Essential oil analyses of the leaves and berries of *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux

Análisis del aceite esencial de las hojas y arcéstidas de Juniperus oxycedrus *L. subsp.* badia *(H. Gay) Debeaux*

Arturo Velasco-Negueruela*, María José Pérez-Alonso*, Jesús Palá-Paúl*, Ana Íñigo*, María Cervera*, Ginés López**

*Universidad Complutense de Madrid, Departamento de Biología Vegetal I
AVN44@bio.ucm.es
**Consejo Superior Investigaciones Científicas, Real Jardín Botánico de Madrid

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ABSTRACT

The steam distilled oil obtained from the leaves and berries of *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux, gathered in Embalse del Quiebrajano (Jaén) was analysed by GC and GC/MS. The oil from the berries was found to contain as major constituents α-pinene (59,8-61,5%) and myrcene (18,5-18,6%). Other characteristic compounds were germacrene D (3,6-1,8%), cadinanes (1,6-1,3%) , muurolanes (0,7-0,5%) and manoyl oxide (0,1%). The oil from the female leaves contained α-pinene (85.2-86.9%) as main constituent and other characteristic components were germacrene D (0,8-2,2%), manoyl oxide (0,1%), cadinanes (0,8-0,9%) and muurolanes (0,3-0,4%). The oil from the male leaves had as mayor compounds α-pinene (70,6-75,5%) and δ-3-carene (8,4-13,1%) and moderate amounts of β-phellandrene (6,7%) together with small quantities of germacrene D (0,8-0,4%), manoyl oxide (0,5-0,2%), cadinanes (0,4-0,4%) and muurolanes (0,1-0,1%).

KEY WORDS

Juniperus oxycedrus subsp. *badia*
Cupressaceae
Essential oil composition
α-pinene
β-phellandrene
Myrcene
δ-3-carene

RESUMEN

Los aceites esenciales obtenidos de las hojas y arcéstidas de *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux recolectadas en el Embalse del Quiebrajano (Jaén) fueron analizados mediante cromatografía de gases (CG) y cromatografía de gases acoplada a espectrometría de masas (CG/EM). El aceite procedente de las arcéstidas contenía como componentes mayoritarios α-pineno (59,8-61,5%) y mirceno (18,5-18,6%). Otros compuestos característicos fueron germacreno D (3,6-1,8%), cadinanos (1,6-1,3%) , muurolanos (0,7-0,5%) y óxido de manoilo (0,1%). El aceite procedente de las hojas femeninas presentaba α-pineno (85,2-86,9%) como mayoritario y otros componentes característicos como germacreno D (0,8-2,2%), óxido de manoilo (0,1%), cadinanos (0,8-0,9%) y muurolanos (0,3-0,4%). El aceite procedente de las hojas masculinas tenía como mayoritarios α-pineno (70,6-75,5%) y δ-3-careno (8,4-13,1%) y porcentajes medios de β-fellandreno (6,7%) junto con pequeñas cantidades de germacreno D (0,8-0,4%), óxido de manoilo (0,5-0,2%), cadinanos (0,4-0,4%) y muurolanos (0,1-0,1%).

PALABRAS CLAVE

Juniperus oxycedrus subsp. *badia*
Cupressaceae
Composición del aceite esencial
α-pineno
β-felandreno
Mirceno
δ-3-careno

1. Introduction

The genus *Juniperus* L., belongs to the *Cupressaceae* family, and comprises 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 Franco (1986) two sections can be recognised for the Iberian Peninsula: Section *Sabina* Spach comprising *J. phoenicea* L. subsp. *phoenicea*; *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman; *J. thurifera* L and *J. sabina* L. Section *Juniperus* L., comprising *J. 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) established 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).

The volatiles from the leaves and berries of *J. oxycedrus* subsp. *badia* have been the subject of several reports. Adams (2000) found in plants from Jaén (Spain), α-pinene (20.7%), germacrene D (8,5%) and manoyl oxide (10,9%) as major constituents of the leaf oils. Adams *et al.* (1999) analyzed the leaf oil of *J. oxycedrus* subsp. *badia* gathered in Jaén (Spain) and found α-pinene (5,1-28,1%), germacrene D (3,4-24,5%) and manoyl oxide (0,2-21,0%) as characteristic constituents. Salido *et al.* (2002) studied the essential oils from the leaves and berries of *J. oxycedrus* subsp. *badia* from Sierra de la Pandera, Jaén (Spain), and found α-pinene (40,0-57,0%) and manoyl oxide (5,0-10,0%) as major components of the leaf oil and α-pinene (65,0%) and germacrene D (39,5%) in the berry oil.

As a part of our work on the essential oils of the Iberian species of the *J. oxycedrus* complex (Velasco-Negueruela *et al*. 2002) we have studied the essential oils from the leaves and berries of *J. oxycedrus* subsp. *badia* by means of gas chromatography and gas chromatography-mass spectrometry.

2. Materials and methods

2.1. *Plant material*

The leaves and berries of *Juniperus oxycedrus* subsp. *badia* were gathered at Quiebrajano (Jaén province), 820 m, 30SVG3565, 15-2-2002. A voucher specimen has been deposited at the Herbarium MACB 83295.

2.2. *Isolation of volatile constituens*

Fresh leaves and ripe berries of *J. oxycedrus* subsp. *badia* 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 and berries were oven dried 48 hours at 100 ºC for the determination of oil yields. These were: Female plant berries (JB1A) 0,32%. Female leaves (JB1H) 0,15%, Female plant berries (JB2A) 0,6%. Female leaves (JB2H) 0,17%. Male leaves (JBL1V) 0,26%. Male leaves (JBL2V) 0,20% based on dried weight of samples.

2.3. *Analyses*

Analytical GC was carried out on a Varian 3300 gas chromatograph fitted with a Silicone DB-1 capillary column (50 m x 0,25 mm), film thickness 0,25 µm; carrier gas N_2, flow rate 1,5 mL/min, split mode, temperature programmed 60 ºC -240 ºC at 3 ºC/min. Injector temperature was 250 ºC. A FID detector at 300 ºC was used. 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 fused silica capillary column coated with crosslinked polymethylsiloxane as stationary phase (Agilent Technologies, 25m x 0,20mm, film thickness 0,33 µm. Carrier gas He, flow rate 1mL/min. Temperature was programmed from 70 ºC to 250 ºC at 4 ºC/min. rate. Samples were injected at 250 ºC, using a 1:20 split ratio. The chromatograph was coupled to a HP 5971 A mass selective detector. Spectra were recorded in the scan mode at 70 eV.

2.4. *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 and WILEY built-in libraries and with those reported in the literature (Libbey 1991, Adams 1995, Swigar & Silverstein 1981, Joulain & König 1998).

3. Results and discussion

The components of the oils, 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 oils from the male leaves (JBL1V, JBL2V) were characterized by high amounts of α-pinene (70,6-75,5%) and δ-3-carene (8,4-13,1%) and moderate amounts of -phellandrene (6,7%) and small quantities of germacrene D (0,8-0,4%) and manoyl oxide (0,5-0,2%). The total of monoterpene hydrocarbons was 94,3-95,9%, oxygenated monoterpenes (2,3-1,3%), sesquiterpene hydrocarbons (1,7-1,0%), oxigenated sesquiterpenes (0,7-1,1%), diterpenes (0,7-0,5%) and linear compounds (0,3-0,2%). The oils from the female leaves (JBL1H, JBL2H) were found to contain α-pinene (86,9-85,2%) as major constituent and small amounts of germacrene D (0,8-2,2%) and manoyl oxide (0,1%). The total of monoterpene hydrocarbons was 92,9-92,4%, oxygenated monoterpenes (2,3-1-2%), sesquiterpene hydrocarbons (2,6-4,4%), oxigenated sesquiterpenes (1,5-1,7%), diterpenes (0,3-0,1%) and linear components (0,4-0,2%). The oils from the berries (JB1A, JB2A) were characterized by high amounts of α-pinene (61,5-59,8%) and myrcene (18,6-18,5%) and lower quantities of germacrene D (3,6-1,8%) and manoyl oxide (0,3-0,1%). The total of monoterpene hydrocarbons was 87,9-87,7%, oxigenated monoterpenes (2,9-5,8%), sesquiterpene hydrocarbons (7,2-4,6%), oxigenated sesquiterpenes (1,6-1,6%), diterpenes (0,4-0,3%) and linear components (t). It is worth mentioning the absence of the four farnesol isomers in the oils from the berries and the presence of these components in the leaves of male and female plants although in very small amounts.

Our results are somewhat different to those of Adams (2000), Adams *et al.* (1999) and Salido *et al.* (2002) in that they found smaller amounts of α-pinene while those of germacrene D and manoyl oxide were major constituents. Comparing these results with those of Adams (1998, 2000), and Adams *et al* (1999) on the major components in the oils from the leaves of *Juniperus oxycedrus* subsp. *oxycedrus* from Spain they found α-pinene (41,3%) and moderate amounts of limonene (4,5%) and -phellandrene (8,2%). The oil from the berries of *J. oxycedrus* subsp. *oxycedrus* collected in Spain was found to contain (Guerra *et al.* 1987) α-pinene (60,6%) and myrcene (25,9%). Our results point out that, from their leaf and berry oil composition, *Juniperus oxycedrus* subsp. *badia* and *J. oxycedrus* subsp. *oxycedrus* are very close taxa. Therefore and taking into account some morphological features of *J. oxycedrus* subsp. *badia* as being large trees with wide leaves and larger berries, this taxon should be considered just as a variety of the *typus* species.

The sesquiterpene alcohols with M^+ 220 and m/z 159(100), RIs 1663, 1668 are probably oxygenated derivatives of calamenenes or calacorenes already found in the oil of *J. communis* (Gaston *et al.* 1988). These two components together with other unidentified sesquiterpene alcohols were considered by Adams *et al.* (1999) and Salido *et al.* (2002) as chemosystematic markers for *J. oxycedrus* subsp. *badia*, but it seems that these components are also present in other species of *Juniperus*.

Table 1. Essential oil composition (%) of the leaves and berries of *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux

Component	**RI**	**JBL1V**	**JBL2V**	**JBL1H**	**JBL2H**	**JB1A**	**JB2A**
n-hexanal	782	t	t	-	-	t	t
2-(E)-hexenal	836	t	t	0,2	t	t	t
tricyclene	908	t	t	-	-	-	-
α-thujene	913	0,2	0,1	0,2	0,2	t	t
α-pinene	926	**70,6**	**75.5**	**86,9**	**85,2**	**61,5**	**59,8**
1-octen-3-ol	930	0,3	0,2	0,3	0,2	t	t
α-fenchene	937	0,6	0,9	t	t	-	-
camphene	937	0,3	0,4	t	t	0,6	0,9
thuja-2,4(10)-diene	938	0,2	0,1	0,2	0,1	0,1	0,2
sabinene	953	0,2	0,2	0,2	0,1	0,1	t
β-pinene	963	**1,4**	**1,3**	**1,4**	**2,3**	**3,8**	**3,8**
myrcene	**963**	**2,9**	**2,1**	**1,5**	**2,4**	**18,6**	**18,5**
α-phellandrene	987	1,2	0,1	0,3	0,1	-	-
δ-3-carene	**994**	**8,4**	**13,1**	**0,5**	**0,1**	-	-
α-terpinene	1002	t	t	0,3	0,1	t	0,2
p-cymene	1010	0,6	t	0,2	t	0,1	t
limonene	**1016**	**t**	**1,2**	**2,2**	**1,3**	**2,4**	**2,8**
β-phellandrene	**1016**	**6,7**	**t**	**0,2**	**0,1**	**t**	**t**
γ-terpinene	1035	0,1	0,1	0,2	0,1	0,1	0,2
m-cymenene	1056	t	t	t	t	t	t
terpinolene	1066	0,9	0,8	0,5	0,3	0,6	1,3
linalool	1074	0,1	t	-	-	t	0,1
campholenal	1103	0,2	0,3	0,3	0,2	0,1	0,2
trans-pinocarveol	1117	0,2	0,2	0,4	0,2	1,1	1,7
cis-verbenol	1119	t	0,1	0,1	t	0,3	0,8
trans-verbenol	1122	t	t	0,1	t	-	-
camphene hydrate	1128	t	t	t	t	-	-
p-mentha-1(7),2-dien-8-ol	1140	0,1	0,1	0,4	0,3	0,2	0,2
borneol	1142	0,1	0,1	0,1	0,1	0,2	0,4
p-mentha-1,5-dien-8-ol l	1146	0,3	0,2	0,1	0,1	0,3	0,2
(Z)-pinocamphone	1148	-	-	-	-	t	t
terpinen-4-ol	1152	t	t	t	t	t	t
p-cymen-8-ol	1158	t	t	t	t	t	t
α-terpineol	**1163**	**0,6**	**0,2**	**0,3**	**0,3**	**0,5**	**1,5**
myrtenal	1165	t	t	t	t	0,1	0,1
myrtenol	1171	t	t	t	t	0,1	0,2
verbenone	1179	t	t	0,1	t	t	t
trans-carveol	1197	t	t	t	t	t	t
thymyl-methyl-ether	1213	t	t	t	t	t	t
bornyl acetate	1265	0,2	0,1	0,3	t	t	0,4
4-terpenyl acetate	1320	t	t	t	t	t	t
α-terpenyl acetate	1328	0,5	t	0,1	t	t	t
α-cubebene	1331	t	t	-	-	0.1	0,3
α-ylangene	1352	-	-	t	t	t	t
α-copaene	1358	t	t	t	t	0,2	0,1

Table 1. Essential oil composition (%) of the leaves and berries of *Juniperus oxycedrus* L. subsp. badia (H. Gay) Debeaux

Component	RI	JBL1V	JBL2V	JBL1H	JBL2H	JB1A	JB2A
β-bourbonene	1361	0,2	0,1	0,1	0,2	t	t
β-caryophyllene	**1400**	**0,2**	**0,1**	**0,3**	**0,4**	**0,5**	**0,3**
β-copaene	1408	t	t	t	0,1	0,1	t
trans-α-bergamotene	1416	-	-	t	t	0,2	0,1
cis-β-farnesene	1423	-	-	t	t	t	t
α-humulene	**1433**	**0,1**	**0,1**	**0,4**	**0,3**	**0,4**	**0,4**
cis-muurola-4(14),5-diene	**1438**	**t**	**t**	**t**	**t**	**t**	**t**
γ-muurolene	**1450**	**0,1**	**0,1**	**0,2**	**0,3**	**0,5**	**0,3**
germacrene D	**1455**	**0,8**	**0,4**	**0,8**	**2,2**	**3,6**	**1,8**
α-muurolene	**1475**	**t**	**t**	**0,1**	**0,1**	**0,2**	**0,2**
viridiflorene	1468	t	t	t	0,1	0,2	0,1
γ-cadinene	**1489**	**0,1**	**0,1**	**0,4**	**0,3**	**0,5**	**0,6**
δ-cadinene	**1497**	**0,2**	**0,1**	**0,3**	**0,4**	**0,7**	**0,4**
α-calacorene	1511	-	-	t	t	t	t
elemol	1513	-	-	t	t	-	-
germacrene B	1536	-	-	t	t	t	t
β-calacorene	1543	-	-	-	t	t	t
n.i $C_{15}H_{24}O$	1545	0,1	0,1	0,1	0,2	0,3	0,2
caryophyllene oxide	1556	0,1	0,1	0,2	0,3	0,4	0,3
n.i $C_{15}H_{24}O$	1580	t	t	t	0,1	0,1	0,1
n.i $C_{15}H_{24}O$	1600	0,1	0,1	0,1	0,1	0,2	0,2
n.i $C_{15}H_{24}O$	1605	0,1	0,2	0,2	0,2	0,1	0,1
epi-α-muurolol (T-muurolol)	1610	t	t	t	t	t	t
α-cadinol	1626	**0,1**	**0,2**	**0,1**	**0,2**	**0,4**	**0,3**
n.i $C_{15}H_{24}O$	1640	0,1	0,1	t	t	-	-
n.i $C_{15}H_{28}O$	1657	0,1	0,1	0,2	0,2	0,4	0,3
n.i $C_{15}H_{24}O$	1660	-	-	t	-	-	-
n.i $C_{15}H_{24}O$	1663	-	-	-	-	t	t
n.i $C_{15}H_{24}O$	1668	t	t	t	t	0,2	0,1
ZE-farnesol	1672	t	t	t	0,1	-	-
ZZ-farnesol	1688	t	t	t	t	-	-
EE-farnesol	1697	t	t	t	0,1	-	-
EZ-farnesol	1717	t	0,2	0,1	0,2	-	-
sandaraco-8(14),16-pimaradiene	1940	-	-	0,1	-	0,1	0,1
manoyl oxide	**1965**	**0,5**	**0,2**	**0,1**	**0,1**	**0,3**	**0,1**
abieta-7,13-diene	1980	t	t	t	t	t	t
abieta-8,12-diene	1988	t	t	t	t	t	t
abietatriene	2029	t	0,1	0.1	t	0,1	0,1
abieta-8(14),12-diene	2055	0,2	0,2	t	t	t	t
Total monoterpene hydrocarbons		**94,3**	**95,9**	**92,9**	**92,4**	**67,9**	**87,7**
Total oxygenated monoterpenes		**2,3**	**1,3**	**2,3**	**1,2**	**2,9**	**5,8**
Total sesquiterpene hydrocarbons		**1,7**	**1,0**	**2,6**	**4,4**	**7,2**	**4,6**
Total oxygenated sesquiterpenes		**0,7**	**1,1**	**1,5**	**1,7**	**1,6**	**1,6**
Total diterpenes		**0,7**	**0,5**	**0,3**	**0,1**	**0,4**	**0,3**
Total linear compounds		**0,3**	**0,2**	**0,4**	**0,2**	**T**	**t**

t = traces (<0.1%).%; in boldface = characteristic components. RI = Retention index on the DB1 column.

Mass spectra of unidentified constituents:

RI 1545 (m/z rel.int.) 220[M+](35), 91(100), 123(95), 41(90). 55(82), 159(52), 187(17), 205)15).

RI 1580, 220[M+](14), 123(100), 91(67), 43(52), 55(34), 149(10), 177(8), 187(5), 205(3).

RI 1600, 220[M+](5), 43(100), 67(80), 93(65), 109(55), 159(43), 164(10), 202(8).

RI 1605, 220[M+](3), 69(100), 41(50), 123(22), 81(15), 179(15), 202(4).

RI 1640, 220[M+](55), 159(100), 43(95), 91(90). 67(50), 121(45), 177(52), 202(8).

RI 1657, 224[M+](5), 43(100), 54(52), 82(47), 125(15), 139(10), 138(8), 206(5)

RI 1660, 220[M+](10), 123(100), 41(82), 55(55), 81(50), 91(50), 159(65), 191(22), 205(5)

RI 1663, 220[M+](50), 159 (100), 91(80), 1787(70), 79(50), 19(50), 205(15).

RI 1668, 220[M+](90), 159(100), 79(60), 93(62), 43(55), 131(37), 177(25), 205(8)

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