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Chemical composition and antimicrobial activity of *Pinus halepensis* from Algeria

RIMA HAICHOUR[1,2], MESSAOUD RAMDANI[1,♥], TAKIA LOGRADA[1], PIERRE CHALARD[3], GILLES FIGUEREDO[4]

[1]Laboratory of Natural Resource Valorisation, Faculty of Life and Natural Sciences, Ferhat Abbas University Setif-1. 19000 Setif, Algeria
Tel./fax.: +213-658-101010, ♥email: ramdanimessaoud@yahoo.com
[2]Department of Natural Sciences, Mohamed Boudiaf University of M'sila. PB 166 M'sila 28000, Algeria
[3]Université Clermont Auvergne, CNRS, SIGMA Clermont, ICCF, F-63000 Clermont–Ferrand, France
[4]LEXVA Analytique, 460 Rue Du Montant, 63110 Beaumont, France

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Abstract. *Haichour R, Lograda T, Ramdani M, Chalard P, Figueredo G. 2020. Chemical composition and antimicrobial activity of Pinus halepensis from Algeria. Biodiversitas 21: 4345-4360.* The chemical composition of *Pinus halepensis* essential oils and their antibacterial activities were investigated. Plant samples were collected in the flowering stage from the East locality of Algeria. The aerial parts of *P. halepensis* from fifteen localities were subjected to a hydro-distillation. The analysis of the chemical composition of essential oils was performed by using GC, and GC / MS. Hydro-distillation of *P. halepensis* produces a pale yellow viscous oil with an average yield of 0.64 ± 0.37%. A total of thirty-seven compounds representing 99.4 ± 0.5% of the total oils were identified in *P. halepensis*. The hydrocarbon compounds were found to dominate essential oils in *P. halepensis*. In addition, the major compounds were caryophyllene-E (29.06 ± 8.5%), myrcene (19.14 ± 6.67%), α-pinene (16.86 ± 3.35%), phenyl ethyl 3-methyl butanoate (5.67 ± 2.47%), α-humulene (4.81 ± 1.43%), terpinolene (3.94 ± 1.6%) and sabinene (3.11 ± 1.84%). The essential oil samples were clustered in five groups based on their chemical composition by UPGMA analysis. The first group includes the Tafrent essential oil population. It is characterized by the phenyl ethyl-3-methyl butanoate – Sabinene – Δ3-carene chemotype. The second group was subdivided into two clusters. The 1st cluster or chemotype was characterized by the myrcene – α-pinene – caryophyllene-E. The second chemotype was characterized by caryophyllene- E – α-humulene – myrcene. The essential oil of *P. halepensis* is an effective antimicrobial against *Staphylococcus aureus*; *Klebsiella pneumonia*; *Salmonella enteric* spp *arizonae*; *Listeria innocua*; *Proteus mirabilis* and *Bacillus cereus*, and not active against *E. coli* and *Pseudomonas aeruginosa.*

Keywords. Algeria, antibacterial activity, bioactive molecules, chemotypes, essential oil, *Pinus halepensis*

INTRODUCTION

Pinus halepensis is a plant species widely distributed around the Mediterranean and over the mountain massifs (Correal-Mòdol and Casals 2012). In Algeria, it is found in all bioclimatic variants, especially in the semi-arid stage, showing high dominance (Quezel and Barbero 1992). Pines are characterized by high levels of fatty acids, vitamin E, polyphenols and natural antioxidants, widely used for cosmetics (Ustun et al. 2006) and the food industry (Chikh Rouhou 2006; Kadri et al. 2014). Aleppo pine resin is commonly used in popular medicine, as a powerful antiseptic, and as a cure for treating infections of respiratory and urinary tracts, gallstones, sinusitis, and rheumatism (Motte-Florac 2000; Berroukche et al. 2014). The chemical composition of *P. halepensis* essential oils has been widely studied (Roussi et al. 1995; Hmamouchi et al. 2001; Lahlou 2003; Macchioni et al. 2003; Dob et al. 2005; Dob et al. 2007; Tumen et al. 2010; Abi-Ayad et al. 2011; Ustun et al. 2012, Gallis et al. 2012; Amri et al. 2013; Amri et al. 2014; Efestathia et al. 2014; Djerrad et al. 2015; Fekih et al. 2015; Mohareb et al. 2017; El Baha et al. 2016; Nam et al. 2016; Rodrigues et al. 2017; Bouyahiya et al. 2019; Mitić et al. 2019) (Table S1).

The essential oils possess bioactive properties due to the mixture of various volatile compounds, including monoterpenes as the main component, which makes an important contribution to pathogen resistance (Sharma et al. 2019). A previous study on the chemical composition of Tunisian Aleppo pine essential oils showed the presence of β-elemene, α-humulene, α-pinene, and β-pinene (Dziri and Hosni 2012). Populations from other places are mainly composed of β-caryophyllene-E, myrcene, and α-pinene (Amri et al. 2014). The essential oil of Aleppo pine populations from Morocco and Italy contains α-pinene, myrcene, and β-caryophyllene-E (Hmamouchi et al. 2001; Macchioni et al. 2003). The population from Italy contained β-caryophyllene-E, myrcene, ρ-cymene, and α-pinene (Vidrich et al. 1988). The essential oil of Aleppo pine from Greece was rich in β-caryophyllene-E, α-pinene, and cembrene (Roussis et al. 1995; Gallis et al. 2012). Corsica population is characterized by β-caryophyllene-E, myrcene, α-pinene, terpinolene, Δ3-carene, and sabinene (Nam et al. 2016). *P. halepensis* essential oils populations of Turkey exhibited the presence of α-pinene and β-pinene (Ustun et al. 2012). The main chemical composition of *P. halepensis* varies widely in different countries, with different various main components. The main compounds of Aleppo pine in Portugal are α-

pinene, β-myrcene, Δ3-carene, and β-caryophyllene (Rodriguez et al. 2017). The main compounds of Aleppo pine in Libya are α-pinene, β-pinene, α-terpineol, and caryophyllene (Mohareb et al. 2016). Besides, the main compounds of Aleppo pine from Egypt and caryophyllene, α-pinene, and thumbergol (El-Baha et al. 2016). The essential oil of Aleppo pine from three different altitudes in Libya are a high percentage of α-pinene, β-pinene, α-terpineol, and caryophyllene (Mohareb et al. 2016). The Greece populations are abundant in β-Caryophyllene-E and thumberguol (Mitić et al. 2019). The chemical composition of *P. halepensis* essential oils from various locations in Algeria have been well documented (Table S2).

The chemical composition of essential oils from populations in some regions in Algeria (Sidi Feradj, Djelfa, and Saïda) showed that the main compounds are β-caryophyllene-Z, α-humulene, aromadendrene (Dob et al. 2007). The essential oil of the Ghazaouet population contains mainly caryophyllene oxide (48.1%) and β-caryophyllene-E (2.9%) (Abi-Ayad et al. 2011). The Tlemcen populations consist of two groups; one is characterized by myrcene, α-pinene, terpinolene, and isovalerate-2-phenylethyl, and the other one is dominated by myrcene, α-pinene and β-caryophyllene-E (Fekih et al. 2014). Aleppo pine was found to be rich in α-pinene, myrcene, terpinolene, and terpinene-4-ol in the Tebessa region (Djerrad et al. 2015), while the essential oils of Tissemsilt populations contain myrcene, iso-valerate of 2-phenylethyl and β-caryophyllene-E as the main compounds (Tazerouti et al. 1993). The Aleppo Pine essential oils contain various bioactive compounds, that have, antioxidant (Ustum et al. 2012; Djerrad et al. 2015; Tumen et al. 2018), antibacterial (Fekih et al. 2014; Ghalem et al. 2014), antifungals (El Baha et al. 2016), anti-inflammatories (Suntar et al. 2012) and anticancer (Simard 2007; Alonso-Castro et al. 2011; Kadri et al. 2014; Bouzenna et al. 2016), activities.

Several previous studies have investigated the antibacterial activities of *P. halepensis* essential oils. The studies showed that Aleppo pine essential oil is active as an antibacterial against *E. coli*, *B. subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus* (Ghanmi et al. 2007; Raho et al. 2014; Fekih et al. 2014; Ashmawy et al. 2018). The previous studies showed that several bacteria are sensitive to the essential oil of *P.halepensis*, i.e., *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Corynebacterium fascians*, *Pseudomonas solanacearum* (Mohareb et al. 2016), *Klebsiella pneumoniae*, *E. coli*, *Morganella morganii*, *Staphylococcus aureus* (Mitić et al. 2019), *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes* (Bouyahya et al. 2019), and *P. aeruginosa* (Abi-Ayad et al. 2011).

The present study aimed to determine the chemical composition of the essential oils of *Pinus halepensis* from the east locality of Algeria, to identify the different possible chemotypes from the studied populations, and to evaluate their antibacterial activity against eight bacterial strains.

MATERIALS AND METHODS

Plant materials

Aerial parts of *Pinus halepensis* were collected when they were in flowering in November 2016. There were five sampling sites in northern Algeria, Boutaleb (south of Setif city), Babor (north of Setif), Ouled Yaakoub forest (Khanechela city), Seriana forest (Batna city) and Jijel (Figure 1).

The geographical coordinates of sampling sites were presented in Table 1. Voucher specimens were deposited in the Herbarium of Biology and Ecology Department, Setif-1 University, Algeria.

Hydro-distillation of aerial part of *Pinus halepensis*

Hydrodistillation of air-dried materials using a Clevenger apparatus type was carried out for 3 hours. The obtained essential oil was dried with sodium sulfate anhydrous. The yield was calculated based on the dry weight of samples.

Essential oil analysis

Chemical composition of essential oils was analyzed by Gas Chromatography (Hewlett-Packard CPG/FID 7890) coupled to Gas Chromatograph apparatus (CPG/MS 7890/5975C). It is equipped with a column A polar (DB5 MS: 40m 0.18mm 0.18µm), programming from 50°C for (5 min) to 5°C/min, until 300°C. Helium was used as the carrier gas (1.0 mL/min), injector in split mode (1:30), and injector and detector temperature of 280°C with 1/100 split. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; Ion source temperature of 180°C; MS data were acquired in the scan mode in the *m/z* range 33450. The chemical components were identified by comparing their mass spectra with the Mass Spectral Library of NIST (Masada 1976; NIST 2002). Their retention time was compared to the retention time of authentic compounds or with literature (Adams 2007).

Antibacterial activity

Essential oils were tested against eight selected bacteria strains resistant to antibiotics, namely *E-coli* ATCC25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumonia* ATCC 700603, *Salmonella enteric* spp. *arizonae* CIP 81-3, *Listeria innocua* clip 74915, *Proteus mirabilis* ATCC 35659, and *Bacillus cereus* ATCC 11778. The *in vitro* antibacterial activity of the essential oil was determined by the microdilution method, according to the Clinical and Laboratory Standards Institute Recommendations (NCCLS).

Bacteria were cultured overnight in physiological saline (0.8% of NaCl). The optical density was observed at 625 nm. Muller-Hinton Agar (MHA) supplemented with 5% sheep blood was poured into Petri dishes, solidified, and dried before bacterial inoculation. Six mm-sterile discs were placed on MHA growth media that had been inoculated with a suspension of test bacteria. Ten µl of essential oil and diluted essential oil (1:1, 1:2, 1:4 and 1:8 v/v of DMSO, DMSO used as a negative control) was transferred to sterile discs. Petri dishes were incubated at 37°C for 18 to 24h aerobically, and the inhibition zones were determined and recorded after incubation complete. The diameter of the inhibition zone around the discs refers to bacterial growth inhibition. All experiments were carried out in three replicates.

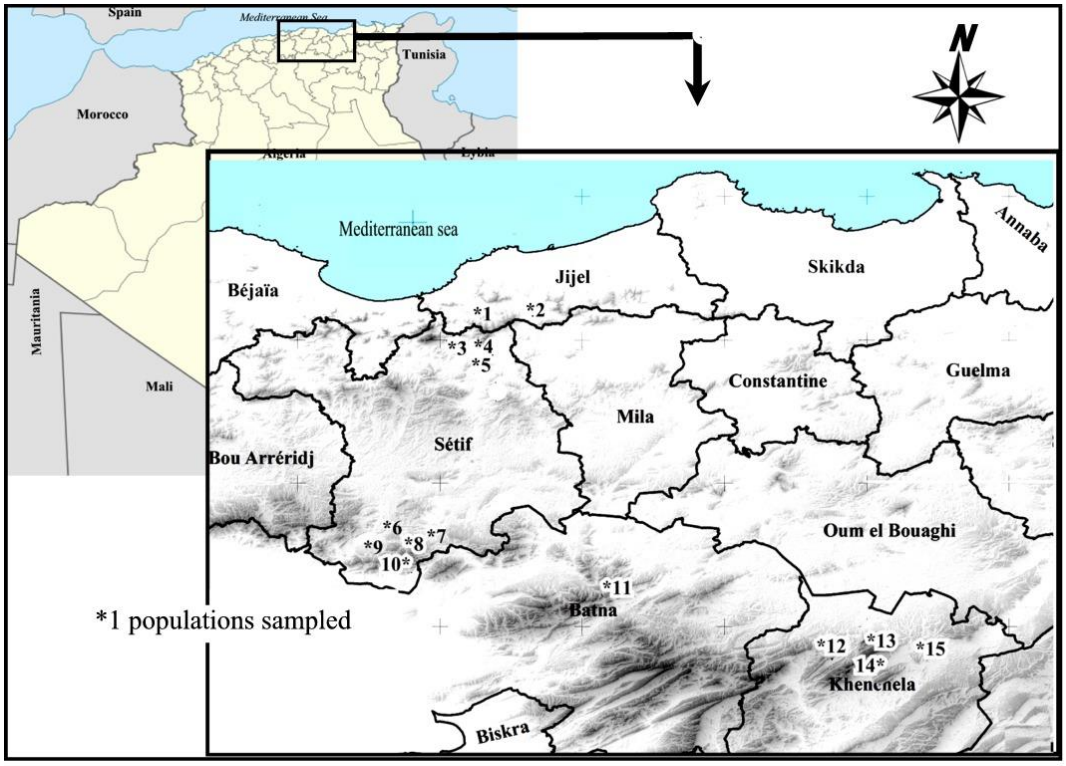

Figure 1. Sampling locations of *Pinus halepensis* in northern Algeria. (For the numbers (*1) see Table 1)

Table 1. Geographical coordinates of the sampling sites in northern Algeria

Localities		Populations	Altitude (m)	Geographic coordinates	
Jijel	1	Bir ghzala	0947	36°36' 48.75"N	5°30' 38.56"E
	2	Iraguene	0767	36°35' 10.32"N	5°34' 51.24"E
Setif North (Babor)	3	Djouada	1568	36°30' 18.90"N	5°29' 50.70"E
	4	Daassa	0861	36°29' 23.64"N	5°32' 21.48"E
	5	Beni Bezez	1133	36°19' 69.03"N	5°41' 50.87"E
Setif South (Boutaleb)	6	Ain laaneb	1433	35°44' 25.54"N	5°21' 04.93"E
	7	Bouriache	0982	35°43' 09.89"N	5°13' 45.34"E
	8	Cheabet Thnia Khrouf	1296	35°42' 06.27"N	5°20' 55.77"E
	9	Chikda	1259	35°42' 56.19"N	5°25' 05.09"E
	10	Sidi amor	1054	35°42' 16.14"N	5°22' 03.08"E
Batna	11	Seriana	1026	35°39' 48.90"N	6°11' 53.30"E
Khanechela (Ouled Yakoub)	12	Boussenane	1178	35°22' 51.60"N	6°54' 05.35"E
	13	Troud	1160	35°23' 53.19"N	6°57' 02.17"E
	14	Tafrent	1562	35°22' 31.72"N	6°57' 30.84"E
	15	Tizi yaala	1224	35°25' 06.33"N	6°83' 23.72"E

The sensitivity of bacteria to the essential oils was categorized into five groups according to the diameter of the inhibition halos): Not sensitive (–) (diameter < 8 mm); Sensitive (+) (diameter ranges from 9 to 14 mm); Very sensitive (++) (diameter ranges from 15 to 19 mm), and Extremely sensitive (+++) (diameter > 20mm) (Ponce et al. 2003). Standard antibiotics were used as positive control: Gentamicin (10 µg), Erythromycin (15 µg), Amoxicillin (25 µg), Meropenem (10 µg) and oxacillin (1 µg).

Statistical analysis

Principal Components Analysis (PCA) examined the relationships between terpene compounds, identified the possible population structure, and the variations in the composition of *P. halepensis* essential oil. Cluster (UPGMA) analysis applied in the original variables and the Manhattan distance analyzed the hierarchical associations between the populations. Statistical significance was determined by the three-way ANOVA and posthoc tests. Statistica (Ver. 10) carried out all statistical tests, and the antibacterial activity data are expressed as mean ± SD, where differences with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The extraction of essential oils from the aerial parts of *P. halepensis* was performed by the hydro-distillation method, using a Clevenger type apparatus. The provided essential oils have different colors ranging from light yellow to transparent, with a very strong odor. The average yield of essential oil was 0.64 ± 0.37%. The highest yield (1.34%) was from the essential oil of the Iraguene population, and the lowest yield (0.34%) was obtained from the Tafrent population. The results indicated that altitude directly affected the yield of *P. halepensis* essential oil. The regression curve confirms yield reduction by increasing altitude (Figure 2).

The essential oils of *P. halepensis* were analyzed by Gas Chromatography and Mass Spectrometry (GC / MS). The identified components are shown in Table 2 according to retention time. Chromatographic analysis of Aleppo pine essential oils showed the presence of thirty-seven identified volatile compounds, representing of 99.40 ± 0.5% of the total essential oil. The major compounds were caryophyllene-E (29.06 ± 8.5%), myrcene (19.14% ± 6.67%), α-pinene (16.86 ± 3.35%), phenylethyl 3-methyl butanoate (5.67 ± 2.47%), α-humulene (4.81 ± 1.43%), terpinolene (3.94 ± 1.60%), sabinene (3.11 ± 1.84%), Δ3-carene (2.18 ± 1.85%) and cembrene (2.15 ± 1.93%), and other compounds of relatively low percentage (limonene, β-ocimene, β-pinene, germacrene-D, α-muurolene and linalool). The essential oil of *P. halepensis* contained 14 monoterpene hydrocarbons (51.46 ± 9.73%), and four oxygenated monoterpenes (1.27 ± 1.47%. The oil of Tizi yaala and Troud regions was found to be rich in myrcene (28.23-24.01%) and α-pinene (23.97-21.08%). Myrcene and α-pinene are minimal amounts in Daassa and Beni Bezez populations (Table 3).

The hydrocarbon sesquiterpenes were 37.12 ± 9.73%, which was dominated by caryophyllene-E. The high content of caryophyllene-E was found in the Beni Bezez (47%), Daassa (40.92%), and Iraguene (36.10%) populations. The lowest content was found in the Tafrent population (15.20%). The oxygenated sesquiterpenes are very low (1.0 ± 0.91%). Three diterpenes (cubitene, cembrene, and α-cembrene-3Z) were also identified in *P. halepensis* essential oil. The total average of oil esters was 5.87 ± 2.62%, which were consisted of bornyl acetate (0.01%), geranyl acetate (0.2%), and phenyl ethyl 3-methyl butanoate (5.67%).

The results of the chemical composition analysis of the *P. halepensis* essential oils show variability. Relationship analysis between populations was carried out by the Principal Component Analysis (PCA), and it showed that the composition of essential oil differed significantly between population, and the major compounds show a few inter-population variabilities (Figure 3).

The three-dimensional spatial projection of the populations based on the three main axes from the PCA (Figure 4) shows that the populations of Tafrent, Troud, and Beni Bezaz are distinctly separated. The rest of the populations are not separated split clearly, and their separation into homogeneous groups is less obvious.

The result of the PCA test and *P. halepensis* populations in two distinct clades are confirmed by UPGMA clusters analysis (Figure 5). This population's clustration in small groups indicates the presence of difference in the chemical composition of the essential oils.

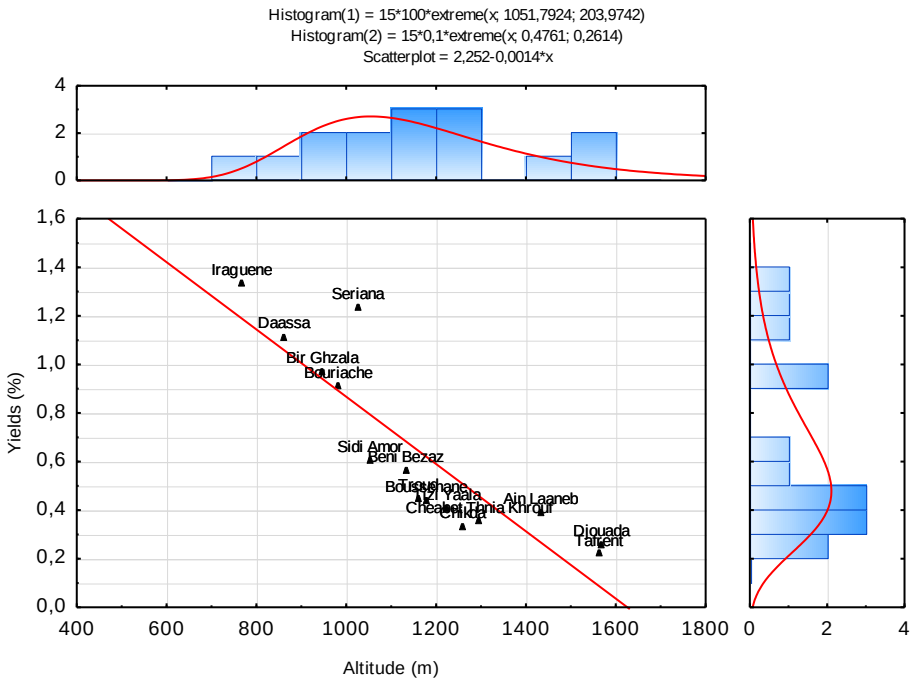

Figure 2. The yield of essential of the *P. halepensis* populations from Algeria

The first clustration separated the population of Tafrent from the other populations. It is based on the presence of volatile compounds of sabinene, Δ3-carene, and terpinolene. The second clade is divided into two sub-branches, i.e. the populations of Beni Bezez and Daassa, forming a subgroup characterized by a high level of caryophyllene-E and α-humulene. The second subgroup is divided into two parts. The first part includes populations of Cheabet Thniyat Khrouf; Bouriache; Bousennene; Ain Laanab; Djouada; Bir ghzala; Iraguene; Chikda and Seriana are characterized by the presence of caryophyllene-E, α-pinene, and α-humulene. The second part includes populations of Sidi Amor, Troud, and Tizi Yaala are characterized by caryophyllene-E, α-humulene, and myrcene. Based on the UPGMA analysis, at least four chemotypes were identified in the *P. halepensis* population in the study area (Table 4).

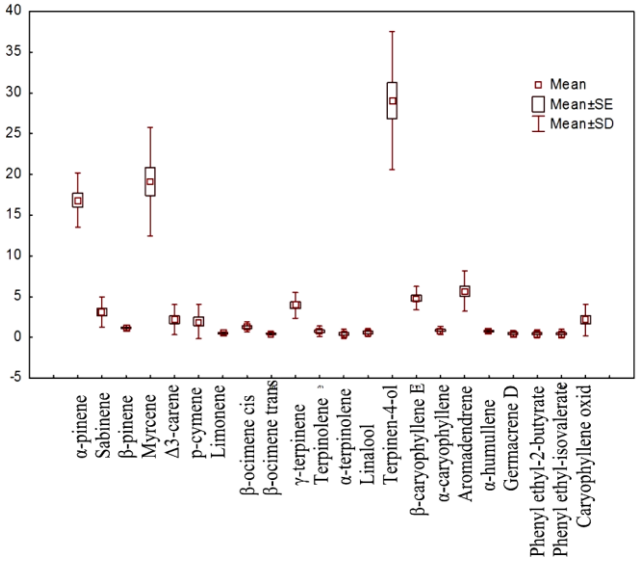

Figure 3. Variability of major components of *Pinus halepensis* essential oils

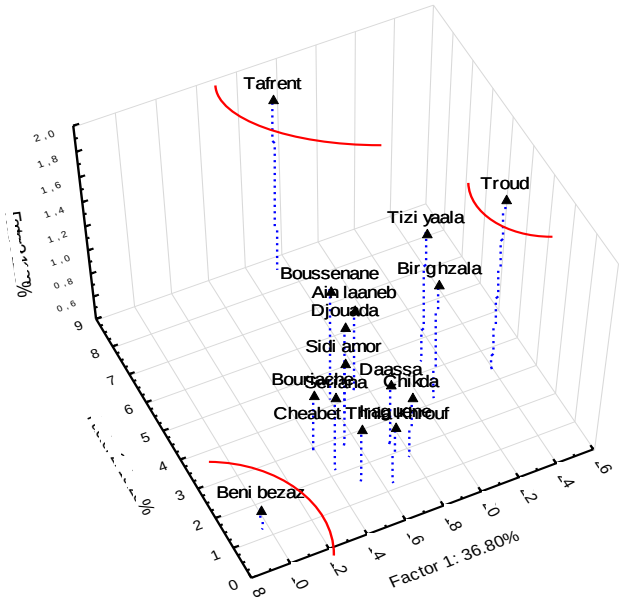

Figure 4. Three-dimensional spatial projection of *Pinus halepensis* populations

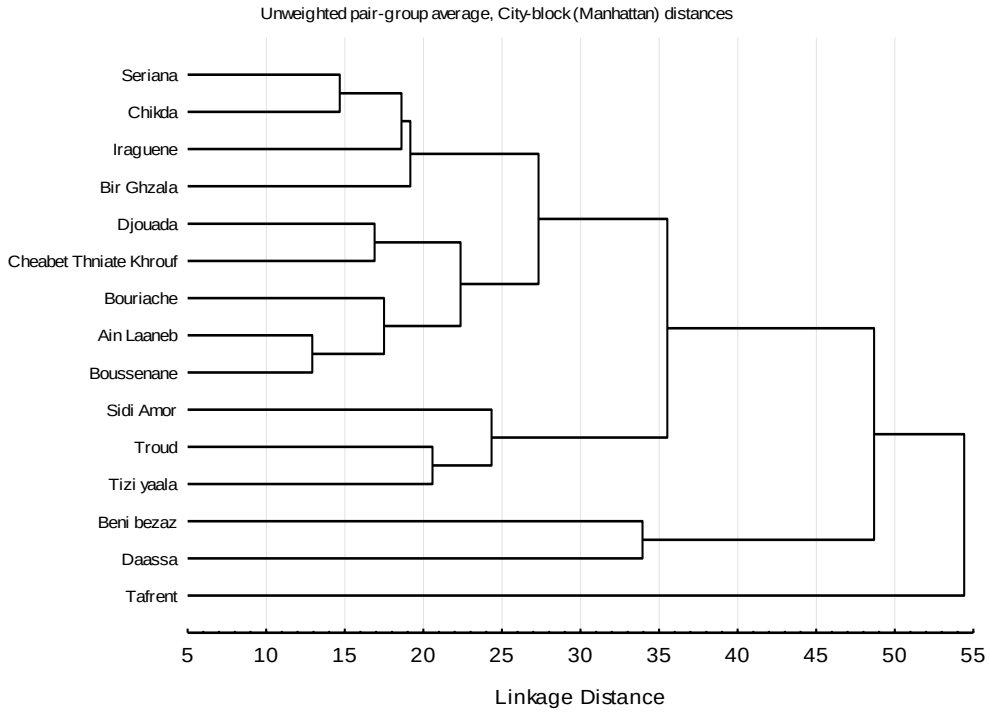

Figure 5. Dendrogram resulting from UPGMA of *Pinus halepensis* populations based on linkage distance

Table 2. Chemical composition of *Pinus halepensis* essential oils

Populations		**Seriana**	**Djouada**	**Beni bezaz**	**Iraguene**	**Daassa**	**Bir ghzala**	**Bouriache**	**Cheabet Thnia Khrouf**	**Ain laaneb**	**Sidi amor**	**Chikda**	**Boussenane**	**Troud**	**Tizi yaala**	**Tafrent**	**Average**	**SD**
Yields		1.24	0.26	0.56	1.34	1.12	0.98	0.91	0.36	0.40	0.61	0.33	0.44	0.452	0.41	0.22	0.64	0.37
Compounds nb	KI*	22	24	17	24	23	24	32	35	28	35	27	29	22	23	26	28	7
Total (%)		99.9	99.2	99.3	99.7	99.4	99.7	98.8	98.5	98.2	98.8	98.7	99.5	99.8	99.2	98.5	99.4	0.5
α-thujene	924	0.38	0.42	0	0.12	0.19	0.22	0.38	0.30	0.37	0.31	0.23	0.46	0.33	0.28	0.35	0.29	0.12
α-pinene	935	14.4	16.30	9.67	16.50	17.37	20.60	13.95	15.14	17.33	15.39	17.86	16.05	23.97	21.08	17.39	16.86	3.35
Camphene	951	0.17	0.16	0	0.18	0.38	0.23	0.12	0.17	0.18	0.15	0.16	0.23	0.26	0.22	0.26	0.19	0.08
Sabinene	974	2.09	3.61	1.21	1.14	2.27	3.10	2.88	1.52	4.17	2.70	1.7	4.15	3.32	4.30	8.54	3.11	1.84
β-pinene	979	1.06	1.18	0.60	0.92	0.94	1.40	0.91	0.89	1.13	1.16	0.95	1.37	1.85	1.51	1.82	1.18	0.35
Myrcene	991	28.1	20.60	13.40	21.30	7.74	20.11	14.58	16.80	13.9	29.25	23.69	14.50	28.23	24.01	10.90	19.14	6.67
Δ3-carene	1009	1.00	1.43	0.60	2.01	0.57	2.60	1.23	0.90	4.28	1.17	1.14	4.30	2.66	1.51	7.33	2.18	1.85
α-terpinene	1017	0.17	0.33	0	0.09	0.66	0.45	0.27	0.19	0.19	0.25	0.14	0.23	0.40	0.43	0.45	0.28	0.17
Cymene-ortho	1020	0	0	0	0	0	0	0	0	0.31	0	0.09	0.25	0	0	0.67	0.09	0.19
Limonene	1030	0.38	0.54	0	0.22	6.61	0.90	4.28	1.44	4.78	0.37	0.84	3.23	0.55	4.35	0.71	1.95	2.11
β-phellandrene	1032	0.45	0.69	0	0.34	0.38	0.68	0.47	0.35	0.71	0.46	0.53	0.65	0.62	0.65	0.74	0.51	0.20
1,8-cineole	1033	0	0	0	0.22	0	0	0	0.03	0	0	0	0	0	0	0	0.02	0.06
β-Ocimene (E)	1045	1.30	1.36	2.42	1.05	2.27	1.46	1.85	1.23	0.95	1.49	0.58	1.60	0.90	0.77	0	1.28	0.63
γ-terpinene	1058	0.34	0.62	0	0.18	0.57	0.56	0.49	0.36	0.37	0.46	0.29	0.42	0.66	0.69	0.71	0.45	0.20
Terpinolene	1085	3.30	5.40	1.51	1.60	2.64	4.51	4.59	2.73	4.18	3.91	2.32	4.20	5.16	5.98	7.03	3.94	1.60
Linalool	1097	0	0	0	1.78	1.70	1.12	1.05	0.89	0.63	1.02	0.24	1.35	1.21	0	0	0.73	0.65
Terpinen-4-ol	1185	0	0.80	0	0	0	0	0.71	0.46	0.43	0.76	0.2	0.70	0	0	2.16	0.42	0.58
α-terpineol	1199	0	0.40	0	0.34	0	0.01	0.19	0.36	0	0.26	0	0	0	0	0	0.10	0.16
Geranyl acetate	1377	0	0	0	0	0	0	0.86	0.20	0	0.23	0	0.40	0	0	1.30	0.20	0.39
α-funebrene-2-epi	1383	0.72	0.38	0	0.68	0.94	0.45	0.57	0.48	0.56	0.52	0.49	0.70	0.46	2.13	0	0.61	0.49
β-caryophyllene (E)	1434	32.1	27.90	47.00	36.10	40.92	31.20	30.47	27.00	25.64	21.70	31.19	31.60	20.32	17.56	15.20	29.06	8.50
β-farnesene (Z)	1453	0	0	0	0	0	0	0.24	0.30	0	0.22	0.1	0	0	0	0	0.06	0.11
α-humulene	1467	5.15	4.82	8.10	6.10	6.61	5.08	5.12	4.59	4	3.54	5.04	5.04	3.43	2.90	2.62	4.81	1.43
Germacrene D	1490	1.10	1.40	1.21	0.00	1.32	0	1.38	0	0.81	1.21	0.95	0.90	0.66	0.92	1.17	0.87	0.50
Phenylethyl 3-M.butanoate	1496	4.50	4.53	7.85	6.46	4.10	4.00	4.58	10.03	8.74	4.47	7.24	2.65	2.97	3.20	9.71	5.67	2.47
α-muurolene	1506	0.79	0.49	0.60	0.60	0.94	0.30	1.04	0.87	0.84	1.05	0.88	1.32	0.77	0.60	0.56	0.78	0.26
Δ-amorphene	1525	0.58	0	0.60	0.50	0	0.07	0.48	0.66	0.66	0.59	0.45	0.11	0.44	0.50	1.49	0.48	0.37
Cadina1,4-diene trans	1543	0.48	0	0.91	0	0	0	0.52	0.29	0.38	0.15	0.22	0.40	0	0	0	0.22	0.27
Elemol	1555	1.30	0	0	0	0	0.02	0.17	0.08	0	0.08	0	0	0	0	0	0.11	0.33
Caryophyllene oxide	1595	0	0.63	1.81	0	0	0	0.54	0.72	0.73	0.49	0.39	0.93	0	0	0.63	0.46	0.50
Guaiol	1605	0	0	0	0	0.02	0.01	0.22	0.50	0	0.37	0	0	0	0.37	0	0.10	0.17
Humulene epoxide II	1622	0	0	0	0	0	0	0	0.29	0	0.34	0	0	0	0	1.10	0.12	0.29
α-muurolol	1665	0	0	0	0.3	0	0	0	0.34	0	0.11	0	0	0	0	0	0.05	0.11
α-eudesmol	1667	0	0	0	0	0.04	0	0.16	1.25	0	0.14	0	0.63	0	0	0	0.15	0.35
Cubitene	1937	0	1.29	0.60	0	0.20	0	0.68	0.71	0.14	0.45	0	0.35	0	1.92	0.54	0.46	0.55
α-cembrene (3Z)	1967	0	0	0	0	0	0	0	0.52	0.13	0.30	0	0	0	0	0.06	0.06	0.15
Cembrene	2059	0	3.90	1.21	1	0	0.60	3.84	5.88	1.61	3.74	0.81	0.73	0.62	3.30	5.02	2.15	1.93

Note: * KI = Kovats index on a HP-5ms (apolar capillary column)

Table 3. Chemical **c**lasses of *Pinus halepensis* essential oils

Populations	**Seriana**	**Djouada**	**Beni bezaz**	**Iraguene**	**Daassa**	**Bir ghzala**	**Bouriache**	**Cheabet Thnia Khrouf**	**Ain laaneb**	**Sidi amor**	**Chikda**	**Boussenane**	**Troud**	**Tizi yaala**	**Tafrent**	**Average**	**SD**
Monoterpene hydrocarbons	53.12	52.64	29.41	45.65	42.59	56.82	46	42.02	52.85	57.07	50.52	51.64	68.91	65.78	56.9	51.46	9.73
Oxygenated monoterpenes	0	1.2	0	2.34	1.7	1.13	1.95	1.74	1.06	2.04	0.44	2.05	1.21	0	2.16	1.27	0.83
Sesquiterpene hydrocarbons	40.92	35.66	58.42	44.2	50.73	37.1	40.56	34.94	32.89	29.54	39.32	40.07	26.08	24.76	21.65	37.12	9.73
Oxygenated sesquiterpenes	1.3	0.63	1.81	0.3	0.09	0.03	1.09	3.31	0.73	1.63	0.39	1.56	0	0.37	1.73	1	0.91
Diterpenes	0	5.19	1.81	1	0.2	0.6	4.52	7.11	1.88	4.49	0.81	1.08	0.62	5.22	5.62	2.68	2.39
Ester	4.5	4.53	7.85	6.46	4.1	4	5.44	10.32	8.74	4.7	7.24	3.05	2.97	3.2	11.01	5.87	2.62

Table 4. Chemotypes identified in *Pinus halepensis* oils

Chemotypes		**Populations**
1	Sabinene - Δ3-carene - terpinolene	Tafrent
2	β-caryophyllene-E - α-pinene - α-humulene	Cheabet Thniyat khrouf; Bouriache; Bousennene; Ain Laanab; Djouada; Bir ghzala; Iraguene; Chikda and Seriana
3	β-caryophyllene-E - α-pinene - myrcene	Sidi Amor; Troud and Tizi Yaala
4	β-caryophyllene-E - α-humulene	Beni Bezez and Daassa

The antibacterial activity of *P. halepensis* essential oils was evaluated by the disk diffusion method against eight bacterial species. The tested bacteria showed high sensitivity to the essential oil of *P. halepensis*, except *E. coli* and *P. aeruginosa* (Table 5). Almost all the essential oils can inhibit the growth of *S. aureus, K. pneumonia, S. enteric, L. innocua, B. cereus,* and *P. mirabilis*.

The results showed that *E. coli* and *P. aeruginosa* were resistant to all undiluted *P. halepensis* essential oils. *S. aureus and K. pneumonia* were the most sensitive bacteria to *P. halepensis* essential oils with a diameter of zone inhibition ranging from 10 to 32mm. However, bacterial sensitivity was decreased in the essential oil diluted of 1/8 Undiluted essential oils from Tafrent, and Cheabet Thniat Khrouf populations were effective against *S. aureus*.

The results in Table 6 showed that sampling locations, doses, and bacterial species, and their interactions are very highly significant ($P<0.001$).

The undiluted essential oil showed the most potent effect compared to the diluted oils. The most effective oils against the tested bacteria were those of *P. halepensis* collected from Boussenen and Ain Laaneb (group a). The oil of *P. halepensis* collected from Daassa, and Beni Bezaz showed the weakest antibacterial activity (group i) (Table 7).

The antibiotic susceptibility test showed that the antibacterial activity of standard antibiotics varied according to the bacterial species. The tested antibiotic (meropenem) showed a higher growth inhibition than the oils against the tested bacteria (group a) (Table 8).

Zone inhibition of *P. halepensis* oils ranges from 5.86 to 3.60 mm at oil concentrations of 100% (undiluted) - 12.5% (diluted 1/8) so that its activity was grouped in the last groups (f, g, h and i). The most sensitive isolate is L. *innocua*, with an inhibition zone average equals of 17.26 mm. The most resistant to *P. halepensis* essential oil was *P. aeruginosa* and *S. enterica* with inhibition zones average of 8.19 and 5.06 mm, respectively (Table 9).

The desirability profile of *P. halepensis* essential oils against the tested bacteria had a prediction value of 0.38287 (Figure 6). The predictive value of essential oils was low for all populations. The oil concentrations used in this study results in low activity against the bacteria tested with a predictive value of 0.3984. The antibiotic oxacillin has a lower activity than that of the prediction. The desirability test has shown that the tested bacterial species have lower values than that of prediction.

Discussion

The yield of *P. halepensis* essential oil varied widely, with an average of 0.64 ± 0.37%. These results are consistent with the results of previous studies conducted in Algeria (Dob et al. 2005, 2007; Fekih et al. 2015; Abi-Ayad et al. 2011; Djerrad et al. 2015); in Marocco (Hmamouchi et al. 2001); in Italy (Macchioni et al. 2003); in Greece (Roussis et al. 1995), in Turkey (Ustun et al. 2012; Tumen et al. 2010), in Portugal (Rodigues et al. 2017) and in Albania (Kreainda et al. 2018). Generally, the yields of *P. halepensis* essential oil varies according to origin (Nam et al. 2014; Mohareb et al. 2016; Djerrad et al. 2015; Fekih et al. 2014). This variability is attributed to ecological factors, geographic origin, climate, altitude, growth stage, picking period, conservation of plant material, and extraction methods (Regnault-Roger 1997; Vekiari et al. 2002). The chemical composition of *P. halepensis* essential oils is complex, and their components are varied. The results of this study are consistent with those obtained in other North African populations and in the Mediterranean basin. The main compounds of essential oil in this study are similar to the oil of the same species collected in Morocco, Italy, Greece, and Corce, with the main compounds, are β-caryophyllene, α-pinene and myrcene (Russis et al. 1995; Lahlou et al. 2003; Macchioni et al. 2003; Efestathia et al. 2014; Nam et al. 2014; Bouyahya et al. 2019.

The results of this study indicated that the concentration of the Δ3-carene was low (2.18 ± 1.85%); however, this compound is the main compound of the Portugal population beside α-pinene and myrcene (Rodrigues et al. 2017). Essential oil from the population of Turkey and Libya showed high content of β-pinene and α- (Ustun et al. 2012; Ahmed et al. 2016). The essential oil of this study showed low content of β-pinene (0.2 – 2.2%). The essential oil of *P. halepensis* population in Greece show the dominance of caryophyllene-E and thunbergol (32.2% and 29.2%, resp.) (Mirtic et al. 2019). Thunbergol was present in the essential oils from Ghazaouet (Abi-ayed et al. 2011) and Egypt (El-Baha et al. 2016), but it was absent in this study.

The results of this study also showed that chemical compounds are different from those obtained from different areas in North Algeria. The main compounds of essential oil from Sidi Ferradj, Djelfa, and Saida were dominated by β-caryophyllène-Z, and humulene (Dob et al. 2005, 2007). The main compounds of essential oil from Djelfa and Batna was y β-caryophyllène-Z and myrcene (Djerrad et al. 2015). The essential oils from Tebessa and Batna were dominated by β-caryophyllène-Z - α-pinene (Djerrad et al. 2015). Besides, the essential oil from the Telemen was dominated by myrcene - α-pinene (Fekih et al. 2015), and the essential oil from the Ghazaouet was dominated by caryophyllene oxide (48%) (Abi Ayed et al. 2016).

The essential oils in this study contain very low of caryophyllene oxide (0.39-1.81%). Determination of essential oil compounds from several populations could be used for determining their chemotypes. Several studies have investigated the antibacterial activity of essential oils obtained from different plant parts of *P.halepensis* (needles, roots, buds or cones) (Hmamouchi et al. 2001; Abi Ayed et al. 2011; Ghanemi et al. 2013; Ghalem 2014; Mohareb et al. 2016; Ashmawy et al. 2018; Bouyahya et al. 2019, Mirtic et al.2019). The antibacterial effect of *P. halepensis* essential oil is by the irreversible damage of bacterial cell wall and membrane, and resulting in leakages of proteins and nucleic acids (DNA & RNA) out of the cell (Montironi et al. 2016). A study by Fekih et al. (2014) showed that the essential oils of *P. halepensis* from Tlemcen are ineffective against *S. aurens, B. cerus, L. monocytagenes, K. pneumoneae, P. aeruginosa, E. coli, P. mirabilis* and *S. typhimurium* strains.

Table 5. Antibacterial activity of the essential oils of *Pinus halepensis*

Populations	**Dilution**	***E. coli***	***P. aeruginosa***	***S. aureus***	***K. pneumoniea***	***S. enteric***	***L. innocua***	***B. cereus***	***P. mirabilis***
Daassa	1	0	0	0	0	0	7±0	0	0
	1/2	0	0	0	0	0	9.7±0.6	0	0
	1/4	0	0	0	0	0	8±0	8±0	0
	1/8	0	0	0	0	0	7±0	8±0	0
Beni bezaz	1	0	0	0	0	0	8±0	6.8±0.8	0
	1/2	0	0	0	0	0	8±0	7.5±0.5	0
	1/4	0	0	0	0	0	0	8.3±0.6	0
	1/8	0	0	0	0	0	0	9±0	0
Tafrent	1	0	0	0	31.7±2	13.7±1.2	0	0	19.3±6.1
	1/2	0	0	15±2	17.33±0.6	14±0	0	0	20±0
	1/4	0	0	12.3±0.6	14±1	0	0	0	18±1
	1/8	0	0	13.3±2.9	10±0	0	0	0	9.7±0.6
Tizi yaala	1	0	0	25±1	30.3±0.6	0	8.5±0.5	0	10.2±0.3
	1/2	0	0	20.2±1.3	17.8±0.8	0	10.5±0.5	0	7.7±0.5
	1/4	0	0	12.8±0.8	7±0	0	7.7±1.2	8.3±0.6	0
	1/8	0	0	10.3±1.5	0	0	0	8±0	0
Troud	1	0	0	25.3±4.2	28.7±0.6	0	8.7±0.6	10±0	0
	1/2	0	0	12±1	14.3±1.2	0	7±1	9±0	9.7±0.6
	1/4	0	0	11.3±1.5	9.3±0.6	0	7.3±1.2	0	0
	1/8	0	0	10.3±0.6	6.3±0.6	0	0	0	0
Sidi amor	1	0	0	30±0	30.7±1.2	0	7.3±0.6	10.3±0.6	8±0
	1/2	0	0	20.3±2.5	14±0	0	9.7±0.6	7.8±0.8	7.2±1
	1/4	0	0	13±1.73	8.3±0.6	0	8±0	0	0
	1/8	0	0	11±1.73	7.3±0.6	0	7±0	0	0
Seriana	1	0	0	20.0±0.5	0	0	0	8±0	0
	1/2	0	0	10.3±0.6	0	0	0	8±0	0
	1/4	0	0	8.3±0.6	0	0	0	8±0	0
	1/8	0	0	0	0	0	0	8±0	0
Ain laaneb	1	0	0	18±2	0	7±0	9±0	11.3±0.6	8.3±0.6
	1/2	0	0	13±1.7	13.3±1.5	7.3±0.6	9.7±0.6	10.5±0.5	8±0
	1/4	0	0	10.3±0.6	9±1	7.3±1.2	9.7±0.6	12.8±0.3	0
	1/8	0	0	8±0	9.7±0.6	7.7±0.6	10±0	11.7±1.2	0
Boussenane	1	0	0	20.7±1.2	9.7±0.6	7.6±0.6	11±1.7	12.3±1.5	8±1
	1/2	0	0	16±2	8.3±0.6	8±0	10.7±0.6	12.3±0.6	7.3±0.6
	1/4	0	0	12.7±2.5	7.7±0.6	7.6±0.6	9±1	15.7±1.2	0
	1/8	0	0	11.7±0.6	7±0	7±0	9.3±1.2	12.3±2.3	0
Bouriache	1	0	0	17±3.6	11.8±0.3	6.6±1.2	10±1.7	13.3±1.3	7.7±0.6
	1/2	0	0	10±1	10.3±0.6	8±0	8±0	8±0	8.3±0.6
	1/4	0	0	9.7±0.6	8.2±0.3	8±0	8.7±2.1	12.3±1	0
	1/8	0	0	7.7±0.6	0	0	8.7±0.6	13±0.8	0
Cheabet Thnia Khrouf	1	0	0	8.7±0.6	0	0	8±0	0	8.3±0.6
	1/2	0	0	15±0	13±1.7	0	8±0	0	8±0
	1/4	0	0	0	12.7±2.1	0	0	0	0
	1/8	0	0	8±0	11.7±2.3	0	0	0	0
Bir ghzala	1	0	0	14.3±1.2	0	0	12±1	9.5±0.5	0
	1/2	0	0	12±1.7	0	0	12±1.7	8.17±0.3	0
	1/4	0	0	14.7±2.5	0	0	0	7.33±0.6	0
	1/8	0	0	10.3±2.3	0	0	0	7±0	7.7±0.6
Djouada	1	0	0	11.7±0.6	0	0	10.3±1.5	10.3±0.6	0
	1/2	0	0	10.3±0.6	7.67±1.2	0	8±0	9.3±0.6	0
	1/4	0	0	8.8±0.3	7±0	0	8.7±2.1	8±0	0
	1/8	0	0	0	7±0	0	8.3±1.5	8±0	8±0
Iraguene	1	0	0	0	0	0	9±0	14.33±0.6	0
	1/2	0	0	0	0	0	8.7±0.6	10±0	0
	1/4	0	0	0	0	0	8.7±0.6	8.17±0.3	0
	1/8	0	0	0	0	0	10±0	8.67±0.6	0
Chikda	1	0	0	20±0	0	0	12±1	17.67±1.7	0
	1/2	0	0	8.7±1.2	7±0	0	12±1.7	0	0
	1/4	0	0	11.3±1.5	8±1	0	0	11.67±0.9	0
	1/8	0	0	8±0	8±0	0	0	9±1.4	0
Antibiotics	Gentamicin	23±0	19.3±0.6	25.7±0.6	20.3±0.6	7.3±0.6	18.3±0.6	0	18.3±0.6
	Eurythmy	10.7±0.6	14.7±0.6	35±1	21.7±0.6	11.3±0.6	30.7±0.6	19.7±0.6	27.3±0.6
	Amoxicillin	22±1	12±1	0	14.3±0.6	0	29.7±0.6	22.7±0.6	30.3±0.6
	Meropenem	35±1	27.7±0.6	30.3±0.6	29.7±0.6	26±0	32.3±0.6	14.3±0.6	32.7±0.6
	Oxacillin	0	0	0	17.7±0.6	0	19.3±0.6	0	10.3±0.6

Table 6. Main and interactions effects of essential oils of *Pinus halepensis*

Source	df	F	P
Main Effects			
Sampling locations	14	143.70	.0000 ***
Doses	8	47297.68	.0000 ***
Species of bacteria	7	12069.2	.0000 ***
Interaction			
Sampling locations * Doses	112	43.37	.0000 ***
Sampling locations * Species of bacteria	98	67.57	.0000 ***
Doses * Species of bacteria	56	3372.36	.0000 ***
Sampling locations * Doses * Species of bacteria	784	25.83	.0000 ***

Note: Very highly significant (P <0.001)

Table 7. The effectiveness of *P. halepensis* essential oils from several sampling sites against eight bacteria species (LSD 0.05 = 0.175)

Rank	Sampling location	Mean inhibition zones	n	Significant groups
1	Boussenan	13.25	216	a
2	Ain laaneb	13.13	216	a
3	Tafrent	12.92	216	b
4	Sidi amor	12.81	216	b
5	Bouriache	12.71	216	bc
6	Tizi yaala	12.59	216	c
7	Troud	12.30	216	d
8	Djouada	11.88	216	e
9	Chikda	11.88	216	e
10	Bir ghzal	11.65	216	f
11	Cheabet Th	11.47	216	g
12	Iraguene	11.13	216	h
13	Seriana	11.04	216	h
14	Daassa	10.73	216	i
15	Beni bezaz	10.69	216	i

Table 8. The effect of oil dilutions on the inhibitory zone of tested bacteria (LSD 0.05 = 0.135)

Rank	Dilution	Mean inhibition zones	n	Significant groups	S*
1	Meropenem	27.5	360	a	+++
2	Erythromycin	21.13	360	b	+++
3	Amoxicillin	16.36	360	c	++
4	Gentamicin	16.04	360	d	++
5	Oxacillin	9.46	360	e	+
6	1	5.86	360	f	-
7	1/2	5.05	360	g	-
8	1/4	3.65	360	h	-
9	1/8	3.04	360	i	-

S*. Statistically significance) (Ponce et al. 2003)

Table 9. Sensitivity groups of tested bacteria to *Pinus halepensis* essential oils

Rank	Species of bacteria	Mean inhibition zones	n	Significant groups	S*
1	*Listeria innocua*	17.26	405	a	++
2	*Staphylococcus aureus*	16.98	405	b	++
3	*Klebsiella pneumoniea*	14.738	405	c	+
4	*Proteus mirabilis*	14.60	405	d	+
5	*Escherichia coli*	9.85	405	e	+
6	*Bacillus cereus*	9.42	405	f	+
7	*Pseudomonas aeruginosa*	8.19	405	g	-
8	*Salmonella enterica*	5.06	405	h	-

Note: LSD 0.05 = 0.12769575886

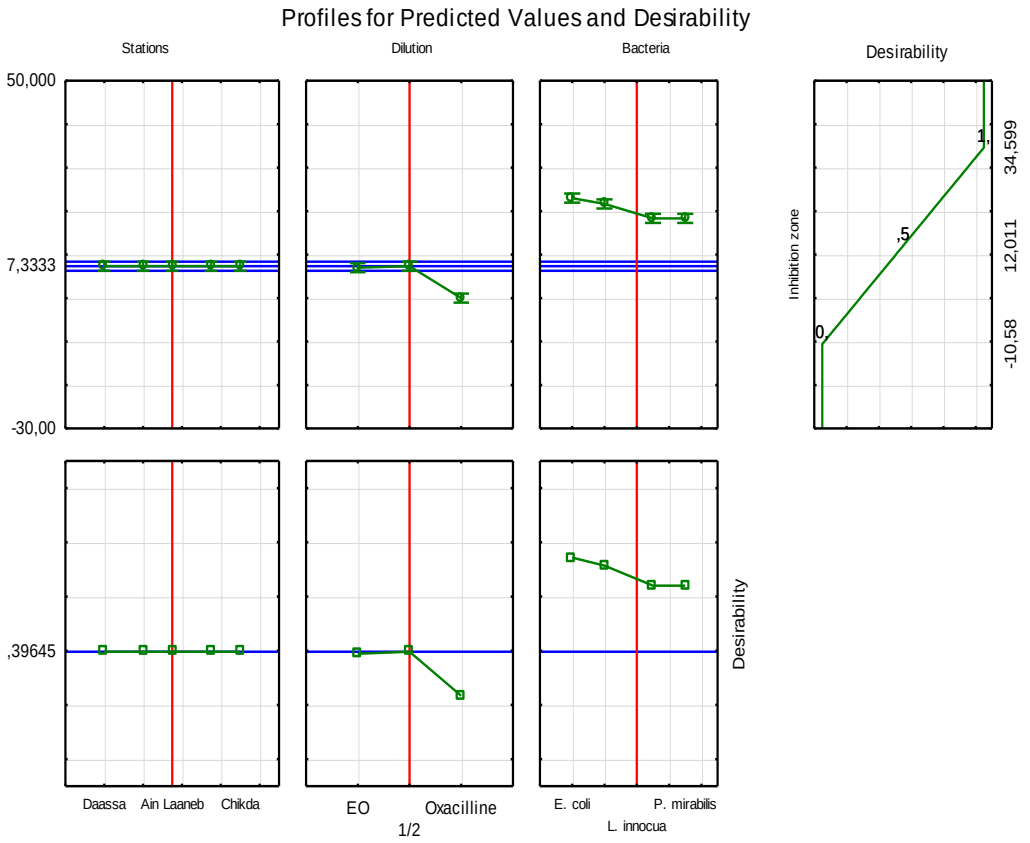

Figure 6. Profile of Predicted Values and Desirability of *Pinus halepensis* essential oils

We have obtained the same results regarding *P. aeruginosa* and K. *pneumoneae* strains. However, this study showed that *K. pneumoneae*, and *S. aureus* are resistant. These results are in line with those reported by Abi Ayad et al. (2011) and Mirtic et al. (2019) who observed that *S. aureus* is more sensitive than *E. coli* and *P. aeruginosa* to *P. halepensis* essential oils; this is likely due to difference in bacterial cell membrane structure between the bacteria.

Previous studies showed that *S.aureus* was sensitive to *Pinus helepensis* essential oils and suggesting that caryophyllene-E (29.06 % of total oil level) is responsible for the antimicrobial activity against this strain (Dahham et al. (2015) and Mitić et al. (2019). The difference in the inhibition diameters could be mainly due to the difference in the chemical composition of essential oil, while the antibacterial compounds are responsible for inhibiting bacterial multiplication, sporulation, and toxin synthesis (Gachkar et al. 2007; Rasooli et al. 2008). The antibacterial activity of essential oils that are composed of many volatile compounds cannot be confirmed their activity based only on the action of one compound (Bajpai et al. 2012).

The antimicrobial activity of essential oils is related to the presence of antimicrobial compounds. The oxygenated compounds in the essential oils are generally more active than the hydrocarbon molecules, which are known to have weak antimicrobial activity (Kalemba and Kunicka 2003). Antimicrobial activity of essential oil might be acted as synergistic among the compounds in the essential oils (Cao et al. 2009; Bounatirou et al. 2007; Peñalver et al. 2005; Chorianopoulos et al. 2004; Sokmen et al. 2004).

Some studies showed that minor compounds might play a role in the antimicrobial activity (Gill et al. 2002; Rota et al. 2008). Nevertheless, the antibacterial activity of the essential oil in this study might be due to the synergy phenomenon between all the volatile compounds. The synergic interactions between various compounds may affect in more pronounced activity (Al-Bayati, 2008; Randrianarivelo et al. 2009; Hmamouchi et al. 2001; Raut et Karuppayil, 2014). Several studies (Billerbeck et al. 2002; Bouzouta et al. 2008; Xianfei et al. 2007; Sandri et al. 2007; Zarai et al. 2011; Al-Bayati, 2008; Raut et Karuppayil, 2014) reported that Gram positive bacteria are more susceptible to essential oils than Gram negative bacteria. It was reported that the antimicrobial activity of essential oils is strongly related to their hydrophobicity (Dorman et al. 2000; Ultee et al. 2002). The cell walls of Gram positive bacteria are mainly composed of peptidoglycans associated with other molecules, like protein or teichoic acid (Nazaro et al. 2013). The cell walls of Gram negative bacteria possess an outer membrane containing hydrophilic lipopolysaccharides (LPS), as a barrier against hydrophobic compounds, such as those present in essential oils (Nikaido et al. 2003; Pandey et al. 2016). The absence of this barrier in Gram (+) bacteria enables direct contact to hydrophobic compounds of

essential oil, with the phospholipid bilayer of the cell membrane. It may result in either an increase in the permeability of the ions and the leakage of vital intracellular constituents or deficiency in the enzymatic system (Sandri et al. 2007; Al-Bayati, 2008; Randrianarivelo et al. 2009; Zarai et al. 2011). Moreira et al. (2005) reported that Gram (-) bacteria could be sensible to essential oils.

Generally, the antimicrobial mechanism is due to series of biochemical reactions in the bacterial cells, which are related to the type and characteristics of the chemical compounds in the essential oil (Nazzaro et al. 2013). The potent antimicrobial power of terpenes compounds, and particularly to phenolic terpenes, have been previously investigated by several authors (Van et al. 2006; Sharma et al. 2019). Several studies have shown the relatively lower antimicrobial efficacy of terpene hydrocarbon compounds (Kalemba et al. 2003). The essential oil contained terpene such as α-pinene, myrcene, β-pinene, linalol, myrcene, β-phellandrene, and germacrene D. Terpene have potent antimicrobial activity against Gram-positive bacteria and pathogenic fungi but have weak activity against Gram-negative bacteria (Hada et al. 2003). Jordan et al. (2013) reported that a high proportion of α-pinene increases the effectiveness of rosemary essential oils against *S aureus*. Terpene alcohols are active against microbial cells due to their hydro-solubility, and their ability to induce significant damage to the microorganism cell walls (Dorman et al. 2000; Carson et al. 2002; Hammer et al. 2003). It is similar to the results of this study that the Tafrent population with the highest content of Terpinen-4-ol.

The α-terpinene was found to inhibit several bacterial species (Dorman and Deans 2000). In this study, the Bir Ghzala population (Jijel city, northeast Algeria) shows the highest content of α-terpinene and active against *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. enterica*, *B. cereus* and *P. mirabilis.* The differences in the efficacy and antibacterial activity could be due to the chemical composition of essential oils, which varies in plant species, geographic region, season, stage, organ and method of extraction (Aprotosoaie et al. 2010).

In conclusion, the essential oils of leaves of *P. halepensis* collected from different altitudes in Eastern Algeria have significantly different yields and chemical composition. There are thirty-seven identified compounds. The main compounds are caryophyllene-E, myrcene, α-pinene, phenyl ethyl 3-methyl butanoate, α-humulene, terpinolene, and sabinene. Nevertheless, the main compounds of whole oils are common with different percentages following the regions and altitudes. The essential oils collected from different locations have different antibacterial activities.

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Table S1. Chemical composition of *Pinus halepensis* essential oils from various countries

Localities	**Greece 1**	**Greece 2**	**Greece 3**	**Greece 4**	**Italy 1**	**Italy 2**	**Morocco 1**	**Morocco 2**	**Morocco 3**	**Turkey 1**	**Turkey 2**	**Libya 1**	**Libya 2**	**Libya 3**	**Corse 1**	**Corse 2**	**Corse 3**	**Egypt**	**Tunisia 1**	**Tunisia 2**	**Tunisia 3**	**Portugal**
Authors*	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**		**L**			**M**		**N**	**O**	**P**	**Q**	**R**
α-pinene	5.3	13.4	8	3.4	18	8.5	23	23	12	18	47	14	7.3	3.7	9.8	22	13	10	9.9	13	10	39
Sabinene	2.4	1.3	1.7	0.4	9.4	6.1	3.7	3.7	0.7	0.1	0	0	0	0	0.7	0.4	6.4	0	1.1	1.2	6.4	0
β-pinene	0.8	1.1	1.9	0.3	2	1.1	3.1	*3.1*	0	47	2.8	20	7.9	1.1	0.8	1.6	1.7	0	10.7	5.1	12	2.3
Myrcene	4.9	6.6	15	5.2	28	13	16	16	24	1.3	6.3	3.8	2	0.8	4.3	3.5	39	0	9.5	21	0	17
Δ3-Carene	0.3	6.9	0.5	1.1	1.7	0.9	0	0	0	0.9	1.7	1.9	0.4	2.8	0.4	2.1	1.5	7.6	0.8	1	0.8	17
p-cymen	0	0	0	0	1.1	11	0.7	0.7	0.7	0	0.4	0	0	0	0.2	0.3	0.2	0	1.2	0.3	0.8	0
Limonene	0.5	5	1	0	1.1	1	1.3	1.3	1	2.3	0.8	0	0	0	0.4	0.8	0.9	0	1.9	1.6	0.6	0
Terpinolène	2.5	3.1	1.9	1	0	0	10	10	1.3	0.3	0	1.4	1.8	1.9	1.1	2.8	7.3	0	0	0	0	0
α-terpinolene	0	3.1	0	0	9.9	0	0	0	0	0	0.1	0	0	0	0	0	0	0	5.4	6.7	0	0
Terpinen-4-ol	0.1	0.7	0.2	0.3	0	0	3.8	3.8	0	0	0	0.4	2.3	1.8	0.3	0.9	1.1	0	0.2	0.6	0.1	0
α-terpineol	0	0.5	0	0.1	0.2	0	0.6	0.6	1.3	0.8	0.3	1	11	2	0.1	0.3	0.2	0	0.1	0.3	0	0
Geranyl acetate	0	0.2	0	0	0.3	0.9	5.3	0	0	0	0	0	0	0	0.1	1.3	0.5	0	0	0	0	0
β-caryophyllene	12	19	19	32	16	26	14	14	28	9.2	11	8.5	25	27	50	31	16	41	0	0	0.1	6.3
α-humullene	2	3.4	3.8	5.9	2.9	0	3.2	3.2	2.8	1.8	2.7	0	0	0	8	5	2.7	0	5.2	2.6	15	0
Germacrene-D	1.2	0.5	0	1	0.1	0	0	0	0.74	8.8	0	8.4	0.6	1.5	0.2	0.4	0.9	0	0	0	0.5	0
Methyl isoeugenol	0	0	0	0	0	5.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenyl ethylisovalerate	4	0	0	0	0	0	0	0	0	0	0	1	7.6	8	2.7	5.7	3.3	0	0	0	0	0
Caryophyllene oxide	0	0	0.3	0.8	0.1	0	1.2	1.2	6.8	0.4	7.5	1.3	1.4	1.9	3.1	1.6	0.7	1.8	0	0.4	0.9	0
α-cadinol	0	0	0	0	0	0	0	0	0	0	0	1.5	1.4	1.9	0	0	0	0	0	6.1	0	0
Cembrene	33	7.6	6.5	2.2	0	0	0	0	0	0	0	0	0	3.9	0.6	2.5	0	0	0	0	0	0
Thumbergol	0	0	0	29	0	0	0	0	0	0	0	0	0	6.2	0	0	0	4	0	0	0	0
β-elmene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0
Longifolene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0

Note: *) A- Gallis et al. (2012); B- Roussis et al. (1995); C- Efestathia et al. (2014); D- Mitić et al. (2019). E- Macchioni et al. (2003); F- Vidrich et al. (1988); G- Hmamouchi et al. (2001); H- Lahlou (2003); I- Bouyahya et al. (2019); J- Ustun et al. (2012); K- Tumen et al. (2010); L- Mohareb et al. (2017); M- Nam et al. (2016); N- El-Baha et al. (2016); O- Amri et al. (2013); P- Amri et al. (2014); Q- Dziri and Hosni 2012; R- Rodrigues et al (2017).

Table S2. Chemical composition of *Pinus halepensis* essential oils from Algeria

Localities	**Sidi Feredj**	**Sidi Feredj**	**Djelfa**	**Saida**	**Djelfa**	**Tissemssilet**	**Djelfa**	**Djelfa**	**Djelfa**	**Djelfa**	**Djelfa**	**Batna**		**Tebessa**		**Tlemcen**	**Ghazaouet**
Authors**	**A**		**B**		**C**				**D**			**E**		**F**		**G**	**H**
α-pinene	1.2	0.7	5.2	6.4	18	6.7	12	12	11	11	14	8.5	7.6	17	21	22	12	0
Sabinene	1.2	0.1	0.8	0.7	3	7	1.5	0.1	0	0	0	1.5	0	0.9	1.2	1.8	4.2	0
β-pinene	0.2	1	5	5.6	2	2	0.6	0.5	0.9	0.2	0.6	1.7	1.9	0.6	3.1	1.6	1.9	0
Myrcene	3.1	0.3	0.2	0.5	3	8.7	24	22	22	20	22	17	17	11	13	14	25	0
Δ3-carene	0.2	0.2	0	0.4	0	0.1	0.4	0.9	0.8	0.8	2.6	1.9	2.7	0	1.3	1.3	1.6	0
p-cymen	0	0	0	0	3	0.3	11	11	11	12	11	15	14	10	9.7	9.3	0.6	0.6
Limonene	0	0.1	0.1	0.1	0	0.8	0.5	0.5	0	0.6	0.6	1.4	3.1	2.7	0.8	1.3	0.9	0
β-ocimene cis	0	0	0	0	0	0	0	0.7	0.9	0.5	0	2.5	2.7	1.4	0	1	0.4	0
β-ocimene trans	0.2	0.1	1.3	1.2	0	2	0	0.9	2.3	0.4	0	3.6	4.2	1.7	0	1.5	1.4	0
γ-terpinene	0	0	0.3	0.3	0	1	1.1	0.2	0.8	0.2	0.5	0.6	0.4	1.5	0.9	1.3	1.4	0
Terpinolene	0	0.8	0.1	2.4	0	0	0	1	0	0.1	0	5.8	2.3	4.6	0	1.1	8.3	0
α-terpinolene	0.1	0	0	0	0	0.2	0	0	0	1	1	0	0	0	0	0	0	0
Linalool	0	0.1	0	0	2	0	0	0	0.9	0	0	0	0	1.2	1.3	0.8	0.4	0
Terpinen-4-ol	0	0.1	0	0.6	1	0	0	0.4	0.9	0.1	0.9	3.4	1.5	1.5	0	0	4.2	0.3
β-caryophyllene Z	40	20	26	25	0	0	26	26	24	25	24	16	17	20	23	21	0	0
β-caryophyllene-E	0	0	0	0	3	7.1	0	0	0.7	0.7	0.1	0	0.5	1.8	1.2	1.4	11	0
Aromadendrene	7.1	7	0	5	0	0	0.4	0.3	0.7	0.4	0.9	1.2	1.6	1.5	0	0.9	0	0
α-humullene	7.9	6.2	0.6	11	1	2.8	0.7	5	0.2	0.6	0.8	1.9	1.5	0.8	1.2	1.7	2.1	3.7
Germacrene D	0.5	0.1	2.2	0.8	0	0.2	0.3	0.5	0.6	0.7	0.9	2.9	1.4	1.8	1.9	1.4	0.1	0
Phenyl ethyl 2-methylbutyrate	0	0	0	0	10	1	0	0	0	0	0	0	0	0	0	0	0	0
Phenyl ethyl-isovalerate	0	0	0	0	8	7.4	0	0	0	0	0	0	0	0	0	0	0	5.8
Caryophyllene oxide	0	0	0	0	0	0	12	13	11	12	11	0	0	0	0	0	0.8	48
Thumbergol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.3

Note: **) A. Dob et al. (2005); B. Dob et al. (2007); C. Tazerouti et al. (1993); D. Djerrad et al. (2015); E. Djerrad et al. (2015); F. Djerrad et al. (2015); G. Fekih et al. (2014); H. Abi ayed et al. (2011).