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ORIGINAL ARTICLE

Composition of the Essential Oils of *Juniperus oxycedrus L.* subsp. *oxycedrus* Growing in Turkey

Türkiye'de Yetişen *Juniperus oxycedrus* L. subsp. *oxycedrus*'un Uçucu Yağ Bileşikleri

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ABSTRACT

In this study, the chemical compositions of the leaves, berries and twigs essential oils of *J. oxycedrus* L. subsp. *oxycedrus*, collected in Turkey, were determined. The oils were analyzed by GC and GC-MS. 15-21 volatile compounds were identified of the leaves, berries and twigs essential oils representing 82.4-98.0% of the total oils. The essential oils were obtained from leaves, berries and twigs by yielding 0.02%, 2.12% and 0.01%, resp. The major compounds were determined manoyl oxide (32.8%) and caryophyllene oxide (11.9%) in leaf oil, myrcene (44.6%), α-pinene (19.9%) and germacrene D (15.5%) in berry oil, manoyl oxide (35.4%) and caryophyllene oxide (16.8%) in twig oil.

Key words: *Juniperus oxycedrus*, GC and GC/MS, Essential oil

ÖZ

Bu çalışmada, Türkiye'den toplanan *J. oxycedrus* subsp. *oxycedrus* türünün yaprak, meyve ve ince dallarından elde edilen uçucu yağlarının kimyasal içerikleri belirlenmiştir. Uçucu yağlar GC ve GC/MS cihazları aracılığıyla analiz edilip, toplam yağın %82.4-98.0 içeriği 15-21 arasında değişen uçucu bileşen ile yaprak, meyve ve ince dal üzerinde tespit edilmiştir. Uçucu yağlar yaprak, meyve ve ince daldan sırasıyla %0.02, %2.12 ve %0.01 verimleri ile elde edilmiştir. Ana bileşenler yaprak yağında %32.8 manoil oksit ve %11.9 karyofillen oksit, meyve yağında %44.6 mirsen, %19.9 α-pinen ve %15.5 germakren D, ince dal yağında %35.4 manoil oksit ve %16.8 karyofillen oksit belirlenmiştir.

Anahtar kelimeler: *Juniperus oxycedrus*, GC and GC/MS, Uçucu Yağ

INTRODUCTION

The genus *Juniperus* L. belongs to the Cupressaceae family, representing about 70 species all over the world, and widely distributed throughout the forests of the temperate and cold regions of the northern Hemisphere, from the far north to the Mediterranean. *Juniperus* L. is represented in Turkey by 7 species and 14 taxa. *Juniperus oxycedrus* has two subspecies – subsp. *oxycedrus* and subsp. *macrocarpa* – in Turkey (1,2).

Several studies have reported the chemical composition of leaves, berries and twigs and their the essential oils (EOs) of *J. oxycedrus* L. subsp. *oxycedrus* (Table 1) (3-16).

In the present work, leaves, berries and twigs EOs of *J. oxycedrus* subsp. *oxycedrus* were investigated to chemical compositions of plants collected from Eskişehir: Hekimdağ in Turkey. In the study, the oils were obtained by hydrodistillation. The oils were analyzed by gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS).

EXPERIMENTAL

Plant material

J. oxycedrus subsp. *oxycedrus* was collected from Eskişehir: Hekimdağ, in Turkey on 15 March 2015. Voucher specimens are kept at the Herbarium of Pharmacy Faculty, Anadolu University, Turkey (ESSE 14987).

Isolation of essential oils

The oils were obtained by hydrodistillation for 3 hours using Clevenger apparatus. The essential oils were stored at 4°C in the dark until analyzed. The oils were analyzed by capillary GC and GC/MS using a Agillent GC-MSD system.

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Table 1. EO yield and chemical composition of *Juniperus oxycedrus* L. subsp. *oxycedrus* (3-16)

Part of plant	Yield %	Major compounds %	Ref.
Leaf	0.7 HD	α-Pinene (42.9%) Limonene (17.8%) Caryophyllene oxide (5.1%) β-Myrcene (3.9%) β-Pinene (3.8%)	3
Leaf	0.01 HD	α-Pinene (17.1%) 13-*epi*-Manoyl oxide (12.5%) (*Z*)-6-pentadecen-2-one (11.5%)	4
Leaf	0.1 HD	*trans*-Pinocarveol (7.0%) *cis*-Verbenol (6.3%) Manoyl oxide (6.0%)	5
Leaf	1.66 HD	α-Pinene (31.25%) Sabinene (5.21%) Limonene (5.02%)	6
Leaf	0.2 HD	Limonene (30.0%) α-Pinene (26.3%) (*Z, E*)-Farnesol (5.1%) Salvial-4 (14)-en-1-one (4.9%)	7
Leaf	0.4±0.14 HD	α-Pinene (49.46%) Germacrene D (8.96%) 13-*epi*-Manoyl oxide (3.62%)	8
Leaf	0.2-0.5 HD	α-Pinene (41.3%) α-Phellandrene (8.2%) *p*-Cymene (6.2%) Manoyl oxide (5.3%)	9
Berry	- SPME	α-Pinene (88.44%) β-Myrcene (6.71%) β-Pinene (2.07%)	10
Berry	0.97 HD	α-Myrcene (23.4%) α-Pinene (16.7%) Citronellol (16.3%) β-Caryophyllene (6.3%)	11
Berry	2.21 HD	Citronellol (26.8%) α-Myrcene (24.3%) α-Pinene (14.4%) Limonene (9.3%)	11
Berry	0.7-1.2 HD	β-Myrcene (56.87±4.6%) α-Pinene (14.84±2.9%) DL-Limonene (5.96±0.6%)	12
Berry	0.4-0.7 HD	β-Myrcene (54.06±6.1%) α-Pinene (10.22±2.7%) DL-Limonene (9.20±1.7%) Germacrene D (8.56±1.7%) (*E*)-Nerolidol (5.94±1.6%)	12
Berry	0.8-1.5 HD	β-Myrcene (49.75±3.8%) α-Pinene (16.50±2.3%) DL-limonene (13.82±2.7%)	12
Berry	0.9-1.5 HD	β-Myrcene (56.97±3.7%) α-Pinene (19.55±3.7%) α-Cadinol (3.92±1.6%) Germacrene D (3.74±0.2%)	12

Table 1 continued

Berry	1.2-1.8 HD	β-Myrcene (45.50±3.0%) α-Pinene (36.64±2.0%) DL-Limonene (5.75±2.1%) Germacrene D (3.65±0.6%)	12
Berry	0.72 HD	α-Pinene (27.4%) β-Myrcene (18.9%) α-Phellandrene (7.1%) Limonene (6.7%)	13
Berry	2.5 HD	α-Pinene (27.4%) β-Myrcene (18.9%)	14
Berry	0.5 HD	α-Pinene (60.60±3.33%) β-Myrcene (24.97±1.76%) γ-Muurolene (5.19%).	15
Berry	0.45 SFE	Germacrene D (13.8%) α-Pinene (10.5%) β-Myrcene (8.1%)	16
Needles	- HD	α-Pinene (92.22%) β-Myrcene (2.46%) β-Pinene (1.79%)	10
Wood	0.68 HD	δ-Cadinene (14.5%) *cis*-Thujopsene (9.2%) α-Muurolene (4.9%) Cadalene (3.7%) Eremophilene (2.5%) α-Cedrol (2.2%)	13

HD: Hidrodistillation, SFE: Supercritical carbon dioxide extraction, SPME: Solid Phase Micro Extraction

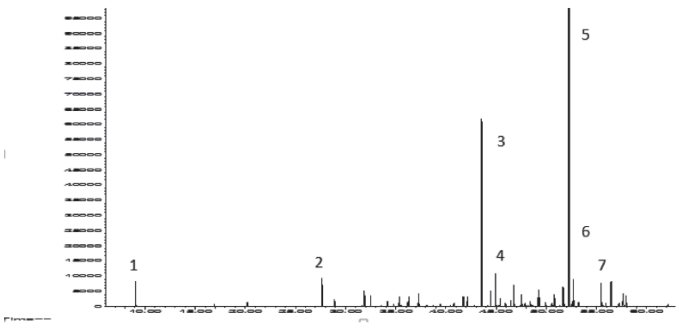

Figure 1. Chromatogram of the twigs essential oil of J. oxycedrus L. subsp. oxycedrus
1. α- Pinene, 2. α- Cubebene, 3. Caryophyllene oxide, 4. Humulene epoxide-II, 5. Manoyl oxide, 6. Caryophyllenol II, 7. Dodecanoic acid

Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry (GC/MS) analysis

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60˚C for 10 minutes and programmed to 220˚C at a rate of 4˚C/min, and

kept constant at 220˚C for 10 minutes and then programmed to 240˚C at a rate of 1˚C/minutes. Split ratio was adjusted 40:1. The injector temperature was at 250˚C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

Table 2. Composition of the EOs of *Juniperus oxycedrus* L. subsp. *oxycedrus*

RRI	Compounds	Twigs %	Berries %	Leaves %
1032	α-Pinene	2.4	19.9	1.4
1132	Sabinene	-	1.4	-
1174	Myrcene	-	44.6	-
1190	Sylvestrene	0.7	-	-
1203	Limonene	-	2.7	-
1213	1,8-Cineole	-	-	-
1218	β -Phellandrene	-	0.7	-
1280	*p*-Cymene	0.5	-	-
1290	Terpinolene	-	0.5	-
1466	α-Cubebene	2.0	0.3	-
1604	Isobornyl acetate	1.6	0.3	1.2
1612	β-Caryophyllene	1.5	4.6	3.7
1668	(*Z*)-β-Farnesene	-	0.5	-
1687	α-Humulene	-	3.1	-
1704	*g*-Muurolene	-	0.3	-
1706	α-Terpineol	1.9	-	-
1726	Germacrene D	-	15.5	5.7
1740	α-Muurolene	1.3	0.7	3.2
1773	*d*-Cadinene	1.4	1.8	4.1
1776	*g*-Cadinene	-	-	1.6
1941	α-Calacorene	0.7	-	1.8
2008	Caryophyllene oxide	16.8	-	11.9
2050	(*E*)-Nerolidol	1.4	-	-
2071	Humulene epoxide-II	3.4	-	4.7
2148	Cedrol	2.9	-	-
2243	Torilenol	1.2	-	2.5
2255	α-Cadinol	-	1.1	-
2256	Cadalene	2.8	-	2.2
2316	Caryophylladienol I	1.8	-	-
2376	Manoyl oxide	35.4	-	32.8
2392	Caryophyllenol II (=*Caryophylla-2(12),6-dien-5b-ol*)	4.5	-	-
2503	Dodecanoic acid *(=lauric acid)*	4.2	-	3.1
2524	Abietatriene	2.5	-	2.5

RRI: Relative Retention Indices calculated against n-alkanes

The GC analysis was carried out using an Agilent 6890 N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions. FID temperature was 300˚C.

Identification of compounds

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

RESULTS AND DISCUSSION

The EOs were obtained from leaves, berries and twigs by yielding 0.02%, 2.12% and 0.01%, respectively. The list of compounds identified in the hydrodistilled leaves, berries and twigs of *J. oxycedrus* L. subsp. *oxycedrus* with their relative percentages and retention indices are given in Table 2.

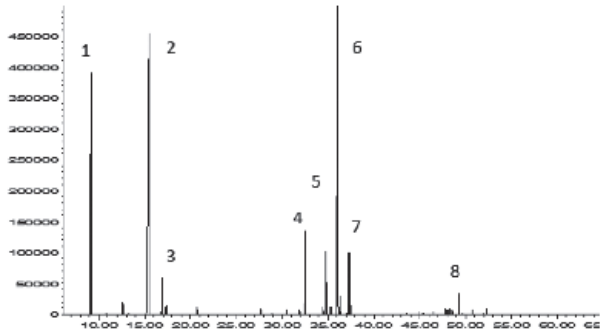

Figure 2. Chromatogram of the berries essential oil of J. oxycedrus L. subsp. oxycedrus

1. α- Pinene, 2. Myrcene, 3. Limonene, 4. β- Caryophyllene, 5. α- Humulene, 6. Germacrene D, 7. d- Cadinene, 8. α- Cadinol

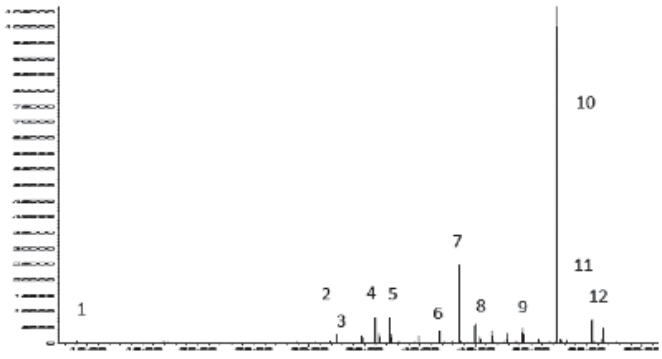

Figure 3. Chromatogram of the leaves essential oil of J. oxycedrus L. subsp. oxycedrus

1. α- Pinene, 2. Bornyl acetate, 3. β- Caryophyllene, 4. Germacrene D, 5. d-Cadinene, 6. α- Calacorene, 7. Caryophyllene oxide, 8. Humulene epoxide-II , 9. Cadalene, 10. Manoyl oxide, 11. Dodecanoic acid, 12. Abietatriene

In our study, 15-21 volatile compounds were identified of the leaves, berries and twigs EOs representing 82.4-98.0% of the total oils. Twig oil composition was not found in previous studies. Manoyl oxide (35.4%) and caryophyllene oxide (16.8%) were identified as major contituents in twig oil (Figure 1), myrcene (44.6%), α-pinene (19.9%) and germacrene D (15.5%) in berry oil (Figure 2), manoyl oxide (32.8%) and caryophyllene oxide (11.9%) in leaf oil (Figure 3).

As seen in previous studies Table 1, the leaf oils were characterized by the presence of α-pinene, t-pinocarveol and limonene as main contituents (3-10). But, in our study, the occurrence of manoyl oxide and caryophyllene oxide was interesting. While some contituents like α-pinene and myrcene were found in berry oil samples (11-15), germacrene D was only in one sample (16).

Medini *et al.* have reported α-pinene, germacrene D, myrcene, abietadiene and *cis*-calamenene as main constituents of the EOs of the berries of *Juniperus oxycedrus* L. subsp. *rufescens* (L.K.) and *Juniperus oxycedrus* L. subsp. *macrocarpa* (S. & M.) Ball. (17).

Sezik et al. have reported manoyl oxide (21.9%) and α-pinene (11.3%) as main constituents in leaf oil of *J. oxycedrus* subsp. *macrocarpa* from Eskişehir (18).

Variability of the oil composition in different populations of the same plant species might be attributed mainly to genetic diversity (19). Chemical composition and the main components of EOs *J. oxycedrus* have differentiate chemotype due to containing different climatic conditions of a large geographical diversity.

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