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ANALYSIS OF ESSENTIAL OIL FROM THE NEEDLES OF *Pinus pinaster* GROWING IN ALGERIA

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*The needle oil of the Algerian maritime pine (*Pinus pinaster *Ait.) growing in natural habitats in Sidi Feradj (Algiers region) was obtained by hydrodistillation in 0.3% yield and analyzed by GC and GC/MS. More than 46 compounds amounting to 65.2% of the total oil were identified. The main components were* β*-caryophyllene (26.6%), allo-aromadendrene (12.5%), and* α*-humulene (4.3%).*

Key words: *Pinus pinaster* Ait., Pinaceae, essential oil composition, sesquiterpene hydrocarbons, β-caryophyllene.

P. pinaster is the most common pine in the Mediterranean region. It is grown on mineral soil, and there are more than 4 million hectares of maritime pine forest in France, Portugal, Spain, Italy, Morocco, Tunisia, and Algeria [1, 6].

Numerous authors [2–18] have extensively studied the different uses of maritime pine trees. Comprehensive investigations of the chemical, biological, and genetic side of this species have been reported [2–7]. The biosynthesis of monoterpenes and sesquiterpenes of *P. pinaster* have been carried out by many investigators [8–10]. The antimicrobial activities of *P. pinaster* have been reported by Chalchat et al. and Hmamouchi et al. [11, 12].

Durel et al. selected the genotype for the vigor trait in maritime pine as the economically important one [5, 13].

To the best of our knowledge, there is no previous report on the chemical composition of the essential oil of *P. pinaster* Ait. from Algeria. The aim of this paper is to extend our knowledge and report preliminary results on the needle oil composition of *P. pinaster* Ait. growing in Algeria.

More than 46 components amounting to 65.2% (area percent) of the total oil were identified and quantified as oil constituents on the basis of retention indices and mass spectra. Table 1 lists the constituents of the essential oils in order of elution in a DB-1 column, their percentage, and their retention indices on the column. The sesquiterpene hydrocarbons had the highest contribution (51.6%). This fraction is dominated by β-caryophyllene (26.6%), *allo*-aromadendrene (12.5%), α-humulene (4.3%), and α-selinene (3.5%). The monoterpene fraction was relatively poor; it represented 1.2% of monoterpene hydrocarbons and 1.8% of oxygenated monoterpene.

Table 2 summarizes previous investigations of authors on the oils hydrodistilled (the three main components) from several populations of *P. pinaster*. We see that our maritime pine oil was dominated by β-caryophyllene (26.6%). These results agree with previous studies on the essential oil of Morocco maritime pine reported by Hmamouchi et al., who found that caryophyllene (22.2%) constitutes an important part of the sesquiterpene hydrocarbons (64.6%) [12]. In contrast to our result, the studies of Gleizes et al. on the essential oils from French maritime pine showed a significant difference in their composition, consisting of 40.2% of sesquiterpene hydrocarbons and 59.8% of monoterpene hydrocarbons [9]. In addition, Pauly et al. examined the needle oil of the same species originating from France and established that it contain 42% monoterpenes, 35% sesquiterpenes, and 23% oxygenated compounds [15]. In 1986, Carmo and Frazao found a significant percent (66%) of monoterpene hydrocarbons in the Portuguese oil of *P. pinaster*, which contained α-pinene (27.3%), and β-pinene (20.2%) [16]. Kubeczka and Schultze examined the majority of the monoterpene hydrocarbons (85.6%) and found that the main compounds were α-pinene (44.1%), β-pinene (29.5%), and myrcene (4.7%) [3]. Dominguez Garrido et al. have reported the main constituents of Spanish maritime pine oil, which are α-pinene, β-pinene, and β-caryophyllene [19].

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TABLE 1. Chemical Constituents of Needle Essential Oil of *Pinus pinaster*

Compound	RI	%	Compound	RI	%
Tricyclene	921	0.7	α-Humulene	1435	4.3
β-Pinene	959	0.3	*allo*-Aromadendrene	1446	12.5
β-Myrcene	976	0.1	Phenyl ethyl 3-methyl butanoate	1448	1.2
δ-3-Carene	999	Tr.	Epi-Cubebol	1453	2.4
Limonene	1017	0.1	α-Selinene	1461	3.5
Undecane	1096	0.1	*trans*-β-Guaine	1470	1.9
trans-Pinocarveol	1111	0.2	β-Bisabolene	1492	0.3
Pinene-hydrate	1114	0.1	α-Methyl-ionone	1518	0.7
Benzyl acetate	1118	0.5	β-Sesquiphellandren-8-ol	1524	0.6
α-Phellandren-8-ol	1135	0.2	Elemol	1558	0.4
Terpinen-4-ol	1163	Tr.	Spathulenol	1576	0.4
Myrtenol	1168	0.4	*trans*-Sesquisabinene hydrate	1580	0.8
Carveol	1188	0.1	Caryophyllene oxide	1596	0.7
Carvone	1210	Tr.	Viridiflorol	1599	0.3
cis-Myrtanol	1213	0.1	Khusimone	1603	0.1
Geraniol	1234	Tr.	Cubenol	1640	0.2
Methyl citronelate	1258	Tr.	α-Bisabolol	1683	Tr.
Tridecane	1294	0.2	2-*cis*,6-*trans*-Farnesol	1690	0.3
trans-Ascoridole	1305	Tr.	Caryophyllene acetate	1720	0.1
α-Cubebene	1328	0.1	*cis*-*trans*-Farnesyl acetate	1812	0.1
Cyclosativene	1345	0.6	Monoterpene hydrocarbons		1.2%
α-Yalangene	1353	0.3	Oxygenated monoterpenes		1.8%
Longicyclene	1362	1.3	Sesquiterpene hydrocarbons		51.6%
Geranyl acetate	1388	2.0	Oxygenated sesquiterpenes		8.9%
β-Caryophyllene	1405	26.6	Total area of identified peaks		65.2%

RI: retention indices calculated against *n*-alkanes.
Tr.: trace (< 0.05%).

TABLE 2. Contents (%) of the Most Dominant Components in the Essential Oils from Several Populations of *Pinus pinaster* as Reported in the Literature

Compound	Morocco (Rabat region) [12]	Italy (Montemarcello) (La Spezia) [18]			Greece [17]	France [11, 15]	Portugal [16]	Germany [3]
	Needles	Needles	Branches	Cones	Needles	Needles	Needles	Needles
β-Caryophyllene	22.2	13.2			15	6.4; 13.0		
α-Pinene	21.4	29	40.4	22	21	43.4	27.3	44.1
γ-Muurolene	7.0							
β-Pinene		22	23.1	29.2		25.1; 16.0	20.2	29.5
Myrcene			12.1					4.7
Longifolene				21				
Germacrene D					19.2	11.5		
Limonene							7.6	

Recently Macchioni et al. found α-pinene (29%) followed by β-pinene (22%) and β-caryophyllene (13.2%). This oil includes 61.2% of monoterpene hydrocarbons and 21.1% of sesquiterpene hydrocarbons [18]. Greece maritime pine essential oil was characterized by α-pinene (21%), germacrene-D (19.2%), and caryophyllene (15%), with β-pinene absent [17].

EXPERIMENTAL

Plant Material. The needles of *Pinus pinaster* Ait were collected in May 2002 at the forest of Sidi Feradj (Algiers). The plant was authenticated in the botanical department, National Agronomic Institute of Algiers, Algeria. The samples were dried in a shade ventiled place.

Oil Isolation. The needles (100 g) were cut into small pieces and separately hydrodistilled for 2 h using a modified Clevenger apparatus with a watercooled receiver in order to reduce hydrodistillation overheating artifacts. The essential oil was taken up in diethyl ether, dried over sodium sulfate, and reduced at room temperature under reduced pressure on a rotatory evaporator. The oil obtained was stored at 4°C.

GC and GC/MS Analysis. GC analysis was performed on a Chrompack CP 9002 chromatograph using fused silica capillary columns with stationary phases DB-1. The various parameters fixed for the 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, with an injection volume of 0.2 μL.

In order to determine retention indices (RI) a series of *n*-alkane (C_5–C_{28}) mixtures was analyzed under the same operative conditions on DB-1 and the sample indices were calculated following Van Den Dool and Kratz [20].

Mass spectra were obtained from GC/MS analysis on a Trace MS Finnigan chromatograph equipped with a 30 m × 0.32 mm i.d.; film thickness 0.25 μm column; the DB-1 capillary column 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. Temperature of the ion source was 200°C. The mass-spectrometer was operating (full scan-mode) in the EI-mode at 70 eV.

Identification of Components. Identification of components was made on the basis of their retentions indices on a nonpolar (DB-1) column and by computerized matching of the acquired mass spectra with those stored in the spectrometer database using the Wiley mass spectra library and with the literature [17, 18, 21].

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