



Chemical Composition of Essential Oils from Needles of *Pinus pinaster* from Italy and Tunisia

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The chemical composition of the essential oil obtained from the needles of *Pinus pinaster* by hydrodistillation was analyzed by gas chromatography-mass spectrometry. Fifty components were detected, of which 41 were identified for a total percentage of about 98 %. The main components of the essential oil of needles from Tunisia were abietadiene (25.4 %), 13-epi-manoyl oxide (16.6 %), abietatriene (10.2 %), β -caryophyllene (11.1 %), 13(16), 14-labdien-8-ol (5.9 %) and α -pinene (5.3 %) whereas the oil from Italy was predominantly composed of limonene (36.7 %), abienol (13.4 %), manoyl oxide (6.5 %) and β -caryophyllene (5.8 %).

Keywords: *Pinus pinaster*, Pinaceae, Tunisia, Italy, Essential oil, Limonene, Abietadiene.

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 and 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. Essential oils from *Pinus* species have been reported to have various therapeutic properties^{1,2}. The chemical compositions of essential oil of many *Pinus* species from different part of the world have been reported by many workers^{3,4}.

Pinus pinaster (maritime pine) is one of the most common pines in the Mediterranean region. There are more than 4 million hectares of maritime pine forest all around the occidental mediterranean basin (Portugal, Spain, France, Italy, Tunisia, Algeria and Morocco). Maritime pine is especially exploited for its wood and resin products. The use of pine needles to produce the essential oil is another possibility for integral profit of pine forests. Several studies have been carried out on the composition of *P. pinaster* essential oil obtained by hydrodistillation of needles, branches or cones. In *P. pinaster* various chemotypes have been distinguished regarding monoterpenes and sesquiterpenes of the wood extractives, needles and cortex and in connection with resin acids of the needles. Genetic variability of *P. pinaster*, based on the use of

terpenes as genetic markers, has been geographically structured in 18 races. They are included in three main groups: Atlantic (Southwest of France, Portugal and Northwest of Spain), Mediterranean (East and Central Spain, Southeast of France, Corse and Italy) and Magrebian (North of Africa)⁵.

The aim of this work was to investigate the chemical composition of the essential oil of *Pinus pinaster* growing spontaneously in two different states in the Mediterranean region (Tunisia: Tabarka and Italy: Sardinia island).

EXPERIMENTAL

Needles of *Pinus pinaster* were collected from Tunisia (Tabarka) and Italy (Sardinia Island). Voucher specimens (numbers: TT 20101 and IS20102) were deposited at the Herbarium of the Department of Botany of the University of Tunis. Vegetal material was air-dried in a hot air-drier at 40 °C with forced ventilation for 2 days. Before utilization, the plant material was ground with a Malavasi mill (Bologna, Italy) taking care to avoid overheating.

Hydrodistillation: Hydrodistillation was performed in a circulatory Clevenger-type apparatus according to the procedure described in the European Pharmacopoeia for 4 h⁶.

Gas chromatography and gas chromatography-mass spectrometry analysis: Analysis of the essential oils was carried out by gas chromatography (GC) and by gas chromatography-mass spectrometry (GC-MS).

Analytical gas chromatography was carried out in a gas chromatograph (Agilent, Model 7890A, Palo Alto, CA), equipped with a flame ionization detector (FID), an autosampler (Agilent, Model 7683B), Agilent HP5 fused silica column (5 % phenyl-methylpolysiloxane), 30 m × 0.25 mm i.d., film thickness 0.25 mm and a Agilent ChemStation software system. Oven temperature was settled at 60 °C, raising at 3 °C/min to 250 °C and then held 20 min at 250 °C; injector temperature: 250 °C; carrier gas: helium at 1 mL/min; splitting ratio 1:10; detectors temperature: 300 °C.

Gas chromatography-mass spectrometry analyses were carried out in a gas chromatograph (Agilent, Model 6890N, Palo Alto, CA) equipped with a split-splitless injector, an autosampler Agilent model 7683 and an Agilent HP5-ms fused silica column; 5 % phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 mm. Gas chromatography conditions used were: programmed heating from 60 to 250 °C at 3 °C/min followed by 20 min under isothermal conditions. The injector was maintained at 250 °C. Helium was the carrier gas at 1 mL/min; the sample (1 mL) was injected in the split mode (1:10). The gas chromatography was fitted with a quadrupole mass spectrometer, MS, Agilent model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200 °C, quadrupole temperature 150 °C, scan rate 3.2 scan/sec, mass range 30-480 u. Software adopted to handle mass spectra and chromatograms was a ChemStation. NIST 05 and HPCH2205 Mass Spectra Libraries were used as references^{7,8}. Samples were run in chloroform with a dilution ratio of 1:100. Compounds were identified by matching their mass spectra and retention indices with those reported in the literature. Moreover, whenever possible, identification has been confirmed by injection of pure compounds. Percentage of individual components was calculated based on gas chromatography peak areas without FID response factor correction.

RESULTS AND DISCUSSION

Forty-one components representing about 98 % of the total oils were identified from the two *P. pinaster* samples analyzed (Table-1).

The oil obtained from the needles of *Pinus pinaster* growing in Tunisia is dominated by hydrocarbon diterpenes, oxygenated diterpenes and sesquiterpene hydrocarbons (40.5 %, 29.2 %, 20.2 %, respectively), the monoterpene fraction was relatively poor and it represented 5.7 % of monoterpene hydrocarbons and 0.8 % of oxygenated monoterpenes. Similar results were reported by Dob *et al.*⁹. They founded that needles oil obtained from *Pinus pinaster* from Algeria contained 51.5 % of sesquiterpene hydrocarbons and only 3 % of monoterpene (1.2 % of monoterpene hydrocarbons, 1.8 of oxygenated monoterpene). However the oil from Italy is dominated by hydrocarbon monoterpenes (40.8 %), 34.0 % of oxygenated diterpenes but only 15 % of sesquiterpene hydrocarbons. The needle oil of the same species originating from France was found to contain 42 % monoterpenes, 35 % sesquiterpenes and 23 % oxygenated compounds¹⁰. These differences could be the basis of further research work aimed at determining whether this variability is caused by endogenous or exogenous factors. Dependence

TABLE-1
RETENTION INDICES, I_R, AND CHROMATOGRAPHIC AREA PERCENTAGES OF CONSTITUENTS OF *Pinus pinaster* ESSENTIAL OILS OBTAINED FROM NEEDLES COLLECTED IN TUNISIA (TABARKA) AND ITALY (SARDINIA ISLAND)

Compound	I _R	Tunisia	Italy
α-Pinene	939	5.3	2.2
β-Pinene	981	0.2	0.7
Myrcene	993	tr	1.2
Limonene	1029	tr	36.7
β-Phellandrene	1030	0.2	tr
Thymol, methyl ether	1237	tr	0.9
α-Copaene	1376	0.3	tr
Longifolene	1406	-	1.2
β-Caryophyllene	1421	11.1	5.8
6,9-Guaiadiene	1446	-	1.0
α-Humulene	1455	2.0	1.2
trans-Cadina-1(6),4-diene	1477	0.6	-
γ-Murolene	1481	2.4	3.6
Phenil ethyl 3-methyl butanoate	1491	0.8	tr
δ-Selinene	1492	tr	1.7
α-Murolene	1499	0.3	tr
γ-Cadinene	1513	0.6	tr
δ-Cadinene	1524	1.5	0.6
(E)-<-Bisabolene	1544	1.3	tr
Caryphyllene oxide	1581	0.9	-
Carotol	1595	-	0.6
Guaiol	1599	-	3.7
Selina-6-en-4-ol	1620	-	0.8
epi-α-Cadinol	1641	0.3	tr
Pentadecanal	1717	-	1.7
n.i.	1894	-	0.9
n.i.	1927	0.2	tr
Manoyl oxide	1990	tr	6.5
13-epi-Manoyl oxide	2013	16.6	tr
Abieta-8,12-diene	2016	4.9	tr
Abietatriene	2054	10.2	tr
Abietadiene	2081	25.4	tr
13(16),14-Labdien-8-ol	2102	5.9	tr
Phytol	2116	-	2.4
n.i.	2123	0.3	-
(11E,13Z)-11813-Labdadien-8-ol	2134	-	3.0
n.i.	2137	0.2	-
Abienol	2150	4.5	13.4
n.i.	2191	0.6	-
n.i.	2193	0.2	-
n.i.	2216	-	1.3
Kaura-5,16-dien-18-ol	2232	1.1	1.0
n.i.	2256	0.8	-
deidro-Abietal	2264	0.9	-
n.i.	2286	0.1	-
Methyl isopimarate	2293	-	0.7
Methyl levopimarate	2304	-	3.6
Methyl dehydroabietate	2338	0.3	1.5
Methyl abietate	2382	tr	0.6
bis(2-Ethylhexyl) phthalate	2551	-	1.4
Total identified		97.6	97.7
Hydrocarbon monoterpenes		5.7	40.8
Oxygenated monoterpenes		0.8	0.9
Hydrocarbon sesquiterpenes		20.2	15.0
Oxygenated sesquiterpenes		1.2	6.9
Hydrocarbon diterpenes		40.5	-
Oxygenated diterpenes		29.2	34.0

Note : I_R: GC retention indices relative to C9-C25 n-alkanes on the HP-5 ms column; tr: traces < 0.1 %; n.i.: not identified

on the geographical origin considerable variations were also found. The results showed the presence of two new chemotypes of *P. pinaster*. In the Italian sample, limonene (36.7 %), abienol

(13.4 %), manoyl oxide (6.5 %) and β -caryophyllene (5.8 %) were the main components; instead, the oil extracted from Tunisian *P. pinaster* is predominantly composed of abietadiene (25.4 %), epi-13-manoyl oxide (16.6 %), β -caryophyllene (11.1 %), abietatriene (10.2 %), α -pinene (5.3 %) and abietatriene (4.9 %). Literature data reported that α -pinene is prominent in the needle oils from Russia (99.8 %), Portugal (97.5 %) and Argentina (83.2 %)^{11,12}; α -pinene and β -pinene are the two major components in the *P. pinaster* oils from Italy (28.9 and 21.7 %), Portugal (27.4 and 20.2 %) and France (44.1 and 29.5 %)^{13,14}; (E)- β -caryophyllene and alloaromadendrene (26.6 and 12.5 %) are the major compounds found in the essential oil from Algeria⁹; samples from Morocco¹⁵ contained mainly α -pinene and (E)- β -caryophyllene (21.4 and 22.2 %) and the needle oil from Greece¹⁶⁻¹⁷ was dominated by α -pinene, germacrene D and (E)- β -caryophyllene (20.9, 19.2 and 14.8 %, respectively).

Conclusion

In conclusion, from the comparison with literature data, it is possible to assert that this species is characterized by an impressive variability in the chemical composition of its essential oil. A study of biological activities can be investigated.

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