Ninth informational supplement: document M100-S9 vol. 19 (1), NCCLS, Wayne, Pennsylvania, USA. 1999, Table 21.
8. Kiehlbauch JA, Hannet GE, Salfinger M, Archinal W, Monserrat C, Carlyn C .Use of the national committee for clinical laboratory standards guidelines for disk diffusion susceptibility testing in New York state laboratories. J. Clin. Microbiol. 2000; 38:3341–3348.
9. Remmal A. Bouchikhi T, Rhayour K, Ettayebi M, Tantaoui EA. Improved method for determination of antimicrobial activity of essential oils in agar medium. Journal of Essential Oil Research. 1993; 5(2):179-184.
10. Valnet, J.; Duraffourd, C.; Duraffourd, P.; Lapraz, J. C. The aromagram: new results and an attempt at interpretation of 268 clinical cases. Plant Med. Phytother.1978; 12(1):43-52
11. Senhaji O, Faid M, Elyachioui M. Inhibitory effect of cinnamon extracts on bacterial antibio-resistant strains. Bio & Santé. 2004; 4(2): 1-10.
12. Michel Vaubourdolle, Infectiologie, Edn 3, Vol. 3. Wolters Kluwer, Le Moniteur internat, Paris, 2007, 475- 497.
13. Satrani B. et al.,. Composition chimique et activité antimicrobienne des huiles essentielles de *Satureja calamintha* et *Satureja alpina* du Maroc. *Ann. Falsif. Expert. Chim. Toxicol.* 2001 ; 94(956) : 241-250.
14. Tchoumbougnang F, Dongmo PM, Sameza ML, Mbanjo EN, Tiako FG, Amvam ZP & Menut C. Activité larvicide sur Anopheles gambiae Giles et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun. Biotechnol. Agron. Soc. Environ. 2009; 13(1) :77-84.
15. VirendraSR, Juyal JP, Blazquez MA. Chemical constituents of essential oil of Pelargonium graveolens leaves. International Journal of Aromatherapy. 2002; 12(4): 216–218.
16. Mosta NM. « Essential oil yield and composition of rosescented geranium (Pelargonium sp) as influenced by harvesting frequency and plant shoot age ». Thesis of doctorat MSC Agronomy, faculty of natural and agricultural sciences, university of Pretoria, South Africa, 2006.
17. Demarne FE, Van der Walt J. Origin of rose-scented Pelargonium cultivar grown on Reunion island. South African Journal of Botany 1989; 55 (2):184–191.
18. Boukhatem MN, Hamaidi MS. Saidi F, Hakim Y. Extraction, composition et propriétés physico-chimiques de l'huile essentielle du Géranium Rosat (Pelargonium graveolens L.) cultivé dans la plaine de Mitidja (Algérie). Nature et Technologie. 2010; 3:37-45.
19. AFNOR. « Recueil de normes : les huiles essentielles. Tome 2, Monographies relatives aux huiles essentielles ».Edn 6, Vol. 2, Paris La Défense AFNOR, Paris, 2000,661-663.
20. Ammar AH, Lebrihi A, Mathieu F, Romdhane M, Zagrouba F. Chemical Composition and in vitro Antimicrobial and Antioxidant Activities of Citrus aurantium L. Flowers Essential Oil (Neroli Oil). Pakistan Journal of Biological Sciences. 2012; 15: 1034-1040.
21. Jain N, Aggarwal KK, Syamasundar KV, Srivastava SK, Kumar S. "Essential oil composition of geranium (Pelargonium sp.) from the plains of Northern India."Flavour and Fragrance J. 2001; 16:44–46
22. Hassane SOS, Ghanmi M, Satrani B, Mansouri N, Mohamed H, El Hajaji H, Chaouch A. Composition chimique et activités antibactériennes, antifongiques et antioxydante de l'huile essentielle de *Pelargonium asperum* Ehrh. *ex* Wilde des Comores. Acta Botanica Gallica: Botany Letters. 2011; 158 (2):225-237.
23. Onawunmi GO, Yisak WA, Ogunlana EO. Antibacterial constituents in the essential oil of Cymbopogon citratus (DC.) Stapf. J Ethnopharmacol. 1984; 12:279-286.
24. Matasyoh JC, Wagara IN, Nakavuma JL, Kiburai AM. Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. African Journal of Food Science. 2011; 5(3):138-142.
25. Ponce AG, Fritz R, Del Valle C, Roura SI. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. LW u. –Technol. 2003; 36: 679-684.
26. Moreira MR, Ponce AG, Del Valle CE, Roura SI. Inhibitory parameters of essential oils to reduce a foodborne pathogen. **LWT**-Food Science and Technology **2005**;**38**(5):**565-570.**
27. Gao Y, Jin YJ, Li HD, Chen HJ, Volatile organic compoundsand their roles in bacteriostasis in five conifer species. J Integr Plant Biol. 2005; 47:499–507.

28. Baratta MT, Dorman HJD, Deans SG, Figueiredo AC, Barroso JG, Ruberto G. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragance Journal. 1998; 13: 235-244.
29. Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios. 1997; 89(358):39-46.
30. Bigos M, Wasiela M, Kalemba D, Sienkiewicz M. Antimicrobial Activity of Geranium Oil against Clinical Strains of Staphylococcus aureus. Molecules 2012; 17:10276-10291.
31. Ghannadi A, Bagherinejad MR, Abedi D, Jalali M, Absalan B, Sadeghi N. Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L'Her and *Vitex agnus-castus* L. Iran J Microbiol. 2012; 4(4): 171–176.
32. Kotan R, Kordali S, Cakir A. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. Z Naturforsch C. 2007; 62(7-8):507-13.
33. Singh D, Kumar TR, Gupt VK, Chaturvedi P. Antimicrobial activity of some promising plant oils, molecules and formulations. Indian J Exp Biol. 2012; 50(10):714-7.
34. Friedman M, Henika PR, Levin CE, Mandrell RE. Antibacterial activities of plant essential oils and their components against Escherichia coli O157:H7 and Salmonella enterica in apple juice. J. Agric. Food Chem. 2004; 52(19):6042-8.
35. Sivropoulou A, Kokkini S, Lanaras T. Antimicrobial activity of mint essential oils. J. Agric. Food Chem. 1995; 43 (9):2384–2388.
36. Kim YS, Shin DH. Volatile constituents from the leaves of Callicarpa japonica Thunb. and their antibacterial activities. J. Agric. Food Chem. 2004; 52(4):781-7.