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Original Article

Essential wood oil of *Cupressus sempervirens* varieties (*horizontalis* and *pyramidalis*)

Cupressus sempervirens (Servi) varyetelerinin (horizantalis ve pyramidalis) odunlarındaki uçucu bileşikler

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ABSTRACT

The essential oils isolated from the woods of *Cupressus sempervirens* var. *horizantalis* and var. *pyramidalis* harvested from natural and cultivated locations in Turkey were characterized by GC-MS analyses. Fifty-one compounds, representing 91.9-95.7% of the oil composition, were identified. The oils obtained from two varieties wood exhibited quite similar composition. Sapwood and heartwood oils were mainly composed of oxygenated monoterpenes (43.7-72.4%), sesquiterpenoids (8.7-36.5%) and diterpenoids (2.2-10.4%). The major compounds were carvacrol methyl ether (38.2-62.6%), α-cedrol(15.7-34.3%), manool (1.75-9.83%), terpinen-4-ol acetate (0.82-4.15%) and bornylacetate (0.26-4.62%). Compared to other Cupressus species, *Cupressus sempervirens* wood can be classified as a carvacrol methyl ether rich species. As a result, the wood oil of Cupressus species can be characterized by the presence of two compounds: α-cedrol and carvacrol or carvacrol methyl ether.

Keywords: Cedrol, carvacrol methyl ether, *Cupressus sempervirens* varieties, monoterpenes, wood essential oil

ÖZ

Türkiye de doğal olarak yetişen ve yetiştirilen *Cupressus sempervirens* (servi) 2 varyetesinin odunlarından izole edilen eterik yağlardaki bileşikler Gaz Kromatografi-Kütle Spektroskopi cihazıyla belirlenmiştir. Taze odunlardan elde edilen eterik yağların analizleri sonucu 51 bileşik tanımlanmış olup, bu bileşikler eterik yağın %91,9-95,7'sini oluşturmaktadır. Çalışmada 2 farklı varyete odunu, öz ve diri odun ayrımı yapılarak incelenmiştir. Sonuç olarak 2 varyete odunu benzer bileşim göstermiştir. Öz ve diri odunların eterik yağ bileşimlerinde oksijenli monoterpenler (%43,7-72,4), oksijenli seskiterpenler (%8,7-36,5) ve diterpenoidler (50,82-4,15) bulunmuştur. Eterik yağlarda en fazla bulunan bileşikler sırasıyla, karvakrol metil eter %38,2-62,6, α-sedrol %15,7-34,3, manool %1,75-9,83, terpinen-4-ol asetat % (0,82-4,15 ve borneil asetattır (%0,26-4,62). Diğer servi odunlarının eterik yağ bileşimleri ile kıyaslandığında *Cupressu sempervirens* odunu, en fazla karvarol metil eter içeren tür olarak tanımlanabilir. Sonuç olarak servi odunlarını karakterize eden 2 terpen türü bileşik olduğu söylenebilir: bunlar alfa-sedrol ile karvakrol metil eter veya karvakroldür.

Anahtar Kelimeler: *Cupressus sempervirens* varyeteleri, karvakrol metil eter, monoterpenler, odun uçucu bileşenleri, sedrol

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INTRODUCTION

Cupressus sempervirens, or Mediterranean Cypress, grows over a wide natural range in the Mediterranean region. In Turkey, Cypresses grow in cemeteries or are cultivated for ornamental purpose. There are two varieties of *Cupressus sempervirens* in Turkey, - *horizontalis* and *pyramidalis*. In Turkey, some of the existing natural stands of *C. sempervirens* var *horizontalis* are found in the Taurus mountains region, Koprulu Kanyon Natural Park (Goker, 1992).

A literature survey shows that there are many papers reporting the composition of essential oils (EO) from the leaves and cones of *Cupressus sempervirens*. Previously, our team investigated the essential leaf oil of two varieties *C. sempervirens* (Uçar et al., 2007). Following this, the neurobiological

effects of extracts from the same varieties were studied (Tumen et al., 2012). The leaf oil composition of *Cupressus sempervirens* samples from other countries, such as Iran (Emami et al., 2006; Asgary et al., 2013), Algeria (Mazari et al., 2010), Egypt (Ibrahim et al., 2009) and Tunisia (El Hamrouni-Aschi et al., 2013) have also been reported. The data relating to essential oil composition of Cupressus woods is very limited. While some literature available on other Cupressus woods such as *Cupressus atlantica* (Barrero et al., 2005), *Cupressus arizonica* (Amri et al., 2014) and *Cupressus tonkinensis* (Thaia et al., 2013), to the best of our knowledge, no reports are available on the essential wood oil compositions of *C. sempervirens* var. *horizantalis* and var. *pyramidalis*. Due to prohibitions on tree felling and its identity as something sacred, cutting down this tree is forbidden in our country.

The aim of this study is to identify the composition of essential wood oils from two varieties of *Cupressus sempervirens*, to show the chemical differences between sapwoods and heartwoods and to fill the gaps in our knowledge of the wood of this species.

MATERIAL AND METHODS

One sample (T2BahCem) from Cupressus *sempervirens* var. pyramidalis was cut from a cemetery as snow turndown, the other two samples (T1Belg, T2Belg) were harvested from Belgrad forest, two samples (T11, T12) of var. *horizantalis* were also taken from Koprulu kanyon. The fresh woods were immediately taken to laboratory and the barks were removed then sapwoods and heartwoods were separated. The fresh woods were then chipped. For the heartwood samples about 200 g of fresh chips were put in a balloon and 3 L of distilled water were added. The hydro distillation continued for approximately 3 hours. The distillate was extracted with petroleum ether than injected into GC-MS.

Analyses were carried out on the GC-MS (Shimadzu, QP 5050A; Shimadzu, Japonya) instrument to identify and quantitate the compounds in the oils.

The identification of most compounds was based on the libraries NIST 21, NIST 107 and WILEY 229. A private MS-data (Uçar library) library and Adam's library (2007) was also used to identify some compounds. Analyses were carried out on 30 m nonpolar fused silica DB-1 and DB-5 columns (0.25 mm, 0.25 µm film thickness), with a helium flow rate of 1.0 mL/min and split ratio of 10:1. The following temperature program was maintained: 5 min at 60°C, 3°C /min to 120°C, 5°C /min to 200°C, 10°C /min to 260°C and 8 min at 260°C.

Compound identification was also verified by comparing the RI (Kovats indices) relative to C5–C24 n-alkanes obtained in a nonpolar DB-5MS column, with those provided in the literature Adams (2007) and Wiley library.

RESULTS AND DISCUSSION

The essential oil constituents from sapwoods and heartwoods of the two varieties *C. sempervirens* are shown in Table 1. Fifty-one constituents were identified in all oils, accounting for 91.9-95.7% of the total oil composition. A comparison of the EO profile of the two *C. sempervirens* varieties of woods showed slight quantitative differences but no qualitative. No differences were observed in the composition of heartwood and sapwood in the same variety whereas variations in the percentages of various components in the essential oils from sapwood and heartwood were detected. Oxygenated monoterpenes dominated the composition of the oils with a content of between 43.7 and 72.4%, followed by sesqui-terpene and terpenoids with content ranging from 8.7 to 36.5%. An interesting finding was the amount of diterpenoids which were higher than monoterpene hydrocarbons in the oils with content 2.2-10.4%, monoterpene hydrocarbons constituents have minor amounts 1.0-3.4%.

Carvacrol methylether (38.2-62.6%), an oxygenated monoterpene, had the highest amount of identified compounds (Figure 1). Other major oxygenated monoterpenes were terpinen-4-ol acetate (0.82-4.15%), bornylacetate (0.26-4.62%), carvacrol (0.09-0.87%), borneol (0.06-1.3%) and terpineol-4 (0.36-1.0%). The only monoterpene hydrocarbon with an amount greater than 1% was limonene (0.09-1.12%).

Furthermore, the second major compound, in sesquiterpenoids fractions of oils, was α-cedrol (15.7-34.3%), followed by the lesser amount α-cedren (0.61-1.48%). Besides monoterpenoids and sesquiterpenoids, diterpenoid compound, manool was also detected at an appreciable level (1.75-9.83%) for oils.

The first study on *C.sempervirens* wood was performed by Piovetti et al. (1981). The petroleum ether extract of wood contained - as the major compounds- carvacrol methyl ether (24.2%), cedrol (16%), 1,7-diepi-β-cedren (2.73%) and β-cedren+β-elemene (1.4%). In the same study, the wood oil of *C.dupreziana* was also investigated and it was discovered that the oil dominated carvacrol methyl ether (61.78%), cedrol (12.28%) and α-cedren (3.85%). Other than this, there is no data available relating to the wood oils of this species. Comparing our results with the previous report revealed some quantitative differences e.g. a higher amount of carvacrol methyl ether and cedrol were detected in our study.

Additionally, our results can be compared with the essential oils of other Cupressus woods. Table 2 shows main compounds in the essential oils of different Cupressus wood alongside our results.

Rushforth et al. (2003) studied the variation among Cupressus species from the eastern hemisphere based on DNAs and they reported that *C. sempervirens, C. dupreziana* and *C. atlantica* form a distinct group. A similar result can be obtained from our study: the resemblance in the chemical composition of *C. sempervirens* and *C. dupreziana* were clearly observed (Table 2). Due to the fact that wood of *C. atlantica* dominated α-cedrol and methyl thymol which is an isomer of carvacrol, *C. atlantica* can be included in this group. Considering the major compounds identified in the wood oil of Cupressus species in Table 2, it is possible to differentiate the species as follow:

Table 1. Composition of volatile compounds in two varieties of *Cupressus sempervirens*

			var. *horizantalis*				C. *sempervirens* var. *pyramidalis*					
			SAP-		HEART		SAPWOOD			HEARTWOOD		
			T11	T12	T11	T12	T2BahCem	T1 Belgr	T2Belg	T2BahCem	T1 Belgr	T2Belg
No	Compound	RI	%	%	%	%	%	%	%	%	%	%
1	tricyclene	923	0.02	0.00	0.08	0.01	0.00	0.01	0.00	0.00	0.06	0.05
2	a-pinene	934	0.04	0.07	0.09	0.04	0.01	0.04	0.06	0.04	0.05	0.04
3	camphene	947	0.02	0.01	0.07	0.01	0.00	0.01	0.05	0.00	0.07	0.04
4	b-pinene	969	0.01	0.00	0.01	0.00	0.04	0.01	0.01	0.07	0.00	0.00
5	myrcene	986	0.20	0.16	0.49	0.38	0.03	0.10	0.39	0.05	0.19	0.31
6	a-terpinene	1010	0.35	0.30	0.36	0.36	0.18	0.14	0.07	0.26	0.19	0.24
7	p-cymene	1013	0.17	0.16	0.15	0.14	0.09	0.18	0.51	0.03	0.10	0.14
8	b-phellandrene	1021	0.02	0.02	0.03	0.02	0.05	0.03	0.07	0.04	0.01	0.02
9	limonene	1023	0.18	0.09	0.38	0.12	0.35	0.18	1.12	0.20	0.36	0.28
10	g-terpinene	1052	0.35	0.28	0.32	0.31	0.22	0.15	0.14	0.20	0.15	0.21
11	cymenene	1074	0.23	0.23	0.34	0.28	0.31	0.29	0.83	0.11	0.13	0.17
12	a-terpinolene	1080	0.70	0.56	1.87	1.26	0.22	0.23	0.93	0.30	0.32	0.44
13	camphor	1116	0.20	0.00	0.69	0.00	0.30	0.05	0.14	0.04	0.07	0.05
14	camphene hydrate	1129	0.05	0.00	0.02	0.00	0.01	0.10	0.09	0.01	0.06	0.03
15	borneol	1147	0.91	0.06	0.55	0.19	0.14	0.65	0.42	0.20	1.03	0.53
16	cymenol	1160	0.06	0.04	0.16	0.14	0.94	0.06	0.05	0.23	0.14	0.30
17	terpineol -4	1161	0.60	0.57	0.52	0.75	1.00	0.92	0.42	0.72	0.36	0.46
18	a-terpineol	1171	0.14	0.10	0.12	0.17	0.21	0.12	0.13	0.17	0.11	0.13
19	neodihydrocarveol	1217	0.02	0.00	0.09	0.12	0.33	0.12	0.00	0.20	0.05	0.11
20	carvacrol ME	1231	60.2	55.4	61.1	59.5	35.0	50.2	62.6	53.8	38.2	49.2
21	carvenone	1233	0.10	0.10	0.20	0.16	0.20	0.01	0.07	0.10	0.05	0.05
22	bornyl acetate	1269	1.79	0.31	1.23	0.26	0.61	4.49	1.03	0.31	4.62	1.91
23	carvacrol	1280	0.41	0.36	0.27	0.68	0.87	0.15	0.20	0.76	0.21	0.09
24	terpinen-4-ol Ace	1284	2.55	2.45	4.14	4.11	2.52	2.38	0.82	4.15	2.88	3.22
25	ME-2isoprop-5-methoxybenzene	1286	0.13	0.15	0.00	0.20	0.20	0.11	0.19	0.40	0.20	0.30
26	eugenol	1327	0.02	0.00	0.02	0.03	0.14	0.01	0.02	0.03	0.01	0.04
27	g-terpinylacetate	1330	0.85	0.80	0.80	0.92	0.51	0.77	0.19	1.02	0.70	0.80
28	a-terpinyl acetate	1332	0.44	0.39	0.57	0.55	0.46	0.46	0.23	0.60	0.44	0.43
29	b-elemene	1383	0.00	0.23	0.02	0.17	0.06	0.17	0.11	0.17	0.25	0.24
30	zingiberene	1397	0.04	0.05	0.04	0.04	0.01	0.04	0.05	0.05	0.05	0.04
31	a-cedrene	1400	1.38	0.93	1.67	0.70	0.61	1.06	0.68	0.75	1.48	1.09
32	b-cedrene	1406	0.45	0.25	0.48	0.20	0.21	0.25	0.28	0.20	0.45	0.27
33	b-caryophyllene	1407	0.05	0.04	0.05	0.05	0.10	0.05	0.07	0.05	0.05	0.03
34	widdrene	1418	0.08	0.45	0.06	0.32	0.42	0.41	0.06	0.33	0.09	0.33
35	b-farnesene	1446	0.08	0.16	0.06	0.11	0.04	0.16	0.03	0.11	0.06	0.12

Table 1. Composition of volatile compounds in two varieties of *Cupressus sempervirens* (continued)

			var. *horizantalis*				C. *sempervirens* var. *pyramidalis*					
			SAP-		HEART		SAPWOOD			HEARTWOOD		
			T11	T12	T11	T12	T2BahCem	T1 Belgr	T2Belg	T2BahCem	T1 Belgr	T2Belg
36	epi-a-cedrene	1467	0.09	0.06	0.10	0.03	0.03	0.04	0.04	0.03	0.08	0.03
37	b-selinene	1472	0.10	0.12	0.01	0.14	0.08	0.16	0.11	0.13	0.21	0.22
38	b-himahalene	1486	0.00	0.00	0.00	0.00	0.18	0.27	0.74	0.00	0.00	0.00
39	b-bisabolene	1495	0.08	0.06	0.06	0.03	0.02	0.08	0.02	0.04	0.08	0.04
40	bsesqphellandrene	1507	0.09	0.08	0.11	0.06	0.39	0.13	0.06	0.07	0.12	0.06
41	a-cedrol	1587	17.8	25.0	15.7	16.8	34.3	21.8	6.05	21.0	31.4	25.8
42	cedr-8-en-15-ol	1642	0.07	0.04	0.07	0.06	0.14	0.16	0.50	0.05	0.14	0.03
43	hexadecanoic acid	1944	0.13	0.06	0.02	0.00	1.38	0.31	4.95	0.00	0.00	0.00
44	procerin	1961	0.02	0.01	0.01	0.12	0.00	0.00	0.00	0.38	0.23	0.20
45	dehydroabietane	2039	0.00	0.02	0.00	0.00	0.02	0.06	0.39	0.00	0.01	0.00
46	manool	2047	3.40	5.51	2.22	4.85	9.83	4.51	1.75	6.26	6.24	6.36
47	linoleic acid	2107	0.03	0.02	0.00	0.00	0.05	0.16	1.82	0.01	0.00	0.00
48	oleic acid	2114	0.05	0.02	0.01	0.01	0.30	0.01	0.80	0.00	0.01	0.01
49	totarol	2282	0.05	0.05	0.00	0.00	0.07	1.70	2.61	0.01	0.03	0.01
50	ferruginol	2297	0.01	0.00	0.00	0.00	0.06	0.07	0.17	0.00	0.04	0.02
51	agathadiol	2328	0.03	0.01	0.00	0.04	0.04	0.00	0.00	0.10	0.10	0.07
	Sum		94.7	95.7	95.2	94.3	93.3	93.6	92.1	93.8	91.9	94.5

Table 2. Main compounds in the essential oils of different *Cupressus* woods

Species (part)	Origin	Major Constituents	References
C. *sempervirens* var. *horizantalis*	Turkey	Carvacrol methyl ether 55.4-61.1%, α-cedrol 15.7-25%, manool 2.22-5.51%, terpinen-4-ol acetate 2.45-4.14%	Our work
C.*sempervirens* var. *pyramidalis*	Turkey	Carvacrol methyl ether 35.4-62.6%, α-cedrol 21-34.3%, manool 1.75-9.83%, terpinen-4-ol acetate 0.82-4.15%	Our work
C. *dupreziana* wood		Carvacrol methyl ether (61.78%), cedrol (12.28%) and α-cedren (3.85%).	Piovetti et al., 1981
C.*sempervirens* var. *horizantalis* branches		α-pinen (46.2%) and δ-caren (22.7%)	Asgary et al., 2013
C. *macrocarpa* heartwood	USA	Carvacrol 82%, terpineol-4-ol 5.58% and nootkatin 5.86%	Lui, 2009
C. *macrocarpa* heartwood		Carvacrol 94.4%,	Zhang et al., 2012
C.*sempervirens* var. *numidica* wood	Tunusia	α-pinen (54.3%, 69.9%), δ-3-caren (11.8 %, 2.8%), carvacrol methyl ether (1.7%, 1.3%), and cedrol (3.7%, 2.3%)	El Hamrouni-Aschi et al., 2013
C. *arizonica* branches		α-pinen 74.6%, myrcene 5.3%, δ-3-caren 4.0%, β-pinen 3.7% and methyl carvacrol 2%	Flamini et al., 2003
C. *arizonica* var. *glabra* wood		α-pinen 40.7%, limonene 3.2 % and umbellulone 2.9%	Abbas et al., 2013
C. *arizonica* wood	Tunisia	α-pinen 76.6%, δ-3-caren 2.3%, limonene 2.6% and cedrol 1.5%	Amri et al., 2014
C.*atlantica* wood		cedrol 45.1%, methyl thymol 15.6% and manool 9.8%	Barrero et al., 2005
C. *funebris* wood		α-cedrol 43.9-72.8%, α-cedren 0.7-3.4%, β-cedren 0.3-2.1% and manool 2.7-7.6%	Adams and Li 2008.
C. *funebris* wood	China	α-cedren 16.9%, cedrol 7.6% and β-cedren 5.7%	Carroll et al., 2011
C. *tonkinensis* wood		α-pinen 42.5%, myrcene 10.2% and cedrol 9%	Thaia et al., 2013

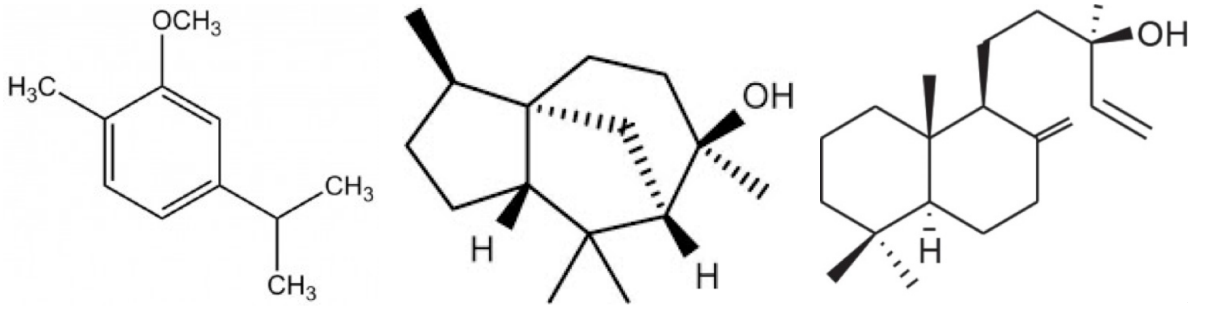

Figure 1. Chemical formula of the main compounds in the essential oil of *Cupressus* woods

First Group: methyl carvacrol and carvacrol rich essential oils; *C.sempervirens* var. *horizontalis*, var. *pyramidalis* (our results), *C. dupreziana* (Piovetti et al., 1981) and *C. macrocarpa* (Lui, 2009; Zhang et al., 2012) may be *C. atlantica* (Barrero et al., 2005),

Second Group: including Cedrol and α-cedren rich essential oil; *C. funebris* (Adams and Li, 2008; Carroll et al., 2011) and *C. atlantica* (Barrero et al., 2005),

Third Group: α-pinen rich essential oil; *C. arizonica* (Flamini et al., 2003; Abbas et al., 2013; Amri et al., 2014), *C. sempervirens* var. *numidica* (El Hamrouni-Aschi et al., 2013) and *C. tonkinensis* (Thaia et al., 2013).

When the above mentioned literatures was taken into consideration, it was noticed that all of the Cupressus wood oils contained more or less α-cedrol and carvacrol or carvacrol methyl ether.

CONCLUSION

The wood oils of Cupressus varieties were investigated by separating sapwood and heartwood which contained similar compounds. The percentages of these compounds were very distinct and the major components in the oils were carvacrol methyl ether, α-cedrol, manool and terpinen-4-ol acetate.

As a result the wood oil of Cupressus species can be characterized by the presence of two compounds: α-cedrol and carvacrol or carvacrol methyl ether. A similar result was reported previously by Piovetti et al. (1981) which stated that the presence of carvacrol methyl ether, cedrol together with α-cedren seems to be a characteristic feature of Cupressaceae.

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