Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(14)60323-6

Chemical composition and antibacterial activity of *Pinus halepensis* Miller growing in West Northern of Algeria

Nadia Fekih[1], Hocine Allali[1*], Salima Merghache[1], Faïza Chaïb[1], Djamila Merghache[3], Mohamed El Amine[1], Nassim Djabou[1], Alain Muselli[2], Boufeldja Tabti[1], Jean Costa[2]

[1]*University of Tlemcen, Laboratory of Natural and Bioactive Products (LASNABIO), Department of Chemistry, Faculty of Sciences, BP 119, 13000 Tlemcen, Algeria*

[2]*University of Corsica, UMR CNRS 6134, Laboratory of Natural Products Chemistry, Campus Grimaldi, BP 52, 20250 Corte, France*

[3]*University of Tlemcen, Laboratory of Physical Chemistry of Antibiotics and Antifungals, Synthesis and Biological Activities, BP 119, 13000 Tlemcen, Algeria*

PEER REVIEW

Peer reviewer

Ramdani Mohammed, Professor, University of Mohamed 1er Oujda – Bd Med VI B.P. 717 Oujda 60000, Morocco.
Tel: +212-7688-9949
Fax: +212-3650-0603
E-mail: moharamdani2000@yahoo.fr

Comments

Essential oils from *P. halepensis* growing in Algeria have been reported to have various therapeutic properties. They are also used as fragrances in cosmetics, flavoring additives for food and beverages. In this study, we provide evidence of its main antimicrobial activity.
Details on Page 102

ABSTRACT

Objective: To find new bioactive natural products, the chemical composition and to sudy the antibacterial activity of essential oil components extracted from the aerial parts of the Algerian aromatic plant *Pinus halepensis* Miller (*P. halepensis*) (needles, twigs and buds).
Methods: The essential oil used in this study was isolated by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia. The chemical composition was investigated using GC-retention indices (*RI*) and GC-MS.
Results: Forty-nine compounds, representing 97.9% of the total collective oil, were identified. Essential oil was dominated by hydrocarbon compounds (80.6%) especially monoterpenes (65.5%). The major compounds from ten oils stations were: myrcene (15.2%-32.0%), α-pinene (12.2%-24.5%), E-β-caryophyllene (7.0%-17.1%), terpinolene (1.8%-13.3%), 2-phenyl ethyl isovalerate (4.8%-10.9%), terpinene-4-ol (1.0%-8.2 %) and sabinene (1.5%-6.3%). The intra-species variations of the chemical compositions of *P. halepensis* aerial parts essential oils from ten Algerian sample locations were investigated using statistical analysis. Essential oil samples were clustered in 2 groups by hierarchical cluster analysis, according to their chemical composition. The essential oil revealed an interesting antimicrobial effect against *Lysteria monocytogenes, Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter baumanii, Citrobacter freundii* and *Klebsiella pneumoniae*.
Conclusions: These results suggest that the essential oil from *P. halepensis* may be a new potential source as natural antimicrobial applied in pharmaceutical and food industries.

KEYWORDS

Pinus halepensis Miller, Essential oils, GC/MS, Chemical variability, Antimicrobial activity

1. Introduction

The genus *Pinus* belongs to the family Pinaceae and comprises about 250 species. It is the largest genus of conifers occurring naturally in the northern hemisphere, especially in the Mediterranean region, Caribbean area, Asia, Europe, North and Central American. The genus *Pinus* has been planted in the temperate regions of the southern hemisphere. They are evergreen and resinous trees growing to 3-80 m tall with needle-like gray-green leaves that grow in pairs[1-3].

The medicinal and aromatic properties of the chemical compounds (*e.g.*, turpentine, resins and essential oil….) of pine make it one of the most popular plants throughout all civilization. Pine is also still widely used in traditional

*Corresponding author: Hocine Allali, University of Tlemcen, Laboratory of Natural and Bioactive Products (LASNABIO), Department of Chemistry, Faculty of Sciences, BP 119, 13000 Tlemcen, Algeria.
Tel/Fax: (+213) 43.28.65.30
E-mail: h_allali72@yahoo.fr

Foundation Project: Supported by the University of Tlemcen and the Ministry of Higher Education and Scientific Research of the Algerian People's Democratic Republic for the granting of financial assistance under the project CNEPRU (Ref. E02020100067/2011-2013).

Article history:
Received 11 Oct 2013
Received in revised form 20 Oct, 2nd revised form 28 Oct, 3rd revised form 14 Nov 2013
Accepted 21 Feb 2014
Available online 28 Apr 2014

therapeutic practice in world and has economic importance[4,5]. In the Northern Mediterranean basin, *Pinus halepensis* Miller (*P. halepensis*) is a pioneer and expansionist species that colonizes abandoned agricultural lands characterized by high biodiversity. Owing to its richness of secondary metabolites, *P. halepensis* may play an important role in plant succession through several processes. For example, secondary compounds (terpenoids and/or phenolic compounds) can affect root symbionts and site quality, by interfering with decomposition, mineralization, and humification[6,7]. *P. halepensis* may inhibit seedling establishment of various species in pine stands, suggesting the allelopathic nature of litter, leaf leachates, and/or root exudates[8,9].

P. halepensis seeds are traditionally used throughout Tunisia and other Arabic countries, for preparing a sweet pudding of group pine seeds, called "Assida-Zgougou". Recently, it has been employed as an ingredient in ice-creams and candies[10]. Essential oils from *Pinus* species have been reported to have various therapeutic properties. They are also used as fragrances in cosmetics, flavoring additives for food and beverages, scenting agents in a variety of household products and intermediates in the synthesis of perfume chemicals[1,2,4,11]. Several phytochemical analyses of *P. halepensis* have been published on terpenes[12,13], turpentine[14] and phenolic compounds[15]. The literature reports some works on the chemical composition of *P. halepensis* essential oil from Italy[6,16], Algeria[5,17-19], Greece[20], Morocco[21] and Turkey[22,23]. Various compositions have been reported.

The first aim of this study was to elucidate the composition of *P. halepensis* essential oil using a combination of GC and GC/MS. The second aim was to characterize the intra-species variation in essential oil composition in natural populations using 10 oil samples from different locations of Algeria and to evaluate the antibacterial activity of essential oil.

2. Materials and methods

2.1. Plant Material

The aerial parts of *P. halepensis* (needles, twigs and buds) were collected in January 2012 from 10 locations from Tlemcen. The plant material was botanically identified by Prof. Noury Benabadji (Laboratory of Ecology and Ecosystem Management of University of Tlemcen, Algeria). Voucher specimens were deposited in the herbarium of the University of Tlemcen. Each fresh aerial part (400-500 g) was submitted to hydrodistillation for 5 h using a Clevenger-type apparatus according to the European Pharmacopoeia[24].

2.2. GC analysis

GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus equipped with a dual flame ionization detection system and two fused-silica capillary columns (60 m×0.22 mm I.D., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylenglycol). The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50), using helium as the carrier gas (1 mL/min); the injection volume was 0.2 μL. Retention indices (*RI*) of the compounds were determined from a software from Perkin-Elmer. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.3. GC-MS analysis

Samples were analyzed with a Perkin-Elmer Turbo mass detector (quadrupole), coupled to a Perkin-Elmer Autosystem XL, equipped with the fused-silica capillary columns Rtx-1 and Rtx-Wax (ion source temperature 150 °C; energy ionization 70 eV). EI mass spectra were acquired over the mass range 35-350 Da (scan time: 1 second). Other GC conditions were the same as described under GC except split 1/80.

2.4. Component identification

Identification of the components was based (i) on the comparison of their GC retention indices (*RI*) on non polar and polar columns, determined relative to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literature data[25,26] and (ii) on computer matching with commercial mass spectral libraries[27-29] and comparison of spectra with those of our laboratory-made library.

2.5. Statistical analysis

Data analyses were performed using principal component analysis (PCA) and cluster analysis (CA)[30]. Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results[31]. Indeed, PCA provides the data for diagrams in which both objects (oil samples) and variables (oil components) are plotted while canonical analysis informs a classification tree in which objects (sample locations) are gathered. PCA was carried out using function 'PCA' from the statistical R software.

The variables (volatile components) have been selected using function from the statistical software. The cluster analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of oil samples.

2.6. Antimicrobial activity

2.6.1. Test microorganisms

Antibacterial activity of *P. halepensis* essential oil was tested against 11 strains of bacteria: Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus cereus* ATCC 10876 (*B. cereus*), *Enterococcus faecalis* ATCC 49452 (*E. faecalis*), *Lysteria monocytogenes* ATCC 15313 (*L. monocytogenes*) and Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Escherichia coli* ATCC 25922 (*E. coli*), *Salmonella typhimurium* ATCC 13311, *Acinetobacter baumanii* ATCC 19606, *Citrobacter freundii* ATCC 8090, *Proteus mirabilis* ATCC 35659, *Klebsiella pneumoniae* ATCC 700603 (*K. pneumoniae*). The microorganisms were obtained from Pasteur Institute of Paris.

2.6.2. Paper-disc diffusion method

Antibacterial activity was tested by the agar-well diffusion

method[32,33]. All bacterial cultures were first grown on MHI agar (Muller-Hinton infusion) plates at 37 °C for 18-24 h prior to inoculation onto the nutrient agar. One or several colonies of similar morphology of the respective bacteria were transferred into API suspension medium (Biomérieux) and adjusted to 0.5 McFarland turbidity standard with a Densimat (Biomérieux). The inoculums of the respective bacteria were streaked onto MHI agar plates using a sterile swab. A sterile filter disc (diameter 6 mm, Whatman paper No.3) was placed. The disc was impregnated by the tested essential oils (10 μL/disc). The treated Petri dishes were placed at 4 °C for 1-2 h and then incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of growth inhibition around the discs after 24 h of incubation at 37 °C. The diameter of the zones of inhibition around each of the discs was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded. The scale of measurement was as follows[34] (disc diameter included): ≥ 8 mm: good activity; 7.5-7.9 mm: average activity; 7-7.4 mm: moderate activity; 6.5-6.9: low activity; ≤ 6.4 mm: no activity.

3. Results

3.1. Sample location and oil yields

We regrouped in Table 1 the major components of *P. halepensis* essential oils reported in literature. Various compositions have been reported, characterized by the occurrence of monoterpenes, sesquiterpenes and phenylpropanoids compounds.

In our study, the aerial parts of *P. halepensis* were collected from 10 locations in West Northern of Algeria. Some information concerning the 10 harvest areas (origins, latitudes, longitudes and essential oil yield) were tabulated in Table 2.

Table 2
Essential oils yields, origins, latitudes and longitudes of ten Algerian *P. halepensis*.

Areas		Samples	Regions	Latitudes	Longitudes	Altitudes	Essential oil yields (%)
Littoral	Area 1	S1	Nedroma	34° 52′ 38″	1°14′ 25″	650 m	0.14
		S2	Hwanet	34° 58′ 13″	1°48′ 10″	650 m	0.20
		S3	Oued Tlala	35° 03′ 51″	1°44′ 59″	197 m	0.40
		S4	Bab Taza	34° 58′ 13″	1° 45′ 16″	662 m	0.22
		S5	Bab El Assa	34° 58′ 08″	2° 01′ 40″	537 m	0.63
		S6	Sidi youchaa	35° 06′ 55″	1° 46′ 47″	111 m	0.30
		S10	Ghazaouet	35° 05′ 59″	1° 50′ 59″	118 m	0.40
Mountain	Area 2	S7	Agadir	34° 53′ 21″	1° 18′ 06″	914 m	0.13
		S8	Amieur	35° 01′ 59″	1° 15′ 20″	706 m	0.14
		S9	Mansourah	34° 52′ 27″	1° 19′ 11″	983 m	0.35

The sample locations were distributed in two areas. Area 1 was considered as littoral zone near to the Mediterranean Sea, while area 2 was a Mountain zone with altitudes up to 700 m. Area 2 has a warm and sub-humid climate while the soil of area 1 is red fersiallitic with vertic character. The yield of essential oils obtained from fresh aerial part in the ten locations of *P. halepensis* ranged from 0.13% to 0.63% and more precisely it is noticeable that higher yields (0.20% to 0.63%) were linked to sample oils from area 1 while lower yields (0.13% to 0.35%) were linked to sample oils from area 2 (Table 2). Volatile oil yield of *P. halepensis* in different parts from Algeria had similar results. Values of 0.3%, 0.52%, 0.8% and 0.9% (in dry weight basis) were found in the Ghazaouet, Saida, Djelfa and Sidi Fredj, respectively[5,17-19].

Table 1
Main components of the essential oils of *P. halepensis* from different origins previously reported.

Plant origin		Algeria[5,17-19]					Greece[20]	Italy[6,16]		Morocco[21]	Turkey[22,23]		
Sites		Ghazaouet	Saïda	Sidi Feradj	Tissemsilt	Djelfa							
Extraction modes		HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD
No	Compounds	%	%	%	%	%	%	%	%	%	%	%	%
2	α-Pinene	nd	6.4	1.2	6.7	17.6	13.4	18.1	8.5	23.3	47.1	18.4	16.4
4	Sabinene	nd	0.7	1.2	7	2.6	1.3	9.4	6.1	3.7	nd	0.1	0.6
5	β-Pinene	nd	5.6	0.2	2.0	1.6	1.1	2.0	1.1	3.1	2.8	46.8	18.7
6	Myrcene	nd	0.5	3.1	8.7	3.2	6.6	27.9	12.5	16.3	6.3	1.3	3.8
8	3-Carene	nd	0.4	0.2	0.1	1.9	6.9	1.7	1	nd	1.7	0.9	16.3
10	*p*-Cymene	nd	nd	nd	0.3	3	nd	1.1	11.4	0.7	0.4	tr	0.1
12	Limonene	nd	0.1	tr	0.8	0.1	5.0	1.1	1	1.3	0.8	2.3	18.7
16	Terpinolene	nd	2.4	nd	nd	nd	nd	nd	nd	nd	nd	0.3	1.8
21	Terpinen-4-ol	0.4	0.6	tr	nd	0.6	0.7	nd	nd	3.8	nd	nd	nd
22	α-Terpinolene	nd	nd	0.1	0.2	tr	3.1	9.9	nd	10.1	1	nd	nd
28	E-β-Caryophyllene	3	nd	nd	7.1	2.7	nd	nd	nd	nd	11.2	9.2	9.5
29	α-Humulene	0.74	10.5	7.9	2.8	1.4	3.4	2.9	nd	3.2	2.7	1.8	1.8
30	2-Phenylethyl isovalerate	nd	nd	nd	7.4	8.4	nd	nd	nd	nd	nd	nd	nd
31	Germacrene D	nd	0.8	0.5	0.2	tr	0.5	0.1	nd	nd	tr	8.8	1.5
37	Caryophyllene oxide	48.2	nd	nd	nd	nd	nd	0.1	nd	1.2	7.8	0.4	0.4
44	Bulnesol	nd	nd	nd	nd	nd	7.6	nd	nd	nd	nd	nd	nd
	Z-β-Caryophyllene	nd	25	40.31	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Aromadendrene	nd	5.4	7.1	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Humulene oxide	6.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Thumbergol	8.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Only the main components were reported; main components are classed by number corresponding to the Table 1; only one sample was studied.
Extraction mode: HD: Hydrodistillation; tr: trace (<0.05%); nd: compounds not detetcted.

3.2. Chemical analysis of *P. halepensis* essential oils

Chemical composition of *P. halepensis* oils from 10 samples were studied using GC and GC/MS (Table 3). Fourty nine compounds, which accounted for 97.9% of oil, were isolated.

Table 3
Chemical composition of *P. halepensis* essential oils from West Northern of Algeria.

Compounds	IR[a] LIT	IR[b] app	IR[c] pol	Collective Oil[d] %	Simple Oils[e] %Min	%Max	Identification
α-Thujene	922	923	1021	0.7	0.4	1.0	*RI*, MS
α-Pinene	931	932	1023	16.8	12.2	24.5	*RI*, MS
Camphene	943	944	1066	0.2	0.1	0.3	*RI*, MS
Sabinene	964	966	1118	4.2	1.5	6.3	*RI*, MS
β-Pinene	970	971	1108	1.9	1.7	2.2	*RI*, MS
Myrcene	970	983	1159	25.2	15.2	32.0	*RI*, MS
α-Phelandrene	997	998	1157	0.1	nd	0.2	*RI*, MS
3-Carene	1005	1006	1147	1.6	0.6	5.5	*RI*, MS
α-Terpinene	1008	1010	1175	0.9	0.1	1.8	*RI*, MS
p-Cymene	1010	1012	1259	0.6	0.2	1.8	*RI*, MS
β-Phelandrene	1021	1021	1204	0.4	0.7	1.4	*RI*, MS
Limonene	1020	1021	1195	0.9	0.6	1.4	*RI*, MS
Z-β-Ocimene	1024	1025	1225	0.4	nd	1.4	*RI*, MS
E-β-ocimene	1034	1036	1241	1.4	0.52	3.4	*RI*, MS
γ-Terpinene	1047	1049	1237	1.4	0.2	2.6	*RI*, MS
Terpinolene	1078	1082	1247	8.3	1.8	13.8	*RI*, MS
Linalool	1080	1084	1529	0.4	0.1	0.8	*RI*, MS
Perillene	1090	1099	1414	0.1	tr	0.1	*RI*, MS
Cis-p-menth-2-en-1-ol	1108	1107	1600	0.2	0.1	0.3	*RI*, MS
Trans-p-menth-2-en-1-ol	1113	1117	1612	0.1	tr	0.2	*RI*, MS
Terpinene-4-ol	1161	1164	1583	4.2	1.0	8.2	*RI*, MS
α-Terpinolene	1179	1175	1688	0.4	tr	0.7	*RI*, MS
Bornyl acetate	1269	1268	1475	0.1	tr	0.3	*RI*, MS
Citronellyl acetate	1331	1333	1645	0.1	tr	0.1	*RI*, MS
Neryl acetate	1342	1342	1409	0.1	0.1	0.2	*RI*, MS
Geranyl acetate	1361	1360	1740	0.2	tr	0.5	*RI*, MS
α-Copaene	1379	1373	1475	0.2	0.1	0.3	*RI*, MS
E-β-Caryophyllene	1424	1418	1583	10.9	7.0	17.1	*RI*, MS
α-Humulene	1456	1449	1651	2.1	1.3	3.4	*RI*, MS
2-Phenylethyl isovanerate	1463	1468	1973	7.7	4.8	10.9	*RI*, MS
Germacrene D	1480	1474	1692	0.2	0.1	0.2	*RI*, MS
α-muurolene	1496	1492	1709	0.2	tr	0.6	*RI*, MS
δ-Cadinene	1516	1513	1738	0.3	tr	0.5	*RI*, MS
E-α-Bisabolene	1532	1532	1740	0.2	0.1	0.3	*RI*, MS
Phenylethyl Tiglate E	1547	1546	2141	0.1	nd	0.2	*RI*, MS
Phenylethyl Tiglate Z	1559	1568	2145	0.8	tr	3.3	*RI*, MS
Caryophyllene oxide	1576	1583	1898	0.8	0.2	2.2	*RI*, MS
Guaiol	1591	1592	2070	0.2	0.1	0.5	*RI*, MS
Humulene epoxyde	1601	1613	2035	0.1	tr	0.1	*RI*, MS
Epi-Cubenol	1624	1625	2043	0.1	0.1	0.2	*RI*, MS
Tau-Cadinol	1632	1633	2163	0.2	tr	0.1	*RI*, MS
T-Muurolol	1634	1638	2141	0.1	0.1	0.3	*RI*, MS
α-Cadinol	1645	1640	2163	0.2	tr	0.3	*RI*, MS
Bulnesol	1659	1666	2195	0.1	nd	0.1	*RI*, MS
Cembrene	1938	1940	2185	0.4	0.1	1.6	*RI*, MS, Ref.
m-Camphorene	1947	1939	2234	0.2	nd	0.4	*RI*, MS, Ref.
Cembrene A	1962	1951	2227	0.1	nd	0.3	*RI*, MS, Ref.
p-Camphorene	1980	1974	1987	0.3	nd	1.1	*RI*, MS, Ref.
Geranyl Linalool	2037	2037	2540	1.5	nd	3.0	*RI*, MS
Total Identification %				97.9			
Yields % (w/w)				0.26	0.10	0.63	
Hydrocarbon compounds				80.6			
Monoterpene hydrocarbons				65.5			
Sesquiterpene hydrocarbons				14.1			
diterpenic hydrocarbons				1.0			
Oxygenated compounds				17.3			
Oxygenated monoterpenes				5.4			
Oxygenated sesquiterpenes				1.8			
Oxygenated diterpenes				1.5			
Non-terpenic oxygenated compounds				8.6			

[a]: Retention indices of literature on the apolar column (RI_{Lit}) reported from König *et al*., 2001; [b]: Retention indices on the apolar Rtx-1 column; [c]: Retention indices on the polar Rtx-Wax column; [d]: Normalized percentages abundance are given on the apolar column except for components with identical *RI* (percentages are given on the polar column), tr=trace (<0.05%). [e]: Minimum and maximum normalized percentages abundance from simple oils; *RI*: Retention indices; MS: Mass spectra in electronic impact mode; Ref.: compounds identified from literature data: König *et al*., 2001).

3.3. Chemical variation of *P. halepensis* essential oils

To identify possible relationships between volatile compound abundances and geographical origins, PCA and CA were applied to a matrix linking essential oil compositions to sample locations. The data mentioned in Table 4 and presented in Figures 1 and 2 were obtained from the correlation matrix and the standardized matrix.

Table 4
Clustering of *P. halepensis* oils samples by statistical analysis.

Components	Group I (S2-S6, S10) Range[b]	Group I (S2-S6, S10) Average[b]	Group II (S1-S7-9) Range[b]	Group II (S1-S7-9) Average[b]
Monoterpene hydrocarbons	54.39		54.55	
α-Pinene	12.2-14.4	13.4	18.0-24.5	21.8
Sabinene	3.5-6.3	5.52	1.5-3.0	2.20
Myrcene	15.2-28.5	23.94	24.1-32.0	27.15
Terpinolene	9.4-13.8	11.53	1.8-5.8	3.42
Oxygenated monoterpenes	5.78		1.85	
Terpinene-4-ol	3.9-8.2	5.78	1.0-2.5	1.85
Monoterpene sesquiterpenes	8.78			
E-β-Caryophyllene	7.0-11.1	8.78	11.0-17.1	14.15
Non-terpenic compounds	10.1		5.76	
2-Phenylethyl isovanerate	8.7-10.9	10.1	4.8-7.0	5.76

[b] Normalized % abundances.

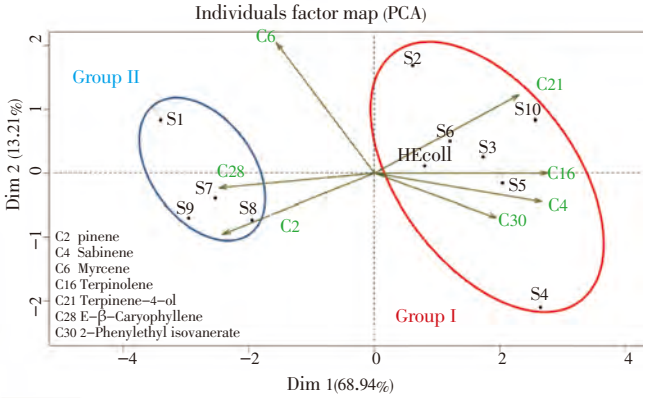

Figure 1. PCA of chemical compositions of *P. halepensis* oils from Algeria.

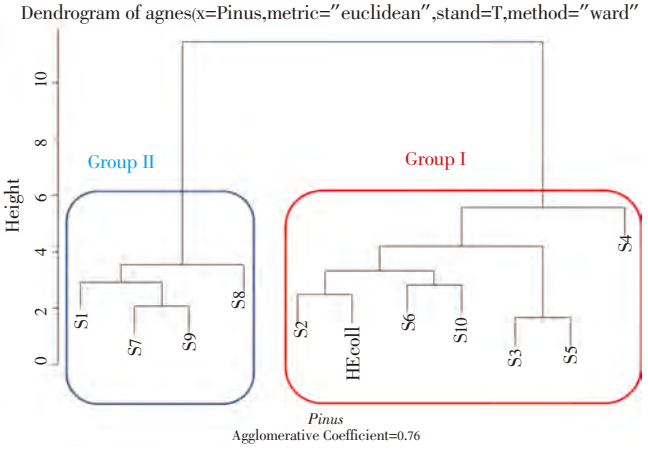

Figure 2. Cluster Analysis of chemical compositions of *P. halepensis* from Algeria.

3.4. Antimicrobial activity

The antibacterial activity of *P. halepensis* essential oil originating from the West Northern of Algeria was evaluated by paper disc diffusion method against 11 bacteria. Table 5 showed that oil has a variable antibacterial activity (8-10 mm) against tested strains. The maximum zone of inhibition was recorded against *L. monocytogenes* (10 mm), *K. pneumoniae* (10 mm), *E. faecalis* (9 mm) and *Acinetobacter baumanii* (9.5 mm). Other hand, the oil was ineffective against *S. aureus*, *B. cereus*, *E. coli*, *Salmonella typhimurium* and *Proteus mirabilis*. According to Sheng-Hsien[34], the essential oil of *P. halepensis* showed good inhibitory effects on some tested microorganisms.

Table 5
Antibacterial activity of *P. halepensis* essential oils from the West Northern of Algeria.

Microorganisms	Diameters of inhibition (mm)
Gram-positive bacteria	
S. aureus	n.a
B. cereus	n.a
E. faecalis	9.0
L. monocytogenes	10.0
Gram-negative bacteria	
P. aeruginosa	8.0
E. coli	n.a
Salmonella typhimurium	n.a
Acinetobacter baumanii	9.5
Citrobacter freundii	8.0
Proteus mirabilis	n.a
Klebsiella pneumoniae	10.0

Essential oil (10 μL/disc) of aerial part of *P. halepensis*; n.a: not active.

4. Discussion

The chromatographic profile of essential oil from *P. halepensis* showed that oils are constituted of 26 monoterpenes, 16 sesquiterpenes, 4 diterpenes and 3 non-terpenic compounds. The oils are mainly composed by hydrocarbon compounds that accounted for 80.6%. The main components were myrcene (25.2%), α-pinene (16.8%), E-β-caryophyllene (10.9%) and terpinoplene (8.3%). However, the oxygenated compounds have the lowest percentage (17.3%), most of them being non-terpenic (8.6%) and monoterpenes oxygenated (5.4%) represented by 2-phenylethyl isovanerate (7.7%) and terpinene-4-ol (4.2%). From a chemotaxonomic viewpoint, it should be noted that *P. halepensis* essential oils are qualitatively similar to those of literature but differ in the amounts of the major components. Indeed, several reports on the composition of oils of other *Pinus* species revealed that monoterpene hydrocarbons were the major constituent in the most of the oils; they often constituted 50% or more of the oil[6,21].

Although the 10 essential oils contained similar types of compounds, there were significant differences in the concentrations of the major components. For instance, the concentrations of α-pinene (C2), sabinene (C4), myrcene (C6), terpinolene (C16), terpinene-4-ol (C21), E-β-caryophyllene (C28) and 2-phenylethyl isovanerate (C30) ranged from 12.2% to 24.5% of oil, from 15.2% to 32.0% of oil, from 1.8% to 13.8% of oil, from 1.0% to 8.2% of oil, from 7.0% to 17.1% of oil and from 4.8% to 10.9% of oil, respectively. The principal factorial plane accounts for 93.56% of the chemical essential oils variance. The F1 axis (68.94%) are positively correlated with oxygenated sesquiterpenes (C4, C16, C21 and C30) and negatively correlated with E-β-caryophyllene (C28) and 2-phenylethyl isovanerate (C30). The plot established using the first two axes suggests that there are two main groups of *P. halepensis* oils. The first group (I) includes oil samples from 6 localities (S2-S6, S10), characterized by more high levels of myrcene C6 (15.2-28.5% of oil), terpinolene C16 (9.4%-13.8% of oil), 2-phenylethyl isovanerate C30 (8.7%-10.9% of oil), sabinene C4 (3.5%-6.3% of oil) and terpinene-4-ol C21 (3.9%-8.2% of oil). The group II includes 4 oil samples (S1, S7-9) was characterized by a high content of α-pinene C2 (18.0%-24.5% of oil), myrcene C6 (24.1%-32.0% of oil) and E-β-caryophyllene C28 (11.0%-17.1% of oil). However, statistical analysis clustered the essential oil samples into two distinct groups linked to the origin of harvest. Group I consisted of oils rich in α-pinene, myrcene, terpinolene and 2-phenylethyl isovanerate, originated from littoral zone (Area 1) and group II consisted of oils rich in α-pinene, myrcene and E-β-caryophyllene, originated from mountains of Tlemcen (Area 2). These results suggested that variation in the compositions of essential oils among populations can be attributed to the growing conditions and environmental factors.

The essential oils of *P. halepensis* showed good inhibitory effects on some tested microorganisms. It would be related to their oxygenated monoterpenes components which constitute more than 16.2% of the oil. The antibacterial activity of essential oil of *P. halepensis* from Ghazaouet (West Northern of Algeria) was evaluated against four strains of bacteria: *S. aureus*, *P. aeruginosa*, *E. coli* and *B. cereus*, using disc diffusion method. The essential oil showed a strong activity against *S. aureus* and *B. cereus*. Contrary, the oil was ineffective against *P. aeruginosa* and *E. coli*[35]. However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Secondly there is some evidence that minor components have a critical part to play in antibacterial activity, possibly by producing a synergistic effect between other components[36,37]. The variation in chemical composition of essential oil might be responsible for the different antibacterial activities.

In conclusion, the comparison of our results with literature shows considerable qualitative and quantitative difference

in yields and composition of *P. halepensis* oils. The variability in oil composition is present even in *P. halepensis* and these variations, sufficient to allow the distinction of different chemotypes, are the results of an adaptive process to particular ecologic conditions (geographical regions, climate conditions, altitude), period of collection of the plant, studied parts of plant, state of plant (fresh or dry) and method of extraction of the essential oil. Bioassay screening of oil showed an activity against *L. monocytogenes* and *K. pneumoniae*. The results of the current study have shown that essential oil of *P. halepensis* is potentially a good source of antimicrobial compounds.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are thankful to Professor Noury Benabadji of the Botanical Laboratory, Biology Department, Abou Bekr Belkaïd University for the identification of the vegetable matter. This study was supported by the University of Tlemcen and the Ministry of Higher Education and Scientific Research of the Algerian People's Democratic Republic for the granting of financial assistance under the project CNEPRU (Ref. E02020100067/2011-2013).

Comments

Background

Plant-derived essential oils have long served as flavoring agents in foods and beverages, and due to their versatile content of antimicrobial compounds, they possess potential as natural agents for food preservation. The antimicrobial activity of essential oils is assigned to a number of small terpenoid and phenolic compounds, which also in pure form have been shown to exhibit antibacterial activity. Essential oils are known to be active against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria. This study was conducted to determine the antibacterial activity of *P. halepensis* essential oils against bacteria.

Research frontiers

The data obtained from the present experiments show a new application of essential oil from aerial parts of *P. halepensis* which is in agreement with its use in traditional medicine.

Related reports

In this present investigation, the authors have followed standard protocols to assess the antimicrobial activity of essential oil from *P. halepensis* growing in Algeria. The results suggest that the essential oil from *P. halepensis* may be a new potential source as natural antimicrobial applied in pharmaceutical and food industries.

Innovations & breakthroughs

To my knowledge, there is no work for antimicrobial activity of essential oil from aerial parts of *P. halepensis* growing in West Northern of Algeria. The present report serves as the first hand information on the fact that this plant is potentially a good source of antimicrobial compounds.

Applications

There is strong interest in the use of naturally occurring compounds which have antibacterial activity for preservation of minimally processed foods. Plant essential oils are a potential source of antimicrobials of natural origin. Essential oils have been evaluated for their effects on the growth of food spoilage and foodborne pathogenic microorganisms, including Gram-positive and Gram-negative bacteria. Possible use of essential oils as food preservatives has been studied.

Peer review

Essential oils from *P. halepensis* growing in Algeria have been reported to have various therapeutic properties. They are also used as fragrances in cosmetics, flavoring additives for food and beverages. In this study, the authors provide evidence of its main antimicrobial activity.

References

[1] Fuentes JL, Vernhe M, Cuetava EB, Sanchez-Lamar A, Santana JL, Llagostera M. Tannins from barks of *Pinus caribaea* protect *Escherichia coli* cells against DNA damage induced by gamma-rays. *Fitoterapia* 2006; **77**(2): 116-120.

[2] Kozan E, Kupeli E, Yesilda E. Evaluation of some plants used in Turkish folk medicine against parasitic infections for their *in vivo* anthelmintic activity. *J Ethnopharmacol* 2006; **108**(2): 211-216.

[3] Quézel P, Santa S. *New flora of Algeria and the Southern disert regions*, Volume I. Paris, France: CNRS; 1963, p. 39-40.

[4] Baba Aissa F. *Medicinal plants in Algeria. Identification, description of active ingredient properties and traditional use of common plants in Algeria*. Algiers: Bouchene and Ad. Diwan; 1991, p. 181.

[5] Dob T, Berramdane T, Chelghoum C. Essential oil composition of *Pinus halepensis* Mill. from three different regions of Algeria. *J Essent Oil Res* 2007; **19**: 40-43.

[6] Macchioni F, Cioni PL, Flamini G, Morelli I, Maccioni S, Ansaldi M. Chemical composition of essential oils from needles, branches and cones of *Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra* from central Italy. *Flavour Frag J* 2003; **18**: 139-143.

[7] Kainulainen P, Holopainen JK. Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. *Soil Biol Biochem* 2002; **34**: 37-42.

[8] Fernandez C, Voiriot S, Mévy JP, Vila B, Ormeño E, Dupouyet S, et al. Regeneration failure of *Pinus halepensis* Mill.: the role of autotoxicity and some abiotic environnemental parameters. *Forest Ecol Manag* 2008; **255**(7): 2928-2936.

[9] Navarro-Cano JA, Barbera GG, Ruiz-Navarro A, Castillo VM. Pine plantation bands limit seedling recruitment of a perennial grass under semiarid conditions. *J Arid Environ* 2009; **73**(1): 120-126.

[10] Cheikh-Rouhou S, Hentati B, Besbes S, Blecker C, Deroanne C, Attia H. Chemical composition and lipid fraction characteristics of Alleppo pine (*Pinus halepensis* Mill.) seeds cultivated in Tunisia. *Food Sci Tech Int* 2006; **12**(5): 407-415.

[11] Kubeczka KH, Schultze W. Biology and chemistry of conifer oils. *Flavour Frag J* 1987; **2**: 137-148.

[12] Asensio D, Owen SM, Llusia J, Penũelas J. The distribution of volatile isoprenoids in the soil horizons around *Pinus halepensis* trees. *Soil Biol Biochem* 2008; **40**: 2937-2947.

[13] Ormeño E, Baldy V, Ballini C, Fernandez C. Production and diversity of volatile terpenes from plants on calcareous and siliceous soils: Effect of soil nutrients. *J Chem Ecol* 2008; **34**(9): 1219-1229.

[14] Mirov NT, Iloff Jr PM. Composition of gum turpentines of pines. XXIII. A report on three mediterranean species: *Pinus pinea* (cultivated in California), *P. halepensis* (from Israel) and *P. brutia* (from Cyprus). *J Am Pharm Asso* 1955; **44**(3): 186-189.

[15] Pasqualini V, Robles C, Garzino S, Greff S, Bousquet-Melou A, Bonin G. Phenolic compounds content in *Pinus halepensis* Mill. needles: a bioindicator of air pollution. *Chemosphere* 2003; **52**(1): 239-248.

[16] Vidrich V, Mechelozzi M, Fusi P, Heimler D. Essential oils of vegetables species of the Mediterranean and Alpine temperate climate areas. Grassi G, Delmon B, Molle JF, Zibetta H, editors. 4th EC Conference on Biomass for Energy and Industry; 1988.

[17] Tazerouti F, Badjah-Hadj-Ahmed AY, Meklati BY, Favre-Bonvin J, Bobenrieth MJ. Analysis of essential oils from needles of *Pinus halepensis* Mill. by gas chromatography and mass spectrometry. *Plantes Medicinales et Phytotherapie* 1993; **26**(3): 161-176.

[18] Dob T, Berramdane T, Chelghoum C. Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria. *Comptes Rendus Chimie* 2005; **8**(11-12): 1939-1945.

[19] Abi-Ayad M, Abi-Ayad FZ, Lazzouni HA, Rebiahi SA, Ziani-Cherif C, Bessiere JM. Chemical composition and antifungal activity of Aleppo pine essential oil. *J Med Plant Res* 2011; **5**(22): 5433-5436.

[20] Roussis V, Petrakis PV, Ortiz A, Mazomenos EB. Volatile constituents of needles of five *Pinus* species grown in Greece. *Phytochem* 1995; **39**(2): 357-361.

[21] Hmamouchi M, Hamamouchi J, Zouhdi M, Bessiere JM. Chemical and antimicrobial properties of essential oils of five Moroccan Pinaceae. *J Essent Oil Res* 2001; **13**(4): 298-302.

[22] Tumen I, Hafizoglu Kilic A, Dönmez IE, Sivrikaya H, Reunanen M. Yields and constituents of essential oil from cones of *Pinaceae* spp. natively grown in Turkey. *Molecules* 2010; **15**: 5797-5806.

[23] Ustun O, Sezer Senol F, Kurkcuoglu M, Erdogan Orhan I, Kartal M, Husnu Can Baser K. Investigation on chemical composition, anticholinesterase and antioxidant activities of extracts and essential oils of Turkish *Pinus* species and pycnogenol. *Ind Corp Prod* 2012; **38**: 115-123.

[24] Council of Europe. *European Pharmacopoeia*. 1st ed. Strasbourg: Council of Europe; 1997.

[25] Jennings W, Shibamoto T. *Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography*. New York: Academic Press; 1980.

[26] König WA, Hochmuth DH, Joulain D. Terpenoids and related constituents of essential oils. Hamburg, Germany: Library of Mass Finder 2.1 University of Hamburg, Institute of Organic Chemistry; 2001. [Online] Available from: http://massfinder.com/wiki/Terpenoids_Library [Assessed on 16 July, 2013].

[27] Mc Lafferty FW, Stauffer DB. *The Wiley/NBS registry of mass spectral data*. New York: Wiley-interscience; 1989.

[28] Mc Lafferty FW, Stauffer DB. Mass Spectrometry Library Search System Bench-Top/PBM version 3.10d, Palisade, Newfield. 6 1994. Wiley registry of mass spectral data.

[29] NIST: National Institute of Standards and Technology. PC Version 1.7 of the NIST/EPA/NIH Mass Spectral Library Perkin Elmer Corporation, Norwalk, CT, 1999.

[30] Brereton RG. *Chemometrics: Data analysis for the laboratory and chemical plant*. New-York: Wiley Interscience; 2003.

[31] Massart DL. *Chemometrics: A textbook*. New York: Elsevier Sciences Ltd.; 1998.

[32] Bagamboula CF, Uyttendaele M, Candan F, Daferera D, Unli GV, Polissiou M, et al. Antimicrobial and antioxidative activities of the essential oils and methanol extracts. *Food Chem* 2004; **84**: 519-552.

[33] Mighri H, Hadjlaoui H, Akrout A, Najjaa H, Neffati M. Antimicrobial and antioxidant activities of Artemisia herba-alba essential oil cultivated in Tunisian arid zone. *Comptes Rendus Chimie* 2010; **13**: 380-386.

[34] Sheng-Hsien L, Ku-Shang C, Min-Sheng S, Yung-Sheng H, Hung-Der J. Effects of some Chinese medicinal plant extracts on five different fungi. *Food Control* 2007; **18**: 1547-1554.

[35] Abi-Ayad M, Abi-Ayad FZ, Lazzouni HA, Rebiahi SA. Antibacterial activity of *Pinus halepensis* essential oil from Algeria (Tlemcen). *J Nat Prod Plant Resour* 2011b; **1**(1): 33-36.

[36] Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, et al. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol* 2000; **88**: 170-175.

[37] Zouari S, Zouari N, Fakhfakh N, Bougatef A, Ayadi MA, Neffati M. Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba alba Asso*. *J Med Plant Res* 2010; **4**(10): 871-880.