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Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria

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Abstract

The chemical composition of the volatile oil extracted by hydrodistillation from the needles of *Pinus halepensis* Miller, grown in natural habitats in Sidi Feradj (Algiers region), was obtained with yield 0.52% and analysed by GC and GC–MS. More than 41 compounds, representing 67.02% of total oil, were identified. The oil was found to be rich in β-caryophyllene (40.31%), α-humulene (7.92%) and aromadendrene (7.1%). ***To cite this article: T. Dob et al., C. R. Chimie 8 (2005)***.

Résumé

La composition chimique de l'huile essentielle extraite par hydrodistillation (rendement: 0,52%) des aiguilles fraîches de *Pinus halepensis* Mill. récoltées à Sidi Feradj (région d'Alger), a été analysée par CPG et CPG–SM. Quarante et un constituants, représentant 67,02% de l'huile totale, ont été identifiés. Les constituants majoritaires sont le β-caryophyllène (40,31%), l'α-humulene (7,92%) et l'aromadendrene (7,1%). ***Pour citer cet article: T. Dob et al., C. R. Chimie 8 (2005)***.

Keywords: Pinus halepensis; Pinaceae; Aleppo pine; Essential oil chemical composition; β-Caryophyllene; α-Humulene

Mots clés : Pinus halepensis ; Pinaceae ; Pin d'Alep ; Composition chimique de l'huile essentielle ; β-caryophyllène ; α-humulene

1. Introduction

The genus *Pinus* (Pinaceae) comprises 250 species and is widespread in the northern hemisphere, especially in the Mediterranean region, Caribbean area, Asia, Europe, North and Central America [1–4].

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 civilisation. Pine is also still widely used in traditional therapeutic practice in world and has an economic importance [5–7].

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The chemical composition of various pine species has been the subject of numerous studies, the majority of the studies focused on North American and Central European species [6,8–10] and only a limited number of chemically oriented reports dealt with Mediterranean pine species [11–13]. Most bibliographical studies of chemical, biological, antimicrobial activities and genetic side of *P. halepensis* have been reported [11–22].

As part of an extensive phytochemical analysis of *P. halepensis* growing in Algeria, we have oriented our first investigation towards the chemical composition of the essential oil obtained from the needles of *P. halepensis* Mill. collected in Algiers region (Sidi Feradj) in Algeria.

2. Experimental

2.1. Plant material

The needles of *P. halepensis* Mill. were collected in May 2002, at the forest of Sidi Feradj (Algiers). The plant was authenticated by Mr A. Beloued in botanical department, National Agronomic Institute of Algiers (N.A.I), Algeria (Herbarium No. P. 105). The samples were dried in shad ventilated place.

2.2. Oil isolation

The needles were cut into small pieces and separately hydrodistilled for 2 h in a Clevenger-type apparatus with water cooled receiver, in order to reduce hydrodistillation overheating artifacts. The essential oil was taken up in diethyl ether and dried over sodium sulphate and reduced at room temperature under vacuum on rotatory evaporator. The oil obtained was stored at (+4 °C) until analysis.

2.3. Analysis of essential oil

2.3.1. Gas chromatography

GC analysis was performed on a Chrompack CP 9002 chromatograph using fused silica capillary columns with two different stationary phases DB-1 and PEG. The various parameters fixed for DB-1 column are: 30 m × 0.32 mm i.d.; film thickness 0.25 μm column; temp. prog., 50 °C for 3 min then 2 °C/min to 260 °C for 5 min; detector heaters 280 °C; injector heaters 250 °C; nitrogen was used as carrier gas at a flow rate of 1 ml/min in the split mode (Split ratio 1:50), with an injection vol. 0.2 μl. For PEG the parameters are: 30 m × 0.32 mm i.d.; film thickness 0.25 μm column; temp. prog., 50 °C for 3 min then 2 °C/min to 220 °C for 15 min; others parameters as the same for the DB-1 column. Components were quantified as area percentages of total volatiles from the GC–FID system without correction factors.

In order to determine retentions indices (RI) a series of n-alkanes (C_5–C_{28}) mixture was analysed under the same operative conditions on DB-1 and PEG columns and the sample indices were calculated following Van den Dool and Kratz [23].

2.3.2. Gas chromatography and mass spectrometry

Mass spectra were obtained from GC–MS analysis on a Trace MS Finnigan chromatograph system equipped with a 30 m × 0.32 mm i.d.; film thickness 0.25 μm DB-1 capillary column it was programmed from 50 °C (3 min) to 260 °C (5 min) at 2 °C/min with helium carrier gas at a flow rate of 1 ml/min and injector heater 250 °C. The mass-spectrometer was operating (full scan-mode) in the EI-mode at 70 eV.

2.3.3. Component identification

Identification of components was made on the basis of their retention indices on non-polar (DB-1) and/or on polar (PEG) columns and by computerised matching of the acquired mass spectra with those stored in the spectrometer data base using Willey mass spectral library and with the literature [13,22–25].

3. Results and discussion

The needles from *Pinus halepensis* collected for the present study were obtained from Algiers region (Sidi Feradj). It's collected from various parts of crowns. Since all of samples were collected in May 2002, the effect of the seasonal variation was diminished [26]. The oil was obtained from the Aleppo pine needles with a yield of 0.52% (v/w).

The chromatographic profile showed a complex mixture of components with a consistent fraction of monoterpenes and sesquiterpenes.

Table 1
Qualitative and quantitative composition of needles oil of *P. halepensis* Miller

Compound [a]	% on DB-1	R. I.[b] on DB-1	R.I. on PEG	Method of identification
Tricyclene	tr	915	–	GC, GCMS
α-Pinene	**1.23**	922	1036	GC, GCMS
Camphene	tr	957	–	GC,GCMS
Sabinene	**1.23**	960	1127	GC, GCMS
β-Pinene	0.23	978	–	GC, GCMS
Myrcene	**3.07**	997	1140	GC, GCMS
α-Phellandrene	tr	1002	1296	GC, GCMS
Hexyle acetate	0.7	1008	1268	GC, GCMS
δ-3-Carene	0.15	1011	1140	GC, GCMS
α-Terpinene	0.11	1021	1181	GC, GCMS
Limonene	tr	1032	–	GC, GCMS
β-Ocimene	0.21	1041	1247	GC, GCMS
γ-Terpinene	tr	1062	1231	GC, GCMS
α-Terpinolene	0.13	1088	–	GC, GCMS
α-Pinene oxide	0.06	1095	1488	GC, GCMS
Linalool	tr	1098	1566	GC, GCMS
Camphor	tr	1143	–	GC, GCMS
Borneol	0.13	1165	1695	GC, GCMS
Terpinen-4-ol	tr	1177	1591	GC, GCMS
p-Cymen-8-ol	tr	1183	1823	GC, GCMS
α-Terpineol	0.07	1189	1684	GC, GCMS
α-Terpinyl acetate	tr	1350	–	GC, GCMS
α-Cubebene	0.17	1351	–	GC, GCMS
Citronellyl acetate	0.19	1354	–	GC, GCMS
α-Yalangene	0.64	1372	1481	GC, GCMS
(Z)-β-Caryophyllene	**40.31**	1404	1591	GC, GCMS
α-Guaiene	0.1	1437	–	GC, GCMS
Aromadendrene	**7.1**	1439	1599	GC, GCMS
α-Humulene	**7.92**	1454	–	GC, GCMS
allo-Aromadendrene	0.65	1461	1685	GC, GCMS
γ-Muurolene	0.06	1477	1715	GC, GCMS
Germacrene-D	0.49	1480	1702	GC, GCMS
Bicyclogermacrene	tr	1494	1723	GC, GCMS
β-Bisabolene	tr	1509	–	GC, GCMS
δ-Cadinene	0.13	1513	1777	GC, GCMS
β-Sesquiphellandrene	0.53	1524	1776	GC, GCMS
Z-Nerolidol	0.07	1534	–	GC, GCMS
Elemol	0.68	1549	–	GC, GCMS
δ-Cadinol	0.23	1622	2152	GC, GCMS
γ-Eudesmol	0.08	1630	–	GC, GCMS
Monoyl oxide	tr	1989	–	GC, GCMS
Monoterpene hydrocarbon	6.48%			
Oxygenated monoterpene	0.36%			
Sesquiterpene hydrocarbon	58.20%			
Oxygenated sesquiterpene	1.06%			

[a] Order of elution on DB-1.
[b] Retention indices. tr: Trace (<0.05%).

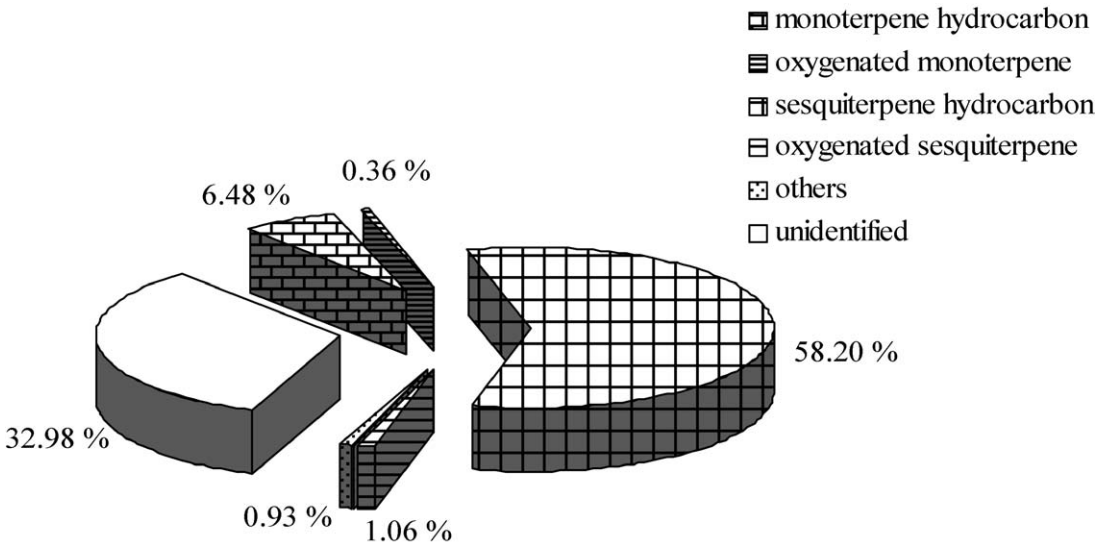

Fig. 1. Chemical composition groups of needles oil of *P. halepensis*.

The list of the compounds, in order of elution on DB-1, and the quantitative data (GC–FID peak area percentages without correction factors), are reported in Table 1. More than 41 oil compounds were identified accounting for 67.02% of the total oil, while 32.98% of the oil remained unidentified (Fig. 1).

Amounting to 67.02% of the total oil, the sesquiterpene hydrocarbons had the highest contribution (58.20%), this fraction dominated by β-caryophyllene (40.31%), followed by α-humulene (7.92%) and aromadendrene (7.10%). The monoterpene was relatively poor; it represented (6.50%) in monoterpene hydrocarbons, it is found to contain a significant percent of myrcene (3.07%), followed by α-pinene (1.23%) and sabinene (1.23%). The oil is characterised by 13 compounds could be detected in traces (< 0.05%).

Table 2 summarises previous investigations of authors on the analysis of the volatile oils from several population of *P. halepensis*. The chemical composition of our *P. halepensis* Mill. oil was dominated by β-caryophyllene, these results agree with data obtained by Roussis et al. who found that monoterpene (41.8%) was dominated in Greece pine oils with remarkable differences concerning the amounts of component: caryophyllene (19.05%) [11]. Vidrich et al. [27] have reported that β-caryophyllene (26.31%) play an important part of the Italy oil. Macchioni et al. [13] found main compounds of the needles oil of Aleppo pine grown in Italy to be: myrcene (27.9%), α-pinene (18.1%) and β-caryophylene (16.4%), with a 73.2% of monoterpenes and 21.2% of sesquiterpenes. When our results were compared with the chemical composition of essential oils obtained from the leaves of *P. halepensis* collected in region of Tessimsilt and Djelfa (Algeria) in 1987, remarkable differences were observed: myrcene (8.65%) and α-pinene (17.56%), dominated in later sites, respectively [19].

Our results differ from those obtained by Hmamouchi et al. [12] who studied the oil composition of needles of the same specie sample in Morocco, in which α-pinene (23.3%) was revealed to be dominant and β-caryophylene (14.2%) present. Several reports on the composition of the needle oils of other *Pinus* species revealed that monoterpene hydrocarbons were the major constituents in the most of the oil, they often constituted 50% or more of the oil [4,12,13].

The comparison of our result with literature (Table 2) shows some qualitative and quantitative differences in compositions 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 adaptative process to particular ecologic conditions.

Table 2
Chemical composition of needles oils of *P. halepensis* as reported in the literature

Constituents	Ouer study	Greece	Italy		Morocco	Algiers [19]	
		[11]	[13]	[27]	[12]	T[a]	D[b]
Tricyclene	tr	–	–	–	–	1.60	0.04
α-Thujene	–	0.16	0.1	–	0.4	–	–
α-Pinene	1.23	13.4	18.1	8.54	23.3	6.66	17.56
Camphene	tr.	0.44	0.3	0.09	0.5	0.17	0.33
Sabinene	1.23	1.27	9.4	6.13	3.7	6.95	2.59
β-Pinene	0.23	1.13	2.0	1.13	3.1	2.04	1.56
Myrcene	3.07	6.62	27.9	12.48	16.3	8.65	3.22
3-Octanol	–	–	–	0.06	–	–	–
α-Phellandrene	tr	0.05	–	0.81	1.6	–	–
Hexyl acetate	0.7	–	–	–	–	–	–
δ-3-Carene	0.15	6.87	1.7	0.87	–	1.88	0.13
α-Terpinene	0.11	0.30	0.5	0.79	1.3	0.65	0.04
p-Cymen	–	–	1.1	11.41	0.7	0.27	3.07
Limonene	tr	5.03	1.1	0.98	1.3	0.8	0.14
β-Phllandrene	–	–	1.0	–	1.27	0.97	0.66
1,8-Cineole	–	–	–	1.67	1.3	–	–
cis-β-Ocimene	–	–	–	–	–	0.11	0.07
trans-β-Ocimene	0.21	–	0.4	–	1.77	2.05	0.04
γ-Terpinene	tr	0.42	0.8	0.24	2.4	1.18	0.02
cis-Sabinene hydrate	–	–	0.1	–	–	–	–
α-Terpinolene	0.13	3.07	9.9	–	10.1	0.19	t
cis-3-Hexene-1-ol	–	–	–	–	–	t	0.09
3,5-Dimethyl-styrene	–	–	–	–	–	t	t
α-Pinene oxide	0.06	–	–	–	–	t	t
Linalool	tr	0.78	0.3	0.41	–	0.39	2.01
Fenchol	–	0.11	–	–	–	–	–
Camphor	tr	–	–	–	–	t	0.05
Borneol	0.13	0.02	–	–	–	–	–
Umbellulone	–	–	0.1	–	–	–	–
Terpinen-4-ol	tr	0.7	–	–	3.8	–	0.59
p-Cymen-8-ol	tr	–	–	–	–	t	0.14
α-Terpineol	0.07	0.54	0.2	–	0.6	0.29	1.48
cis-piperitol	–	–	–	–	–	t	–
Methyl chavicol	–	–	–	5.06	–	–	–
Decanale	–	–	–	0.83	–	–	–
Fenchyl acetate	–	0.28	–	–	–	–	–
Nerol	–	–	–	–	–	–	0.04
D-Citronellolo	–	–	–	1.26	–	–	–
L-Citronellolo	–	–	–	0.41	–	–	–
Methyl thymyl ether	–	0.10	–	–	–	–	–
Geraniol	–	–	–	–	–	t	t
2-Phenyl ethyl acetate	–	–	–	–	2.5	–	0.12
Bornyl acatate	–	–	–	–	–	t	0.16
Carvacrol	–	–	–	1.72	–	–	–
δ-Elmene	–	0.03	–	–	–	–	0.20
α-Terpenyl acetate	tr	0.01	–	–	–	–	–
α-Cubebene	0.17	–	0.1	–	–	t	t
Citronellyl acetate	0.19	–	–	–	–	–	–
Eugenol	–	–	–	0.60	–	–	–
Neryl acetate	–	0.36	–	–	–	–	–
α-Yalangene	0.64	–	–	–	–	0.24	0.21

(continued on next page)

Table 2
(continued)

Constituents	Ouer study	Greece	Italy		Morocco	Algiers [19]	
		[11]	[13]	[27]	[12]	T[a]	D[b]
α-Copaene	–	–	0.4	–	0.5	t	0.03
Z-3-Hexenyl-isovalerate	–	–	–	–	–	t	t
Geranyl acetate	–	0.19	0.3	0.86	5.3	t	0.29
β-Elemene	–	–	–	–	–	t	0.20
Methyl eugenol	–	–	–	–	–	t	t
(*Z*)-β-Caryophyllene	40.31	–	–	–	–	–	–
(*E*)-β-Caryophyllene	–	–	–	–	–	7.07	2.69
β-Caryophyllene[c]	–	19.05	16.4	26.31	14.2	–	–
α-Gurjunene	–	1.18	–	–	–	–	–
α-Guaiene	0.1	–	–	–	–	–	–
α-Cedrene	–	–	–	0.08	–	–	–
Aromadendrene	7.1	–	–	–	–	–	–
cis-carane-trans 2-ol	–	–	–	–	–	t	0.54
α-Elemene	–	–	–	–	–	t	0.58
α-Humullene	7.92	3.36	2.9	–	3.2	2.77	1.38
Allo-aromadendrene	0.65	–	–	–	–	–	–
Calarene	–	0.39	–	–	–	–	–
Methyl *iso*-eugenol-2	–	–	–	–	–	0.27	t
(*E*)-β-Farnesene	–	–	0.2	–	–	t	0.03
9-epi-(*E*)-Caryophyllene	–	–	0.1	–	–	–	–
γ-Muurolene	0.06	–	–	–	–	0.29	0.19
Germacrene D	0.49	0.5	0.1	–	–	0.21	0.03
δ-Selinene	–	–	–	–	–	t	t
Phenyl ethyl-3-methyl butanoate	–	–	1.2	–	–	–	–
Epi-Cubebol	–	–	0.2	–	–	–	–
Bicyclogermacrene	tr	–	–	–	–	–	–
Methyl *iso*-eugenol-l	–	–	–	5.06	–	0.17	t
α-Muurolene	–	0.53	0.4	–	0.5	0.29	0.19
Bicycloelemene	–	–	–	–	–	t	t
β-Bisabolene	tr	–	–	–	–	–	–
trans-γ-Cadinene	–	–	0.3	–	–	–	–
Calamenene	–	–	–	–	–	t	0.18
δ-Cadinene	0.13	0.55	0.3	–	1.0	0.87	0.47
β-Sesquiphellandrene	0.53	–	–	–	–	–	–
Levomenol	–	–	–	–	–	t	0.03
Ethyl laurate	–	–	–	–	–	t	t
Terpenyl-*n*-butyrate	–	–	–	–	–	t	1.48
β-Phenyl ethyl-isobutyrate	–	–	–	–	–	t	t
Phenyl ethyl 2-methyl-butyrate	–	–	–	–	–	0.97	10.29
Phenyl ethyl-isovalerate	–	–	–	–	–	7.37	8.38
Elemol	0.68	0.36	–	–	–	t	–
Nerolidol	0.07	–	–	–	–	t	–
Caryophyllene oxide	–	–	0.1	–	1.2	–	–
Globulol	–	0.01	–	–	–	–	–
Phenyl ethyl-tiglate (*E*)	–	–	–	–	–	t	t
Guaiol	–	1.05	0.3	–	–	0.20	0.17
Phenyl ethyl *n*-hexanoate	–	–	–	–	–	t	t
Humulene epoxyde II	–	–	–	–	–	0.17	0.17
Cubenol	–	–	–	–	–	t	–
β-Eudesmol	–	–	–	–	–	–	t
α-Eudesmol	–	0.41	0.1	–	–	–	–

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Table 2
(continued)

Constituents	Ouer study	Greece [11]	Italy		Morocco	Algiers [19]	
			[13]	[27]	[12]	T[a]	D[b]
Cadinol	–	–	–	–	1.1	t	0.17
α-Cadinol	–	–	–	–	–	0.18	0.15
δ- Cadinol	0.23	–	–	–	–	–	–
trans-Cadinol	–	–	–	–	–	t	0.17
γ-Eudesmol	0.08	–	–	–	–	–	–
Phenyl ethyl-tiglate (*Z*)	–	–	–	–	–	t	t
Torreyol	–	–	–	–	–	t	t
Aristolene	–	1.09	–	–	–	–	–
Butanoic acid, 3-methyl, 2-phenyl ethyl ester	–	6.57	–	–	–	–	–
Kaureuol	–	–	–	–	–	t	t
Diethyl-phatalate	–	–	–	–	–	–	0.08
Cembrene	–	7.62	–	–	–	–	–
(11*E*,13*Z*) Labdadien-8-ol	–	0.30	–	–	–	–	–
Neocembrene	–	–	–	–	–	–	–
Manoyle oxide	tr	–	–	–	–	t	–

tr: trace (< 0.05%). t: trace [19].
[a] T: Tissemsilt.
[b] D: Djelfa.
[c] Correct isomer not identified.

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