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

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ABSTRACT

Objective: To find new bioactive natural products, the chemical composition and to study 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 ($\text{RI}$) 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 monoterpene (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%-13.4%), 2-phenyl ethyl isovalerate (4.8%-10.9%), terpinene-4-ol (1.9%-8.2%) and sabine (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 baumannii, 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

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therapeutic practice in world and has economic importance. 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, food and beverages, scenting agents in a variety of household works on the chemical composition of reported to have various therapeutic properties. They are products and intermediates in the synthesis of perfume halepensis also used as fragrances in cosmetics, flavoring additives for P. halepensis seeds are traditionally used throughout Tunisia (terpenoids and/or phenolic compounds) can affect root symbions and site quality, by interfering with decomposition, mineralization, and humification. P. halepensis may inhibit seedling establishment of various species in pine stands, suggesting the allelopathic nature of litter, leaf leachates, and/or root exudates.

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. 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. Several phytochemical analyses of P. halepensis have been published on terpenes, turpentine and phenolic compounds. The literature reports some works on the chemical composition of P. halepensis essential oil from Italy, Algeria, Greece, Morocco and Turkey. 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 Benahadji (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.

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 (polyethylene glycol). 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 and (ii) on computer matching with commercial mass spectral libraries 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). Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results. 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
phenylpropanoids compounds. The antibacterial activity. Each experiment was carried out in inhibition around each of the discs was taken as measure of after 24 h of incubation at 37°C. The scale of measurement was as follows [34] (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.

| Essential oil yields (%) |
|--------------------------|

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 0.34%, 0.49%, 0.8% and 0.9% (in dry weight basis) were found in the Ghazaouet, Saida, Djelfa and Sidi Fredj, respectively [31,38].

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 detected.
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). Forty 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                        | Collection (GC/MS) Sample Name | n-MS | MS IR | Identification |
|----------------------------------|--------------------------------|------|-------|----------------|
| α-Thujene                        | 922                             | 923  | 1021  | 0.7            |
| α-Pineene                        | 931                             | 932  | 1025  | 0.1            |
| Camphene                         | 943                             | 944  | 1066  | 0.2            |
| Subhene                          | 964                             | 966  | 1118  | 0.4            |
| β-Pineene                        | 970                             | 971  | 1108  | 1.9            |
| Myrcene                          | 970                             | 983  | 1159  | 25.2           |
| α-Phellandrene                   | 997                             | 998  | 1157  | 0.1            |
| 3-Gerene                         | 1005                            | 1006 | 1147  | 1.6            |
| α-Terpine                         | 1008                            | 1010 | 1175  | 0.9            |
| p-Cymene                         | 1010                            | 1012 | 1259  | 0.6            |
| β-Phellandrene                   | 1021                            | 1023 | 1208  | 0.1            |
| Z,β-Orincene                     | 1020                            | 1023 | 1195  | 0.6            |
| E,β-ocimene                      | 1034                            | 1035 | 1241  | 1.4            |
| α-Terpine                         | 1047                            | 1049 | 1273  | 1.4            |
| Terpinolene                      | 1078                            | 1082 | 1247  | 8.3            |
| Linalool                         | 1080                            | 1084 | 1259  | 0.4            |
| Perillol                         | 1021                            | 1099 | 1414  | 1.0            |
| cis-3-methyl-2-en-1-ol           | 1108                            | 1107 | 1600  | 0.2            |
| trans-3-methyl-2-en-1-ol         | 1113                            | 1117 | 1612  | 0.1            |
| Terpinolene-4-one                | 1161                            | 1163 | 1843  | 4.2            |
| α-Terpinolene                    | 1179                            | 1175 | 1688  | 0.4            |
| Bornyl acetate                   | 1269                            | 1268 | 1475  | 0.1            |
| Cinnamyl acetate                 | 1331                            | 1331 | 1654  | 0.1            |
| Neryl acetate                    | 1342                            | 1342 | 1408  | 0.1            |
| Geranyl acetate                  | 1361                            | 1360 | 1740  | 0.2            |
| α-Copaene                        | 1379                            | 1373 | 1475  | 0.2            |
| β-Caryophyllene                  | 1424                            | 1418 | 1563  | 10.9           |
| α-Humulene                       | 1456                            | 1449 | 1651  | 2.1            |
| 2-Phenylethyl isocoumarate       | 1463                            | 1468 | 1975  | 7.7            |
| Germacrene D                     | 1480                            | 1474 | 1692  | 0.2            |
| α-Nonadiene                      | 1496                            | 1492 | 1700  | 0.2            |
| δ-Cadinene                      | 1516                            | 1513 | 1738  | 0.3            |
| α-Eudesmol                       | 1532                            | 1532 | 1740  | 0.2            |
| Phenethylidyl Tiglate E          | 1547                            | 1546 | 2144  | 0.1            |
| Phenethylidyl Tiglate Z          | 1559                            | 1568 | 2145  | 0.1            |
| Caryophyllene oxide              | 1576                            | 1503 | 1806  | 0.8            |
| Guaiol                           | 1591                            | 1592 | 2070  | 0.2            |
| Humulene epoxide                 | 1601                            | 1631 | 2055  | 0.1            |
| Epi-Carbalol                     | 1624                            | 1625 | 2043  | 0.1            |
| Tsa-Cadinol                      | 1632                            | 1633 | 2163  | 0.2            |
| Tα-Muxanol                       | 1634                            | 1634 | 2144  | 0.1            |
| α-Cadinol                       | 1645                            | 1640 | 2163  | 0.2            |
| Eρ-Camphorone                    | 1659                            | 1666 | 2195  | 0.1            |
| Cembrene                         | 1938                            | 1940 | 2185  | 0.4            |
| α-Camphorone                     | 1947                            | 1939 | 2334  | 0.2            |
| Cembrene A                      | 1962                            | 1951 | 2227  | 0.1            |
| p-Camphorone                     | 1980                            | 1974 | 2187  | 0.3            |
| Geranyl Linalool                 | 2017                            | 2017 | 2401  | 1.5            |

Total Identification % 97.9

Yields % (n/c) 0.26

Hydrocarbon compounds 80.6

Monoterpene hydrocarbons 65.5

Sesquiterpene hydrocarbons 14.1

Diterpene hydrocarbons 1.0

Oxymetabolites 17.3

Oxymethonoterpene 5.4

Oxymetho sesquiterpenes 1.4

Oxymetho diterpenes 1.5

Non-terpene oxymetabolites 8.6

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) | Group II (S1–S7–9) |
|--------------------------------|----------------------|--------------------|
| Monoterpene hydrocarbons       | 54.59                | 54.55              |
| α–Pineene                      | 12.2–14.4            | 13.4               |
| Sabinene                       | 3.5–6.3              | 5.52               |
| Myrcene                        | 15.2–28.5            | 23.94              |
| Terpinolene                    | 9.4–13.8             | 11.53              |
| Oxygenated monoterpenes        | 5.78                 | 1.85               |
| Terpinene–4-ol                 | 3.9–8.2              | 5.78               |
| Monoterpene sesquiterpenes     | 8.78                 | 10.1               |
| E–β–Caryophyllene              | 7.0–11.1             | 8.78               |
| 2–Phenethyl isovanerate        | 10.1                 | 5.76               |
| Non-terpene sesquiterpenes     | 10.1                 | 4.8–7.0            |

Normalized % abundances.

![Figure 1](image1.png)

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

![Figure 2](image2.png)

**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 baumannii* (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 baumannii*       | 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 terpinolene (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%) is 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) with 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.

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