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Germacrene-D, a characteristic component of the Essential Oils from the Leaves of *Juniperus oxycedrus* ssp. *macrocarpa* (S. et Sm.) BalI Growing in El Kala, Algeria

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ABSTRACT

The present study evaluates the chemical composition of the essential oils of Juniperus oxycedrus ssp. macrocarpa (Cupressaceae) from El kala region (sub-humid) in Algeria. Leaves essential oils were analyzed by GC/MS. Fifty four compounds were identified. The J. oxycedrus leaves oil was characterized by a high content of Germacrene D (21%), 1,5-Dodecadiene (8,42 %), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)- (10,94 %). and 1H-Naphtho[2,1-b]pyran-3-acetic acid, dodecahydro-3,4a,7,7,10a-pentamethyl-, methyl ester (8,77 %).

Keywords*: Juniperus oxycedrus*; Essential oil; Chemical composition; GC/MS.

INTRODUCTION

Essential oils are important natural products used for their flavor and fragrances in food, pharmaceutical and perfumery industries. They are also sources of aroma chemicals. Essential oils have a complex composition, containing from a dozen to several hundred components. The great majority of components identified in essential oils include terpenes and propenylphenols which are also important components of some essential oils [1].

Among many others, well-known families rich in essential oil bearing species are Apiaceae, Asteraceae, Hypericaceae, Lamiaceae, Lauraceae, Myrtaceae, Pinaceae, Piperaceae, Rutaceae, Santalaceae, Zingiberaceae, Zygophyllaceae and Cupressaceae [2–6].

The genus *Juniperus* (Cupressaceae) consists of approximately 67 species and 28 varieties. The genus is divided into three sections: *Caryocedrus* Edlicher (with only one species); *Juniperus* (syn: *Oxycedrus* Spach with 12 species) and *Sabina* (Miller) Spach (with 55 species) [7].

The genus *Juniperus* (Cupressaceae) is represented in the flora of Algeria by five species, namely : *J. Oxycedrus L.,J. Sabina L., J. thurifera L., J. phoenica L. and J. communis L.,*

[8]. *Juniperus oxycedrus* is a shrub or small tree growing wild in stony places of the Mediterranean and Near East countries [9].

In folk medicine *J. oxycedrus* was used for the treatment of various diseases, such as hyperglycaemia, obesity, tuberculosis, bronchitis and pneumonia [10]. Leaves and stems of *J. oxycedrus ssp. macrocarpa* have been found to

reduce the blood pressure of normotensive rats, to inhibit the response to histamine, serotonin and acetylcholine, and to exhibit significant anti-inflammatory activity [11].

MATERIALS AND METHODS

Plant material

The leaves of *J. oxycedrus* L. were collected in last Mai 2011 (fructification stage) in El Kala (sub humid area), Algeria. The plant was identified by Dr. Haou sihem , department of life sciences and nature, University Larbi Ben M'hidi, Oum el Bouaghi Algeria. A voucher specimen was deposited at the life sciences and nature Department, University Larbi Ben M'hidi, Oum el Bouaghi, Algeria under the code number ZA 130.

Extraction

Essential oils were obtained by hydrodistillation of 100g of dried flowers using a Clevenger-type apparatus for 3 h. diethyl ether (10 ml) was used as the collector solvent as reported in literature. After evaporation of the solvent, the oil was dried over anhydrous sodium sulfate and stored in sealed vials protected from the light at −20 °C before analyses. The oil sample was subsequently analyzed by GC-MS .

Identification of components

Gas chromatography/mass spectrometry (GC/MS)

The oil was analyzed by GC/MS using a Agilent 5973EI mass selective detector coupled with an Agilent GC6890A gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30m x 0.32mm, épaisseur de film 0.25µm). Operating conditions: The carrier gas flow was 1.6 ml He/min, column pressure was 100 Kpa. The injector and detector temperatures were 220°C and 250°C respectively. The column temperature was held at 60°C for 1 min, then raised from 60°C to 200°C at 10°C/min and held there for 5 min and from 200°C to 240°C at 10°C /min and held there for 6 min. The program was run in the splitless mode with a mass range of 50–400 u, and the scan interval was 0.5 s. Detector voltage was set at 1.5 kV.

Identification of components

Identification of oil components was achieved on the basis of their retention indices RI, (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library (NIST database). The concentration of the identified compounds was computed from the GC peak total area without any correction factor.

RESULTS AND DISCUSSION

The hydrodistillation of the Leaves of *J. oxycedrus* L. yielded 0.02 % of yellowish oil and possessed a strong odor. The composition and percentage of the compounds are summarized in Table 1. They are listed by their order of retention times. Fifty two compounds were identified in the essential oils, representing **85.7%** of the total oil. The main constituents of the essential oil were Germacrene D (21.3 %), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)- (10.9 %), 14,15-Dinorlabdane, 8,13-epoxy- (8.8 %), 5-Dodecadiene (8.4%) and some other compounds were only present in minor amounts. The essential oils were dominated by a large amount of sesquiterpenes (29.1 %) and oxygenated sesquiterpenes (28,2), while the hydrocarbon monoterpenes are 15.1 %.

In total, essential oil composition of *J.oxycedrus* L. was considered as a rich source of sesquiterpenes. The composition of leaf essential oils of section *Juniperus* is generally much simpler and dominated by simple monoterpenes, in contrast to the essential oils of section *Sabina*, where oxygenated monoterpenes (e.g. camphor) and sesquiterpenes (e.g. cadinols, cedrol) are the major constituents (Adams, 1991b) [12,13]

In a previous study the essential oil of the section *Juniperus* is dominated by α-pinene (41.3%), with moderate amounts of α-phellandrene, p-cymene, β-phellandrene, limonene, myrcene, α-terpineol, (E)- nerolidol and manoyl oxide (Table 1). In contrast, *J. oxycedrus* subsp. *macrocarpa* has large amounts of sabinene (26.5%) and α-pinene (22.6%). The oil of *J. oxycedrus* subsp. *badia* has a large amount of α -pinene (20.7%), very little sabinene (0.1%) and considerable amount of manoyl oxide (10.9%) along with several, apparently unique, unknown sesquiterpenes (see Adams et al., 1999, for discussion) [14].

Chatzopoulou and Katsiotis (1993) reported that the leaf essential oil of *J. communis* from northern Greece was dominated by α-pinene (41.3%) and sabinene (17.4%), whereas Caramiello et al. (1995) listed sabinene as the dominant component (41.4%) with 13.4% α-pinene, but The oils of *J. taxifolia* and *J. taxifolia* var. *lutchuensis* were dominated by α-pinene (47.5, 46.6%) with moderate amount of myrcene, and b-pinene[15,16].

Table 1 : Percentage composition of the leaves essential oils of the *J. oxycedrus*

Peak	Chemical constituents	Rt	%
1	3-Cyclohexene-1-methanamine, à,à,4-trimethyl-	20.490	1.1
2	α-Copaene	22.374	0.1
3	Cyclohexasiloxane, dodecamethyl-	22.481	0.1
4	Cyclobuta[1,2:3,4]dicyclooctene, hexadecahydro-, cis,trans-	22.673	0.3
5	α-Cubebene	23.127	0.3
6	Elixene	23.261	0.2
7	(-)-Isoledene	23.654	0.1
8	Caryophyllene	24.454	1.2
9	α-Caryophyllene	26.289	1.8
10	+)-Epi-bicyclosesquiphellandrene	26.801	0.1
11	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	27.449	0.1
12	Germacrene D	27.963	**21.3**
13	4a-Methyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	29.727	1.4
14	Andrographolide	30.161	0.4
15	ë-Cadinene	30.464	3.4
16	1-Heptatriacotanol	30.676	0.2
17	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	30.773	0.1
18	α-Calacorene	30.880	0.1
19	ç-Eudesmol	31.669	1.6
20	Elixene	31.811	0.2
21	Caryophyllene oxide	32.829	0.7
22	Nerolidol	33.054	0.2
23	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	33.307	0.5
24	6-[(2Z)-2-Butenyl]-1,5,5-trimethyl-1-cyclohexene	33.476	0.9
25	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	34.047	1.5
26	Caryophyllene oxide	34.496	1.1
27	Andrographolide	35.160	1.7
28	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	37.876	2.3
29	α-Cadinol	38.209	1.0
30	Methyl (Z)-5,11,14,17-eicosatetraenoate	39.358	2.2
31	1,5-Dodecadiene	40.318	**8.4**
32	2,3-Dihydrofarnesol	41.502	0.3
33	2-Pentadecanone	42.148	0.3
34	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	43.128	**10.9**
35	Heptadecane	43.439	0.9
36	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	45.268	0.9
37	Heptadecane	47.984	0.1
38	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (E,E)-	48.454	0.5
39	Androst-2,16-diene	49.999	0.2
40	Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl-	50.491	0.1
41	Naphthalane	52.206	1.7
42	14,15-Dinorlabdane, 8,13-epoxy-	53.154	**8.8**
43	Pentadecanoic acid	53.616	0.7
44	Isopimaradien	54.020	0.1
45	1-Naphthalenepropionic acid	54.134	0.2
46	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	55.067	0.8
47	Eicosane	55.326	0.1
48	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	56.267	0.1
49	1-Naphthalenepropionic acid	56.797	0.1
50	1-Naphthalenepropionic acid	58.365	0.9
51	Thunbergen = Cembrene	59.108	2.4
52	α-Naphthal	60.853	0.2
Total			**85.7%**

Akimov et al. (1976) reported that the leaf oil of *J. oblonga* was dominated by sabinene (26.9%) and α-pinene (16.1%) with moderate amounts of sabinol (7.8%), myrcene (7.3%), c-terpinene (6.1%) and limonene (5.7%)[17].
There are some reports on the monoterpenes of the leaves [18]. The most complete report on the leaf oil was of *J. oxycedrus* subsp. *macrocarpa*[19] from the island of Elaphonissos (S. Greece). The leaf oil was dominated by α-pinene (26.94%) and cedrol (13.88%) with moderate amounts of dihydro-p-cymen-8-ol (8.49%), α-terpineol (6.6%) and d-cadinene (4.55%). Akimov et al. (1976) [20] reported that *J. oxycedrus* from the Crimea contained 10.2% limonene, 7.8% α-pinene and minor amounts of other terpenes, in contrast to the essential oils of *J. oxycedrus* subsp.

macrocarpa growing in Algeria which is characterized by a dominance of Germacrene D (**21.3**%), but in other species from different origin presented by small amounts such as *J. brevifolia* (Islands)0.2% , , *J. communis* (Sweden)0.7%, *J. sibirica* (Mongolia)0.3% , *J. oblonga* (Starvopol Province, Russia)1.2% , *J. oxycedrus* (Spain) 1.0% , *J. navicularis* (Portugal)0.2%, *J. formosana* (China) *2.3*%, *J. rigida 2.3*% (China) , but 00 % in two species *J. conferta* (origin Japan), *J. cedrus* (Islands) [21], except in *J. oxycedrus ssp. Badia* berries collected in september from Spain, the percentage of Germacrene D varried from 4.2% % (green unripe fruits) September collection to 39.5% berry (red-brown mature fruits) while September collection of the J. *oxycedrus ssp. Badia* varied from 0.4 to 2.6 % [22].

Table 2: main classes of essential oils components of *J. oxycedrus*

classes	percentage
ketones	0.3
esters	2.7
Sesquiterpenes hydrocarbons	29.1
hydrocarbons	14.0
Monoterpene Hydrocarbons	15.1
Oxygenated Monoterpenes	1.6
Aldehydes	0.2
Oxygenated Sesquiterpenes	28.2
diterpene Hydrocarbons	3.3
Oxygenatedditerpene	2.1
acids	1.9
others	1.5

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