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Leaf essential oils analysis of Juniperus navicularis Gandoger

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Resumen

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El aceite esencial obtenido de las hojas de *Juniperus navicularis* Gand., recolectadas en el SO de Portugal, fue analizado mediante cromatografía de gases (CG) y cromatografía de gases acoplada a espectrometría de masas (CG/EM), utilizando también los índices de retención de Kováts. El aceite contenía como componentes mayoritarios α-pineno (30,8%), α-felandreno (11,1%) y limoneno + β-felandreno (27,2%). Otros compuestos característicos fueron los cadinanos + muurolanos (2,7%) y también se encontraron (*E*)-nerolidol (4,8%) y α-*epi*-bisabolol (0,6%).

Palabras clave: *Juniperus navicularis*, Cupressaceae, composición del aceite esencial, α-pineno, α-felandreno, limoneno + β-felandreno.

Abstract

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The steam distilled oil obtained from the leaves of *Juniperus navicularis* Gand., gathered in SW Portugal was analysed by CG and GC/MS in combination with retention indices. The oil was shown to contain α-pinene (30,8%), α-phellandrene (11,1%) and limonene + β-phellandrene (27,2%) as major constituents. Other characteristic compounds were cadinanes + muurolanes (2,7%). In addition to these components, (*E*)-nerolidol (4.8%) and α-*epi*-bisabolol (0,6%) were also present.

Key Words: *Juniperus navicularis*, Cupressaceae, essential oil composition, α-pinene, α-phellandrene, limonene + β-phellandrene.

INTRODUCTION

The genus *Juniperus* L., belongs to the *Cupressaceae* family, comprising about 50 species of useful aromatic and medicinal plants, found from the northern hemisphere to the mountains of tropical Africa and West Indians (Mabberley, 1998). According to Amaral Franco (1986) two sections can be recognised for the Iberian Peninsula:

Section *sabina* Spach comprising *Juniperus phoenicea* L. subsp. *phoenicea*; *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman; *J. thurifera* L and *J. sabina* L .

Section *juniperus* L., comprising *Juniperus communis* L. subsp. *communis*; *J. communis* L. subsp. *hemisphaerica* (K. Presl) Nyman; *J. communis* L. subsp. *alpina* (Suter) Celak.; *J. oxycedrus* L. subsp. *oxycedrus*; *J. oxycedrus* L. subsp. *badia* (H. Gay) Debeaux; *J. oxycedrus* L. subsp. *macrocarpa* (Sm.) Ball and *J. navicularis* Gand. Recently ADAMS (2000) based on leaf essential oils and RAPDs (Random amplified polymorphic DNAs) stablished the following species status for the *Juniperus oxycedrus* complex:

J. oxycedrus L.; *J. badia* H. Gay; *J. macrocarpa* Sibth & Sm. and *J. navicularis* Gand. (= *J. oxycedrus* L. subsp. *transtagana* Franco).

This last taxon is a dwarf shrub inhabiting the maritime sands (dunes near the coast) and endemic to the Sado District (Sado River Estuary) of SW Portugal (Rivas-Martínez *et al*., 1990). In this work we have examined the leaf oil volatiles of *J. navicularis* by GC and GC/MS in combination with retention indices.

MATERIAL AND METHODS

Plant material

The leaves of *Juniperus navicularis* were gathered in Portugal, Alcacer do Sal, between Batallha and Murta, 15 Km from Comporta in the Sado river estuary. A voucher specimen10750 GL has been deposited at the Herbarium of the Real Jardín Botánico de Madrid, Spain. Plant material was identified by Prof. Dr. Ginés López González from the Real Jardín Botánico de Madrid, Spain.

Isolation of volatile constituents

Fresh leaves of *J. navicularis* were steam-distilled for 8 hours in an all glass Clevenger type apparatus and the plant material was suspended in a chamber above the boiling water The oils were dried over anhydrous sodium sulphate and stored at 4 ºC in the dark. The extracted leaves were oven dried 48 hours at 100 ºC for the determination of oil yields. The yield was 0,6% based on dried weight of sample.

Analyses

Analytical GC was carried out on a Varian 3300 gas chromatograph fitted with a Silicone DB-1 capillary column (50 m × 0,25 mm), film thickness 0,25 μm; carrier gas N_2, flow rate 1,5 mL/min, split mode, temperature programmed 60 C° - 240 °C at 3 °C/min. Injector temperature 250 °C, detector used FID, detector temperature 300 °C. Injection volume for all samples was 0,1 μL. GC/MS analyses were carried out on a Hewlett Packard 5890 gas chromatograph fitted with a phase bonded poly (5% diphenyl 95% dimethylsiloxane) silicone PTE5 capillary column (30 m × 0,25 mm.), film thickness 0,25 μm. Carrier gas He, flow rate 1,5 mL/min. Temperature program regimen was 70 °C, 2 min and then programmed to 250 °C at 2 °C/min. Injector temperature 250 °C. The chromatograph was coupled to a HP 5971 A mass selective detector (70 eV).

Component identification

Most constituents were identified by comparing their retention indices with those of authentic standards.The latter were either purchased, synthesized or identified in oils of known composition. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer data base using the NBS54K.L and WILEY.L built-in libraries and with those reported in the literature (LIBBEY, 1991; ADAMS, 1995; SWIGAR & SILVERSTEIN, 1981; JOULAIN & KÖNIG, 1998).

RESULTS AND DISCUSSION

The components of the oil, the percentage of each constituent and the retention indices are summarized in Table 1. The components are arranged in order to GC elution on the Silicone columns. The pinane hydrocarbons of the oils of *Juniperus navicularis* were dominated by α-pinene (30,8%) and lower amounts of β-pinene (3,3%). The total of pinane components was 34,1%. The major p-menthane constituents were found to be α-phellandrene (11,1%) and limonene + β-phellandrene (27,2%) and lower amounts of α-terpinene (0,5%), p-cymene (3,0%), γ-terpinene (0,5%%), terpinolene (3,4%), terpinen-4-ol (0,9%) and α-terpineol (1,1%). The total of p-menthane constituents was as follows: p-menthane hydrocarbons (42,7%), p-menthane aromatics (3,0%) and p-menthane alcohols (2,0%). The most important sesquiterpene components were shown to be γ-muurolene (0.1%), β-caryophyllene (0,1%), α-muurolene (0,2%), γ-cadinene (0,2%), δ-cadinene (0,6%), (*E*)-nerolidol (4,8%), 1-epi-cubenol (0,1%), T-muurolol + T-cadinol (0,7%), α-cadinol (0,8%) and epi-α-bisabolol (0,6%). Manoyl oxide was found as 0,1%.

According to bibliography the chemical pattern in the oils of *J. oxycedrus* was to produce pinane hydrocarbons as major components (ADAMS, 2000; VANTHOR-

PE *et al., 1973;* HOSTER, 1974 ADAMS *et al.,* 1999: ADAMS, 1998), in the oils of *J. badia* the main compounds were found to be pinane hydrocarbons, germacrene D and manoyl oxide (ADAMS *et al.,* 1999), in an oil of *J. macrocarpa* gathered in Spain (ADAMS, 2000; ADAMS, 1998; ADAMS *et al.,* 1999) pinane and sabinane hydrocarbons were present as principal constiuents and in the oil of this last species gathered in Greece (STASSI *et al*., 1995) the major components were shown to be α-pinene, cedrol and p-cymen-8-ol . Lastly in the oil of *J. navicularis* gathered in Lisbon, Portugal (ADAMS, 2000; ADAMS, 1998) the main constituents were found to be α-pinene (22,9%), sabinene (8.2%), myrcene (8,6%) α-phellandrene (8,0%) and limonene (14,3%). According to our results the leaf oil of *J. navicularis* was characterized by the production of pinane hydrocarbons and the p-menthanes limonene + β-phellandrene and α-phellandrene. Our results are very similar to those of Adams (ADAMS, 2000) and these have been included for comparison in Table 1.

Table 1
Essential oil composition (%) of *Juniperus navicularis* Gandoger

Component	*RI*	*%*	*Adams (2000)*
Tricyclene	908	t	0,1
α-thujene	913	0,3	2,1
α-pinene	926	**30,8**	**22,9**
1-octen-3-ol	930	t	—
α-fenchene	937	0,1	—
camphene	937	0,5	0,2
sabinene	953	0,5	8,2
β-pinene	963	**3,3**	**3,5**
myrcene	963	5,1	8,6
3-octanol	968	t	—
δ-2-carene	983	0,3	1,2
α-phellandrene	987	**11,1**	**8,0**
δ-3-carene	994	t	—
α-terpinene	1002	**0,5**	**0,9**
p-cymene	1010	**3,0**	**2,6**
limonene + β-phellandrene	1016	**27,2**	**17,8**
(Z)-β-ocimene	1010	t	—
(E)-β-ocimene	1017	0,5	0,4
γ-terpinene	1035	**0,5**	**1,6**
n-octanol	1048	t	—
terpinolene	1066	**3,4**	**2,9**
linalool	1074	0,5	0,2
endo-fenchol	1088	t	t
exo-fenchol	1092	t	—
(E)-sabinol	1105	t	—
camphor	1126	t	—

Table 1
Essential oil composition (%) of *Juniperus navicularis* Gandoger (Continuation)

Component	*RI*	*%*	*Adams (2000)*
camphene hydrate	1128	t	—
(E)-pinocamphone	1135	t	—
borneol	1142	0,2	—
(Z)-pinocamphone	1148	t	—
terpinen-4-ol	1152	**0,9**	**2,8**
p-cymen-8-ol	1158	t	t
α-terpineol	1163	**1,1**	**1,1**
myrtenal	1165	t	—
myrtenol	1171	t	—
thymyl-methyl-ether	1213	t	—
carvacryl-methyl-ether	1222	t	—
9-decen-1-ol	1230	t	—
2,4-decadien-1-ol	1233	t	—
thymol	1251	t	—
carvacrol	1282	t	—
α-cubebene	1331	t	—
hexadecanal	1332	t	—
α-copaene	1339	0,1	t
β-bourbonene	1361	t	—
β-elemene	1368	t	—
β-caryophyllene	1400	0,1	0,7
cadina-3,5-diene	1426	t	—
bicyclosesquiphellandrene	1428	t	—
α-humulene	1433	0,1	0,3
cis-muurola-4(14),5-diene	1438	t	—
cadina-1(6),4-diene	1442	t	—
γ-muurolene	1450	**0,1**	**0,1**
germacrene D	1451	t	0,2
β-selinene	1459	t	—
alpha-muurolene	1475	**0,2**	**0,3**
β-bisabolene	1482	t	—
γ-cadinene	1489	**0,2**	**0,6**
7-epi-α-selinene	1492	t	—
δ-cadinene	1497	**0,6**	**1,9**
trans-cadina-1,4-diene	1995	**t**	—
α-cadinene	1507	**t**	—
α-calacorene	1511	t	—
elemol	1513	t	—
germacrene B	1518	0,2	—
(E)-nerolidol	1528	**4,8**	**4,2**
β-oplopenone	1574	t	—
1,10-diepi-cubenol	1579	t	—
1-epi-cubenol	1598	**0,1**	—

Table 1
Essential oil composition (%) of *Juniperus navicularis* Gandoger (Continuation)

Component	*RI*	*%*	*Adams (2000)*
γ-eudesmol	1602	0,1	—
epi-α-muurolol (T-muurolol) + epi-α-cadinol (T-cadinol)	1610	**0,7**	1,5
α-muurolol (torreyol)	1610	**t**	**0,3**
α-eudesmol	1623	t	—
α-cadinol	1626	**0,8**	**2,2**
epi-α-bisabolol	1651	**0,6**	—
manoyl oxide	1965	**0,1**	0,1

t = traces (<0,1%).% in boldface = characteristic components. RI = Retention index on the DB1 column.

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