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Antibacterial Activity of Essential oils of *Juniperus phoenicea* from Eastern Algeria

Messaoud Ramdani[1*], Takia Lograda[1], Hafsa Silini[2], Azzedine Zeraib[1], Pierre Chalard[3,4], Gilles Figueredo[4], Meriem Bouchaala[1] and Samra Zerrar[1]

[1]Laboratory of Natural Resource Valorisation, Sciences Faculty, Ferhat Abbas University, 19000 Setif, Algeria. [2]Laboratory of Microbiology, Sciences Faculty, Ferhat Abbas University, 19000 Setif, Algeria. [3]Clermont Université, Université Blaise Pascal, BP 10448, F-63000 Clermont Ferrand. [4]CNRS, UMR 6296, ICCF, F-63171 AUBIERE, France. [5]LEXVA Analytique, 460 rue du Montant, 63110 Beaumont, France.

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ABSTRACT

The present study evaluates the chemical composition and antimicrobial activity of essential oils (EOs) of *Juniperus Phoenicea* of five localities from eastern Algeria. The analysis and identification of the components of the Eos was performed using the (GC-MS). The average yield of essential oil of the samples is 0.82%. The chemical composition of the EOs of *J. Phoenicea* is dominated by the presence of a major product, α-pinene (36.3-55.9%). Three components are represented with large concentrations, terpinolene (0-13%), Δ3-carene (0-12.4%) and the β-phellandrene (0-7.3%). Our investigation allows us to support the species *Juniperus phoenicea* of eastern Algeria has several variability quantitative and qualitative. The antimicrobial activity of the essential oils of *J. phoenicea* was evaluated against nine bacteria. The results showed a variable degree of antibacterial activity being the population Elhadjaz most effective.

INTRODUCTION

The genus *Juniperus* is an important component of arid and semi-arid ecosystems throughout the northern hemisphere (Farjon, 1992; Adams, 2008). Previously, from the genus *Juniperus* some terpenoids have been isolated (Fang *et al.*, 1992, 1996; Barrero *et al.*, 2000, 2004, 2006; Lee and Cheng, 2001; Nakanishi *et al.*, 2005; Martin *et al.*, 2006; Okasaka *et al.*, 2006; Mansouri *et al.*, 2010; Seca *et al.*, 2008), neolignans (Nakanishi *et al.*, 2004) and flavonoids (Yuldashev and Rasulova, 2001; Inatomi *et al.*, 2005).The species of *Juniperus* is considered as an important medicinal plant largely used in traditional medicine. The seed decoction of *Juniperus* is used as folk medicine for kidney diseases, and as a diuretic and abortive in Uzbekistan (Karryev, 1967). The isolation and anti-inflammatory activity of some diterpenoids of *J. polycarpus* (El-Sayed, 1998) and several studies about the essential oil of *J. Seravschanica* have been published (Adams, 1999).

* *Corresponding Author*
Email : ramdanimessaoud@yahoo.com
Phone: (213)36835894; Fax: (213)36937943.

Juniperus phoenicea is an evergreen tree indigenous to the North Africa and belongs to the family *Cupressaceae*. The leaves of *J. phoenicea* species are used in the form of decoction to treat diarrhea, rheumatism (Bellakhder, 1997) and diabetes (Bellakhder, 1997; Allali *et al.*, 2008). The mixture of leaves and berries of this plant is used as an oral hypoglycaemic agent (Amer *et al.*, 1994), whereas the leaves are used against bronco-pulmonary disease and as a diuretic (Bellakhder, 1997).

There are many papers report on the chemical composition of leaves and berries essential oils of *J. phoenicea* grown in north Mediterranean basin (Adames *et al.*, 1996; Rezzi *et al.*, 2001; Ennajar *et al.*, 2010; Salido *et al.*, 2002). In Morocco (Barrero *et al.*, 2004; Derwich *et al.*, 2010, 2011; Mansouri *et al.*, 2011a, b; Ait Ouazzou *et al.*, 2012); in Egypt (El-Sawi *et al.*, 2006, 2007), in Tunisia (Akrout 1999; Bouzouita *et al.*, 2008; Ennajar *et al.*, 2007; Medini *et al.*, 2007), in Algeria (Dob *et al.*, 2008; Kilani *et al.*, 2008; Bouzebata and Hadef, 2009; Mazari *et al.*, 2010; Bekhechi *et al.*, 2012), in the Canary Islands and Madeira (Adams *et al.*, 2009), in Portugal (Cavaleiro et al, 2001), in North Africa (Barrero *et al.*, 2006).

All oils of *Juniperus phoenicea* have a high content of α-pinene. The population of Mehdia is individualized by the presence of significant levels of β-pinene, Δ3-carene, limonene, terpinolene and the α-terpinyl acetate.

The population of Spain is isolated by a high rate of manoyl oxide (22%), as well as Tarifa population in Spain with a rate of 6.6% of myrcene (Adames *et al.*, 2009). The essential oil composition of *J. phoenicea* is depending of organ, season and during methods (Ennajar *et al.*, 2007, 2010). The chemical variability of *J phoenicea* has also been investigated, although little is known about their antimicrobial activity. *J. phoenicea* showed an important bacteriostatic and bactericidal effect (Ait Ouazzou *et al.*, 2012)

In the present study, the aim was to identify the chemical composition of the oils of *J. phoenicea* obtained from plants growing in the eastern Algeria as well as to evaluate their antimicrobial activity.

MATERIALS & METHODS

Plant material

Juniperus phoenicea is collected from five localities in eastern Algeria, Boutaleb (Setif), Boussâada (M'sila), Menâa and T'kout (Batna), and Elhadjab (Biskra) (Figure 1). Aerial parts were collected during the flowering stage in October 2012. The air dried materials were subjected to hydro-distillation for 3 h using a clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, Setif University, Algeria.

Extraction of the essential oil

100 g of the air-dried aerial parts of five populations were subjected to hydro distillation for 3 h with 500 ml distilled water using a Clevenger-type apparatus. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on dried weight of the samples was calculated.

Essential oil analysis

The essential oils were analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 μm), programming from 50°C (5 min) to 300°C at 5°C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the *m/z* range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library (Masada, 1976; NIST, 2002) and those described by (Adams, 2001) as well as on comparison of their retention indices either with those of authentic compounds or with literature values (Adams, 2001).

Antibacterial Activity

The antimicrobial activities of the essential oils were evaluated against both Gram positive (*Enterobacter cloacae* ATCC 13047, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Staphylococcus aureus* ATCC 25923) and six Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas syringae, Salmonella sp, Serratia liquefaciens* ATCC 27592, *Serratia marcescens* ATCC 14756, *Shigella* sp). The bacterial inoculums was prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) and MH agar supplemented with 5 % sheep blood for fastidious bacteria were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 μl of mother solution and diluted essential oil (1:1, 1:2, 1:4, and 1:8 v:v of DMSO). DMSO was used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24h aerobically (Bacteria). After incubation, inhibition zone diameters were measured and documented.

Statistical analysis

Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek for hierarchical associations among the populations. The cluster analyses were carried out using STATISTICA 10 software.

RESULTATS AND DISCUSSION

The hydrodistillation of the essential oil of *Juniperus phoenicea* gave a viscous liquid with a yellowish color and strong odor of juniper. The average yield of essential oil of our samples is 0.82%, the highest rate is observed in the essential oil of the populations of T'kout and Elhadjaz (0.92%), while the population of Menâa is characterised by the lowest yield (0.70%).

The analysis and identification of the components of the essential oil of *J. Phoenicea* was performed using the (GC-MS). The compounds identified in these oils and their relative abundances are presented in order of their appearance in Table 1. These analyses led to the identification of 73 components. The chemical composition of the essential oil of *J. Phoenicea* is dominated by the presence of a major product, α-pinene with an average (48.08%), the highest content was observed at the Elhadjaz population 55.9% and the lowest was recorded at station of T'kout with 36.5%.

Three components are represented with large concentrations in the essential oil. The oil of Boussaâda is characterised by terpinolene (13%). The Δ3-carene caracterise the populations of T'kout, Menâa and Elhadjaz with a rate (12.4%, 5.4% and 3%) respectively. The populations of Boutaleb and T'kout contains a percentage of (7.3 - 4.4%) of β-phellandrene. The chemical composition of this species contains other

components of a lower rate, linalool tetrahydro- in Menâa, Elhadjaz and T'kout. Our populations contain a low rate, but more than 1%, of β-caryophyllene, germacrene-D and germacrene-B.

The classification of our populations, according to their chemical kinship relations, is based on the construction of groups. The UPGMA based on the unweighted pair-group average distance and the City-block (Manhattan) (Figure 2), has divided our populations in to four groups.

We note the individualization of our populations studied. The population of T'kout is rich in Δ3-carene (12.4%); Boussâada is characterized by a high rate of terpinolene (13%), while the β-phellandrene with a rate (7.3%) characterizes the population of Boutaleb. Both populations, Menâa and Elhadjaz, are grouped by the presence of appreciable levels of Δ3-carene and linalool-tetrahydro. The antibacterial activity of the essential oils was evaluated against nine microorganisms, using disc diffusion method. The disc diameters of zone of inhibition of essential oils for the microorganisms tested are grouped in the Table 2. The results showed that the oils inhibited the growth of bacterial strains produced a zone diameter of inhibition from 7 - 45 mm, depended on susceptibility of the tested bacteria.

The essential oil of T'kout population has no effect, on the bacteria (*Enterobacter cloacae* ATCC 13047; *Escherichia coli* ATCC 25922, MRSA; *Pseudomonas syringae; Serratia marcescens* ATCC 14756 and *Shigella* sp), by against its effect is very significant on *Salmonella sp* with antibacterial activity than the effect of gentamicin. The action of the oil on bacteria *Serratia liquefaciens* ATCC 27592 and *Staphylococcus aureus* ATCC 25923 is moderate to low. The Bacteria **(***Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 25922, MRSA, *Pseudomonas syringae*, *Serratia marcescens* ATCC 14756, *Salmonella* sp and *Serratia liquefaciens* ATCC 27592) are resistant to the essential oil of Menâa population. This oil has a moderate antibacterial activity against *Shigella* sp and *Staphylococcus aureus* ATCC 25923. The essential oil of the population of Elhadjaz shows a significant activity on all the bacteria tested. The bacteria **(***Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 25922, *Pseudomonas syringae*, *Salmonella sp* and *Shigella* sp) are resistant to essential oils of the population of Boutaleb. The rest of the tested bacteria are very sensitive to this oil. The dilutions of essential oil used have a moderate activity on all bacteria. The essential oil of the Boussâada population has significant activity against bacteria (*Enterobacter cloacae* ATCC 13047, *Salmonella sp*, Serratia marcescens ATCC 14756, *Shigella* sp and *Staphylococcus aureus* ATCC 25923); while the rest of the bacteria are resistant to the oil concentrate. All the tested bacteria are sensitive to oil dilution of Boussaâda population

Our returns of the essential oil are low compared to those of the literature. This yield is 1.96% in Egypt (El-Sawi *et al.*, 2007), in Morocco, the yield is 1.62% (Derwich *et al.*, 2011), in Portugal, Spain and Greece the yield is low (Adams et al, 1996). Bouzouita *et al.*, (2008) found a yield of 0.5% in Tunisia. This difference in essential oil content is related to several factors, such as the geographical area of collection, climate, stage of development and the season.

The comparison of the chemical components of the essential oil of our samples with those of *Juniperus phoenicea* oils shows that α-pinene is the major product of the oil. The highest rate of α-pinene is found in the population of Spain (Palma) and Morocco (Adames *et al.*, 2009; Mansouri *et al.*, 2011), while the lowest rate is found in the population of Spain (Tarifa)(Adames *et al.*, 2009). The β-phellandrene is the second product; the population of Tarifa in Spain is individualized by a rate of 31.5% (Adames *et al.*, 2009). The Δ3-carene is substituted by low levels except for the population of Morocco (Mahdia) (20.64%)(Mansouri *et al.*, 2011) and the Algerian population (T'kout) with a rate of 12%. the terpinolene, limonene oxide and manoyl characterize, with a high rate, each one of populations, Boussaâda (Algeria), Morocco (Mansouri *et al.*, 2011) and Spain (Adames et al. 2009).

The essential oil of *Juniperus phoenicea* exhibits antimicrobial activity against all strains tested. The inhibition zones were lower than those of antibiotics, which showed wide inhibition zones at very low concentrations. The reference antibiotic showed no activity in the three Gram positive bacteria (*Enterobacter cloaceae*). It showed significant activity with all bacteria tested. Although the concentrations of the oil of Elhadjaz population were generally higher than the standard antibiotic (gentamicin), they showed marked antibacterial activities as evidenced by their zones of inhibition. The antimicrobial activity is likely to be associated with the high concentration of a-pinene. The results show that the Gram-negative bacteria are more resistant than the Gram-positive bacterium. It has been shown that Gram-positive bacteria are more sensitive than Gram- as was shown by (Bouzouita *et al.*, 2008, Ait Ouazzou *et al.*, 2012). *Enterococcus feacalis* was the most sensitive microorganism with the highest inhibition zone (15.6 mm) to the essential oil of *J. phoenicea*. On the other hand, that *Pseudomonas syringae* was resistant at these essential oils as reported by (Mazari *et al.*, 2010).

CONCLUSION

Analysis of the chemical composition of the essential oil of *Juniperus phoenicea* has allowed identifying 73 compounds. The majority compounds are the α-pinene, Δ3-carene, β-phellandrene, myrcene, linalool-tetrahydroxy-, germacrene-D and β-phellandrenedrene.

The Essential oil was found to be active against all the bacterial strains. Dilution of the essential oil affected the effectiveness in some cases. That is, the activity of the oil varies with its concentration and kind of bacteria. The oil of T'kout and Mena population has no antibacterial activity; bat the oil of Elhadjaz population is very active against all the bacteria tested. For this renewed interest, the present study provides additional data of the chemical composition and antibacterial activity of the EOs of *J. phoenicea* obtained from aromatic plants growing in Algeria.

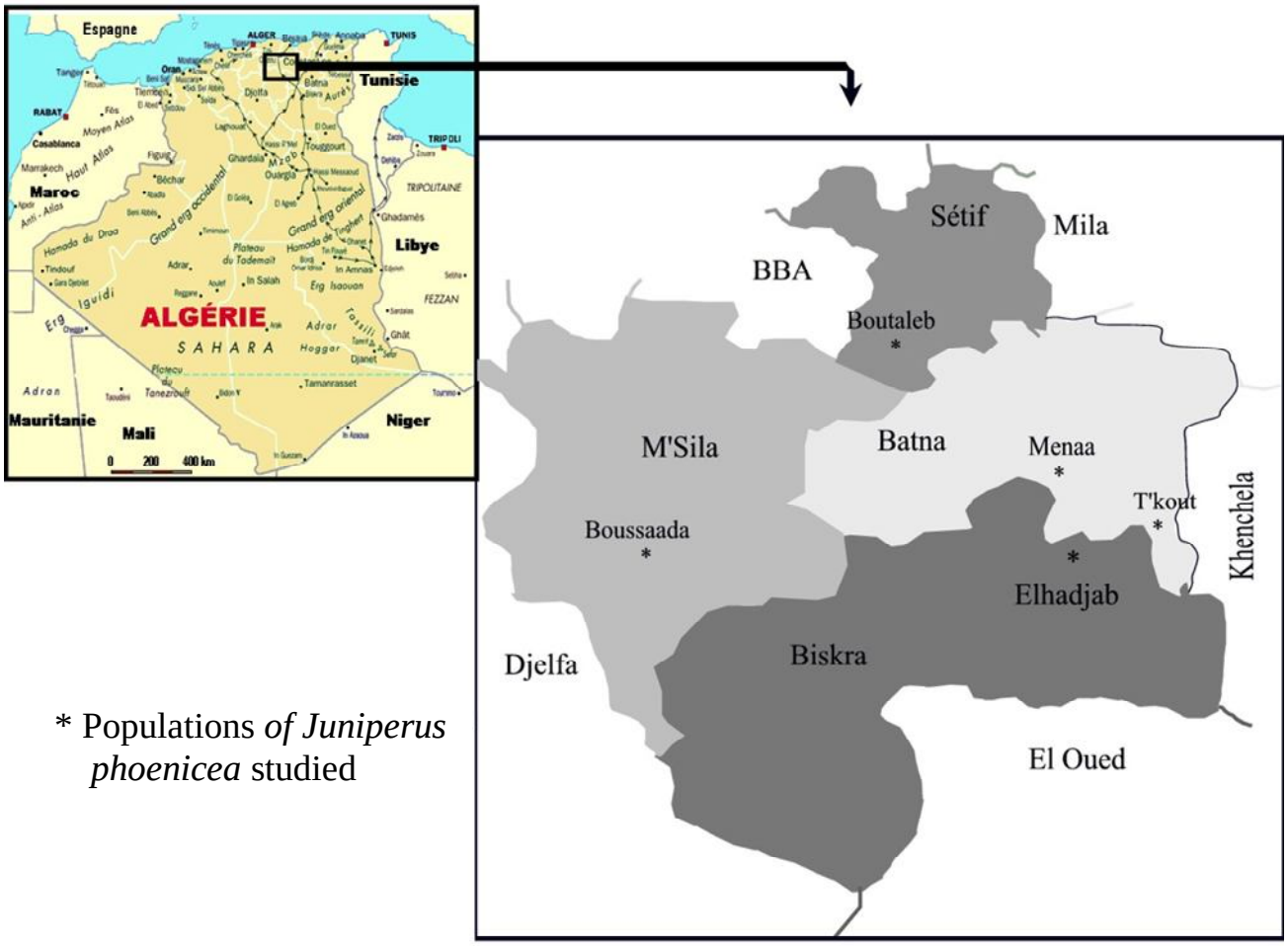

Fig. 1: Populations of *Juniperus phoenicea* studied.

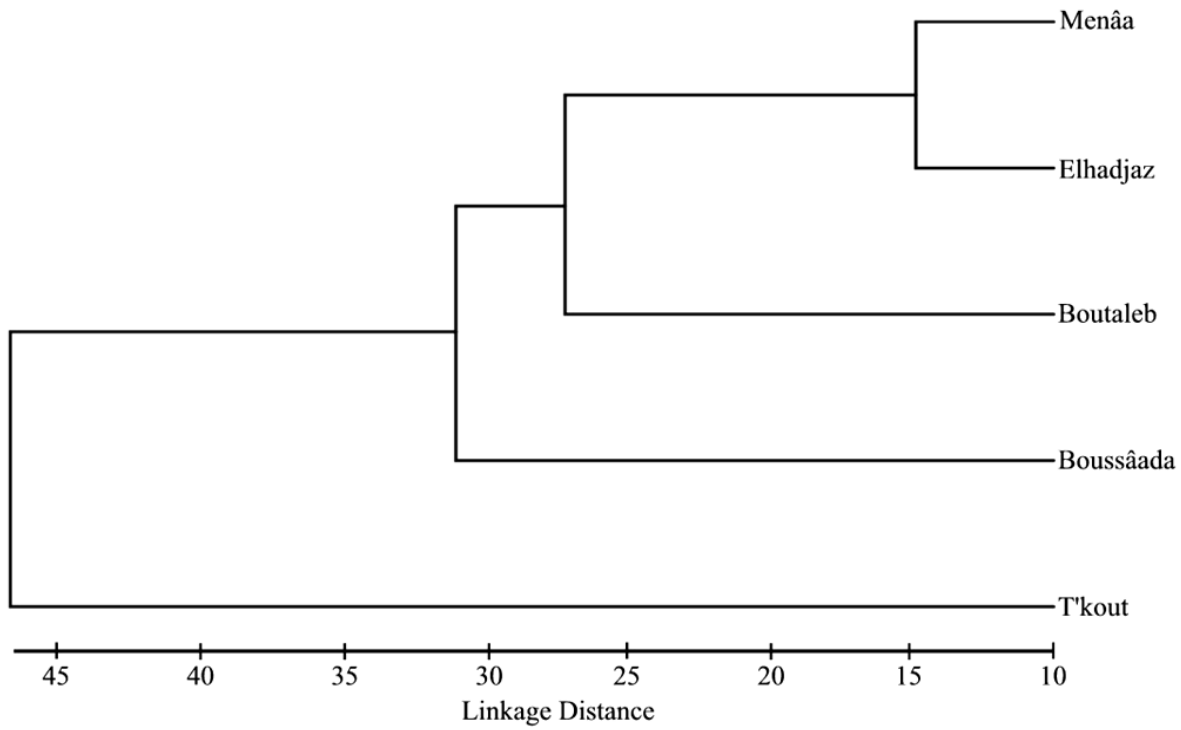

Fig. 2: UPGMA of *Juniperus phoenicea* populations.

Table. 1: Chemical composition of *Juniperus phoenicea* populations.

Populations	KI	Menâa	Elhadjaz	T'kout	Boutaleb	Boussâda
Yield (v/w)		0.7	0.92	0.92	0.8	0.75
Nb of Compounds		53	44	44	47	47
Total (%)		88.5	91.4	86	85	85
Tricyclene	920	0.3	0.2	0.2	0.3	0.2
α-Pinene	935	**47.2**	**56**	**36.5**	**53.7**	**47.1**
Fenchene	945	-	-	-	0.1	0.8
Camphene	948	0.4	0.4	0.3	0.4	0.3
Verbenene	952	0.1	-	0.1	0.1	0.1
Sabinene	971	0.1	-	0.3	-	-
β-Pinene	975	0.8	0.8	0.8	1	0.7
Myrcene	988	1.8	1.8	1.9	2.4	1.5
Δ-3-Carene	1006	**5.4**	3	**12.4**	-	-
Isosylvestrene	1009	-	-	-	1.6	0.4
Para cymene	1022	0.4	0.3	0.4	0.8	0.5
Limonene	1027	1.3	0.7	-	0.6	0.8
β-Phellandrene	1028	-	0.8	4.4	**7.3**	1.7
γ-Terpinene	1056	0.3	0.3	0.1	0.3	0.2
Linalool oxide (trans)	1069	-	-	0.1	0.3	0.2
Terpinolene	1085	-	-	-	0.1	**13**
Cymenene	1088	0.1	-	0.2	0.5	0.2
Linalool	1097	-	-	-	1.7	0.8
Linalool tetrahydro-	1099	3.6	3.2	1.8	0.5	0.1
α-campholene aldehyde	1125	0.2	0.3	0.3	0.2	0.3
Trans-Pinocarveol	1140	0.2	0.4	0.5	-	-
Trans-Verbenol	1145	0.2	0.9	0.8	-	-
α-Phellandren-8-ol	1150	-	-	0.1	0.3	0.1
p-mentha-1,5-dien-8-ol	1172	0.1	0.2	0.4	-	-
Pinocamphone cis	1175	-	-	-	0.2	0.3
Terpinene-4-ol	1180	0.1	0.2	0.3	0.2	0.1
α-terpineol	1195	0.5	0.7	1	-	-
Safranal	1197	-	-	-	0.1	0.9
Nopol	1203	0.1	-	-	0.3	0.2
β-Fenchyl acetate	1217	0.1	-	-	0.7	0.4
Citronellol	1226	-	-	-	0.4	0.3
2,4-Decadien-1-ol	1314	0.5	0.2	0.4	-	-
γ-Terpinene	1332	0.2	-	0.3	-	-
Δ-Elemene	1336	0.3	0.3	0.3	-	-
Piperitone	1343	-	-	-	0.6	0.2
Pseudopinene	1347	0.8	0.8	3.3	-	-
α-Terpinyl acetate	1349	-	-	-	0.7	0.4
Carveyle acetate cis	1368	-	-	-	0.4	0.4
β-Bourbonene	1385	0.2	-	0.2	0.1	0.2
β-elemene	1390	0.5	0.4	0.4	0.4	0.4
β-caryophyllene	1421	1.7	0.9	1.1	1	1.7
g -Elemene	1430	0.4	0.4	0.4	0.4	0.6
Germacrene-D	1438	1.5	1.6	1.5	1.4	1.5
Citronellyle Propanoate	1446	-	-	-	0.3	0.1
α-cubebene	1451	0.6	0.6	0.6	-	-
α-humulene	1458	1.1	0.9	0.7	0.6	0.8
Muurola 4,14,5-dienecis	1494	-	-	-	0.5	0.5
Epi-bicyclo-sesquiphellandrene	1495	1.6	1.1	1.3	-	-
Cadina-1,4-Diene	1496	-	-	-	0.1	0.2
Calarene (+)	1497	1.2	1.1	0.7	-	-
α-muurolene	1499	0.7	0.4	0.4	0.3	-
α-selinene	1511	0.3	0.2	0.3	0.1	0.2
α-amorphene	1514	0.2	3.2	0.1	-	-
Δ-cadinene	1520	1.8	-	3	0.2	0.2
Cis-calamenene	1523	1.8	1.4	1	-	-
Valencene	1539	0.3	0.2	0.1	-	-
Elemol	1550	0.7	0.6	0.8	0.4	0.8
Muurol-5-ene-4-α-ol cis	1561	-	-	-	1.5	2.3
Germacrene B	1562	1.7	1.9	1.7	0.9	1.2
α-amorphene	1566	0.5	0.3	0.2	-	-
Citronellyl propionate	1572	0.2	0.5	0.2	-	-
Germacrene D-4-ol	1579	0.2	0.2	0.3	0.2	0.2
Caryophyllene oxyde	1585	0.6	0.5	0.6	0.4	0.8
Ethyl laurate	1593	0.5	0.5	0.9	-	-
Humulene-1,2-epoxyde	1613	-	-	-	0.2	0.3
α-Cedrene	1631	**2.8**	-	-	-	-
α-gurjunene	1635	0.2	-	0.2	-	0.2
(+)-β-guaiene	1658	0.8	0.5	0.7	-	-
Hexenyl cyclopentanone	1698	0.5	0.6	0.8	0.2	0.6
Manoyl oxide	1997	0.1	1.6	0.2	-	-

Table. 2: Inhibition diameter (mm) of essential oil of *Juniperus phoenicea*.

Bacteria	Gent	Populations																			
		T'kout				Menâa				Elhadjaz				Boutaleb				Boussâada			
		E	*Dilution*			E	*Dilution*			E	*Dilution*			EO	*Dilution*			E	*Dilution*		
		O	½	¼	1/8	O	½	¼	1/8	O	½	¼	1/8		½	¼	1/8	O	½	¼	1/8
Enterobacter cloacae ATCC 13047	0	0	0	0	0	0	0	0	0	8	8	9	10	10	0	10	10	7	7	9	9
MRSA	14	0	0	0	0	0	0	0	0	27	25	30	22	0	0	9	8	0	0	9	7
Staphylococcus aureus ATCC 25923	25	8	7	0	0	25	15	9	8	45	35	11	15	11	12	9	7	13	30	23	10
Escherichia coli ATCC 25922	14	0	0	0	0	0	0	0	0	35	25	18	19	0	8	0	7	0	9	7	7
Pseudomonas syringae	10	0	0	0	0	0	0	0	0	15	9	8	8	0	0	7	8	0	7	9	0
Salmonella sp	16	25	18	15	15	0	0	0	0	30	20	17	15	0	0	10	9	7	8	8	8
Serratia liquefaciens ATCC 27592	10	7	8	0	9	0	0	0	0	12	11	11	11	11	8	8	9	0	9	0	0
Serratia marcescens ATCC 14756	12	0	0	0	0	0	0	0	0	13	10	9	12	7	0	8	8	7	7	8	9
Shigella sp	15	0	0	0	0	15	10	10	8	30	30	35	25	0	0	7	8	12	8	8	7

MRSA = Methicillin-resistant *Staphylococcus aureus*; Gent. = Gentamicine; EO = Essential oil

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