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Research Article

Chaerophyllum libanoticum Boiss. Et Kotschy: The fruit essential oil, composition, skin-whitening and antioxidant activities

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ABSTRACT

This study was aimed to evaluate the essential oil composition of *Chaerophyllum libanoticum* fruits and its potential uses in the cosmetic industry. The essential oil was analyzed by Gas Chromatography and Gas Chromatography-Mass Spectrometry (GC-GC/MS) systems, simultaneously. The yield of essential oil was calculated as 0.22 % (*v/w*). Major components of the oil were characterized as limonene (26.7%), *p*-cymene (25.5%), and β-phellandrene (7.0%). In addition, antioxidant and antityrosinase activities of the essential oil were evaluated. The oil exhibited moderate antioxidant activity (TEAC). In the DPPH$^{\bullet}$ assay, the oil was tested at 5 mg/mL concentration, and the inhibition ratio was calculated as 31.3 ± 1.1%. At 1 mg/mL of concentration, TEAC (mmol/L) value was determined as 0.027 ± 0.008. As evidence to its skin whitening properties, the oil inhibited the tyrosinase 17.7 ± 1.6 % at 1 mg/mL..

Keywords: *Chaerophyllum libanoticum*, Apiaceae, Essential oil, Antioxidant, Antityrosinase

1. INTRODUCTION

Apiaceae is considered as one of the most important families due to its economic value in the field of pharmaceutical, cosmetic, flavor, and fragrance industries and is represented by about 3780 species in 434 genera [1,2]. The genus *Chaerophyllum* L. is represented in the Flora of Turkey by 15 species [3], and the members of the genus have a distinctive aromatic character [4]. *Chaerophyllum* Boiss. et Kotschy. is known as "Mentik" in Southern parts of Turkey, and is consumed as food like some other *Chaerophyllum* species [5].

Essential oil components are responsible for the characteristic fragrance of *Chaerophyllum* species, and various studies have been reported on the volatile composition of different parts and origins [6-11]. Previous investigations on biological properties of *Chaerophyllum* species revealed that they possess various biological activities such as antimicrobial [7,10], antioxidant [7,10], anti-inflammatory [7], angiotensin-converting enzyme (ACE) inhibition [12], glutathione-*S*-transferase inhibition [13], cytotoxic properties [14].

Essential oils have been used in cosmetic preparations due to their health benefit effects in skin disorders related to their antioxidant, antimicrobial and anti-inflammatory activities. Recently, tyrosinase, a key enzyme in the production of melanin, has become

one of the most important targets in the cosmetic industry [15]. Overproduction of melanin leads to hyperpigmentation, age spots, melasma, and this process might be related to increased oxidative stress on the skin. Essential oils, their components and essential oil-bearing plants are considered natural cosmetic ingredients. They may also possess antioxidant and antityrosinase properties.

This present study was aimed to characterize volatile constituents of *Chaerophyllum libonoticum* Boiss. Et Kotschy fruit essential oil collected from Osmaniye. The essential oil was analyzed by GC-FID and GC/MS system, simultaneously. Furthermore, antioxidant and antityrosinase activities were determined by *in vitro* methods.

2. MATERIALS AND METHODS

2.1. Plant Material

Chaerophyllum libanoticum was collected from Osmaniye; Zorkun, Mitisin Plateau at an altitude 1340 m in Turkey, on October 14, 2018 (Ahmet Duran, 10733).

2.2. Chemicals and Reagents

All chemicals and solvents were of high purity and at least of analytical grade. 1,1- diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) (Aldrich), 2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (Sigma-Aldrich), Trolox ((*S*)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Sigma-Aldrich), gallic acid (Sigma), ascorbic acid (Sigma-Aldrich), L-DOPA (Sigma), kojic acid (Sigma-Aldrich), tyrosinase from mushroom (Sigma-Aldrich), dipotassium hydrogen phosphate (Merck), sodium dihydrogen phosphate dihydrate (Merck), sodium sulfate anhydrous (Sigma-Aldrich), potassium persulfate (Sigma-Aldrich), methanol (Merck), ethanol (Sigma-Aldrich), and *n*-hexane (Sigma-Aldrich) were purchased.

2.3. Isolation of Essential Oil

The air-dried fruits of *Chaerophyllum libanoticum* were subjected to hydrodistillation, using a Clevenger-type apparatus for 3h. The obtained oils were kept at +4°C in the dark until the experiments.

2.4. GC-FID Analysis

GC analyses were performed using an Agilent 6890N GC system. FID temperature was set to 300°C and the same operational conditions were applied to a triplicate of the same column used in GC/MS analyses. Simultaneous auto injection was employed to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms.

2.5. GC/MS Analysis

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60m x 0.25mm, 0.25µm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. The interphase temperature was at 280°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

2.6. Identification of Compounds

The components of essential oils were identified by comparison of their mass spectra with those in the in-house Baser Library of Essential Oil Constituents, Adams Library [16], MassFinder Library [17], Wiley GC/MS Library [18], and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of n-alkanes. Alkanes were used as reference points in the calculation of relative retention indices (RRI) [19]. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.7. DPPH Radical Scavenging Activity

DPPH$^{\bullet}$ scavenging activity was determined by the method of Ağalar and Temiz, 2021 [20]. 0.2 mM DPPH solution was prepared in a dark environment with methanol. The experiment was carried out in 96-well microplates. Incubation was carried out for

30 minutes at room temperature and in the dark (A). For the sample control, 100 µL of methanol was added instead of DPPH (B). 0.1 mM DPPH as a blank control (C), methanol (D) as solvent control, and Vitamin C and gallic acid as a positive control were used. Absorbances were measured at 517 nm, and inhibition percentages were calculated according to the formula below (Eq.1).

% inhibition: [(C-D) -(A-B) /(C-D)] x 100 (Eq.1)

2.8. Trolox Equivalent Antioxidant Capacity (TEAC)

The trolox equivalent antioxidant capacity of the essential oils was performed *via* the method described by Re et al. (1999) using $ABTS^{+\bullet}$ radical [21]. 7 mM $ABTS^{+\bullet}$ radical and 2.5 mM potassium persulfate were dissolved in water and kept in the dark for 16h at room temperature. Essential oils were dissolved in ethanol at 1 mg/mL concentration. Trolox were prepared in absolute ethanol with 2.5, 2, 1.5, 1, 0.5, 0.25, 0.125 and 0.0625 mM concentrations. 990 µL $ABTS^{+\bullet}$ solution and 10 µL samples were mixed. Absorbances were measured at 734 nm for 30 min, and the results were expressed as Trolox equivalent antioxidant capacity (mmol/L Trolox).

2.9. Tyrosinase Inhibition

The tyrosinase inhibitory activity of the oil was evaluated by using L-DOPA [20]. Tyrosinase and L-DOPA were prepared in the phosphate buffer (100 mM, pH 6.8). Essential oils were dissolved in buffer containing 5% DMSO. For each sample solution, four wells designated as A, B, C and D each contained a reaction mixture (40 µL) as follows: A, 20 µL buffer (pH 6.8) and 20 µL tyrosinase (200 U/mL); B, 40 µL buffer; C, 20 µL tyrosinase (200 U/mL) and 20 µL sample; D, 20 µL sample and 20 µL buffer. The contents of each well were mixed and incubated at 37 ˚C for 10 min. Then, 5 mM of L-DOPA (160 µL) was added. After second incubation at 37 ˚C for 10 min, the absorbance at 475 nm of each well was measured. The percentage inhibition of the tyrosinase activity was calculated by the following equation 2.

% inhibition: [(A-B)-(C-D)/(A-B)] x 100 (Eq. 2)

2.10. Statistical Analysis

All the experiments were carried out in triplicate, and data were expressed as means ± standard deviation (SD). Statistical analysis were performed using Sigmaplot 14.0 software (Systat Software, Inc., San Jose, CA, USA). IC_{50} values were calculated by regression analysis.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of *C. libanoticum* Oil

The essential oil obtained from the fruits of *C. libanoticum* was analyzed by GC-GC/MS systems, simultaneously. The essential oil yield was calculated as 0.22% (*v/w*).

Sixty-four constituents were characterized as representing 95.9% of the essential oil. Limonene (26.7%), *p*-cymene (25.5%), and β-phellandrene (7.0%) were found to be the most abundant compounds.

β-Pinene (5.1%), cryptone (4.1%), hexadecanoic acid (3.4%), α-pinene (2.7%), octanal (2.0%), γ-terpinene (1.4%), *trans*-pinocarveol (1.3%), and myrtenol (1.0%) were present in relatively high amounts (Table 1).

In the essential oil, monoterpene hydrocarbons (69.9%) were the most abundant group. Also, the percentage of oxygenated monoterpenes (12.7%) and other compound groups (11.5%) were considerable. In addition, sesquiterpene hydrocarbons (0.7%) and oxygenated sesquiterpenes (1.0%) were present in relatively low amounts. (Figure 1).

Several studies identified essential oil compositions of *Chaerophyllum* species. Previous reports on volatile constituents of *Chaerophyllum* species growing wild in Turkey are given in Table 2. Studies were mainly focused on the aerial parts and fruits of these species. Essential oils obtained from different species were characterized with various major components. Demirci et al. (2007) detected that the fruit essential oil was rich in limonene (15.9 %) and β-phellandrene (17.6 %) [10]. Our results revealed

Table 1. Chemical composition of the fruit essential oil of *C. libanoticum*

RRI	Compounds	%	IM
1032	α-Pinene	2.7	t_R, MS
1118	β-Pinene	5.1	t_R, MS
1132	Sabinene	0.3	t_R, MS
1174	Myrcene	0.1	t_R, MS
1183	Pseudolimonene	0.3	MS
1194	Heptanal	0.2	MS
1203	**Limonene**	**26.7**	t_R, MS
1218	**β-Phellandrene**	**7.0**	t_R, MS
1246	(Z)-β-Ocimene	0.2	t_R, MS
1255	γ-Terpinene	1.4	t_R, MS
1280	***p*-Cymene**	**25.5**	t_R, MS
1290	Terpinolene	0.4	t_R, MS
1296	Octanal	2.0	t_R, MS
1400	Nonanal	0.1	MS
1435	γ -Campholene aldehyde	0.1	MS
1452	*p*-Cymenene	0.2	MS
1458	*cis*-1,2-Limonene epoxide	0.1	MS
1463	Heptanol	0.1	t_R, MS
1468	*trans*-1,2-Limonene epoxide	0.4	MS
1474	*trans*-Sabinene hydrate	0.1	t_R, MS
1476	4,8-Epoxyterpinolene	0.7	MS
1499	α-Campholene aldehyde	0.5	MS
1535	β-Bourbonene	0.2	tR, MS
1548	(*E*)-2-Nonenal	0.8	MS
1553	Linalool	0.5	t_R, MS
1565	8,9-Limonene epoxide-I	0.3	MS
1570	8,9-Limonene epoxide-II	0.1	MS
1571	trans-p-Menth-2-en-1-ol	0.7	MS
1586	Pinocarvone	0.2	MS
1607	Thymol methyl ether	0.1	t_R, MS
1611	Terpinen-4-ol	0.3	t_R, MS
1617	*trans*-Dihydrocarvone	tr	t_R, MS
1638	*trans-p*-Menth-2,8-dien-1-ol / *cis-p*-Menth-2-en-1-ol	0.7	MS
1648	Myrtenal	0.4	MS
1655	(*E*)-2-Decenal	0.3	MS
1670	*trans*-Pinocarveol	1.3	t_R, MS
1678	*cis-p*-Menth-2,8-dien-1-ol	0.5	MS
1690	Cryptone	4.1	MS
1706	α-Terpineol	0.4	t_R, MS
1744	Phellandral	0.7	MS
1751	Carvone	0.8	t_R, MS
1783	β-Sesquiphellandrene	0.2	MS
1786	*ar*-Curcumene	0.3	MS
1804-	Myrtenol	1.0	MS
1811	*trans-p*-Mentha-1(7),8-dien-2-ol	0.3	MS
1815	*p*-Mentha-1,3-dien-7-al	0.1	MS
1845	*trans*-Carveol	0.6	t_R, MS
1864	*p*-Cymen-8-ol	0.9	t_R, MS
1867	*cis*-Carveol	0.3	t_R, MS
1896	*cis-p*-Mentha-1(7),8-dien-2-ol	0.1	MS
2029	Perilla alcohol	0.2	MS
2113	Cumin alcohol	0.3	t_R, MS
2131	Hexahydrofarnesyl acetone	0.2	t_R, MS
2144	Spathulenol	0.2	t_R, MS
2198	Thymol	0.1	t_R, MS
2239	Carvacrol	0.2	t_R, MS
2241	ar-Turmerol	0.2	MS
2278	Torilenol	0.1	MS
2296	Myristicine	0.1	MS
2369	Eudesma-4(15), 7-dien-1β-ol	0.3	MS
2392	Caryophyllenol II	0.2	MS
2931	Hexadecanoic acid	3.4	MS
	Grouped compounds (%)		
	Monoterpene hydrocarbons		69.9
	Oxygenated monoterpenes		12.8
	Sesquiterpene hydrocarbons		0.7
	Oxygenated sesquiterpenes		1.0
	Others		11.5
	TOTAL %		**95.9**

RRI: Relative retention indices calculated against n-alkanes; %: calculated from the FID chromatograms; tr: Trace (<0.1 %). Identification method (IM): t_R, identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

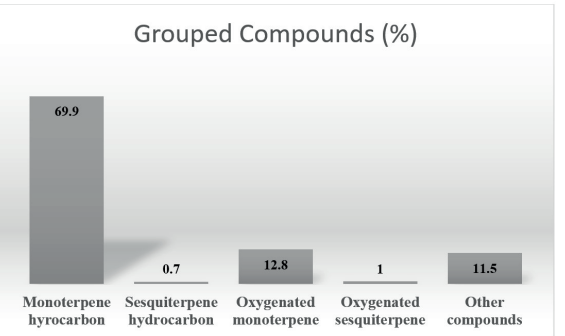

Figure 1. Compound group distribution of the essential oil

Table 2. Main constituents of the essential oils of *Chaerophyllum* species growing wild in Turkey

Plant	Collection place	Parts	Main constituents	Reference
C. aksekiense	Antalya	Fruits	Heptacosane (10.1%), humulene epoxide II (7.8%), (*E*)-β-farnesene (6.2%), caryophyllene oxide (6.0%),	[6]
C. aromaticum	İstanbul	Aerial parts	Sabinene (28.1%), terpinolene (16.7%), γ-terpinene (16.1%)	[7]
C.byzantinum	Bursa	Aerial parts	Sabinene (30.0%), *p*-cymen-8-ol (16.0%), terpinolene (11.5%)	[8]
C. crinitum	Bitlis	Aerial parts	(*E*)-β-Ocimene (38.1%), terpinolene (12.7%)	[9]
C. libanoticum	Osmaniye	Fruits	Limonene (15.9 %), β-phellandrene (17.6 %)	[10]
C. macrospermum	Hakkari	Aerial parts	Terpinolene (21.4%), myristicin (18.9%), *p*-cymen-8-ol (11.9%)	[9]

that the *p*-cymene ratio was higher than that of β-phellandrene. Moreover, Gnannadi et al. (2011) reported that the main constituents of the essential oil obtained from flowering aerial parts of *C. macropodum* were *trans*-β-ocimene (34.5%), *trans*-β-farnesene (11.8%), *cis*-β-ocimene (10.4%), and p-cymene [11]. A study on *C. aromaticum* showed that γ-terpinene (21.0-33.8%), β-phellandrene (14.3-30.0%) and β-pinene (14.3-17.8%) were the main compounds in the fruit essential oil [22].

3.2. Antioxidant Properties of *C. libanoticum* Oil

The antioxidant activity of the essential oil was investigated by two assays, namely DPPH radical scavenging activity and Trolox equivalent antioxidant capacity (TEAC), and the results are given in Table 3. The essential oil of *C. libanoticum* at a 5 mg/mL concentration inhibited DPPH radical by 31.3 ± 1.1 %. The effects were relatively weak when compared with positive controls (vitamin C, IC_{50} value of 9.3 ± 0.01 µg/mL, gallic acid, IC_{50} value of 1.93 ± 0.02 µg/mL). Furthermore, the essential oil presented 0.027 ± 0.008 Trolox equivalent antioxidant capacity at 1 mg/mL concentration.

Demirci et al. (2007) evaluated the DPPH$^{\bullet}$ scavenging activity of *C. libanoticum* fruit essential oil, and they reported the IC_{50} value with >30 mg/mL [10]. Kurkcuoglu et al. (2018) found that the essential oil obtained from aerial parts of *C. aromaticum* inhibited DPPH radical by 2.06% at 20 mg/mL [7]. Ebrahimabadi et al. (2010) evaluated the DPPH radical scavenging activity of *C. macropodum* leaf and flower's essential oils and extracts. The results revealed that the extracts had higher activity than the essential oils [23].

3.3. Tyrosinase Inhibition of *C. libanoticum* Oil

Tyrosinase is a rate-limiting enzyme for the biosynthesis of melanocytes. Thus, it is considered the primary target for skin hyperpigmentation disorders. In the present study, tyrosinase inhibition of the essential oil was tested at 1 mg/mL of concentration, and it exhibited considerable effect (Table 3). However, the results were found to be weak when compared to standard, kojic acid. To the best of our knowledge, tyrosinase inhibition of the essential oil of *Chaerophyllum* species was evaluated for the first time.

4. CONCLUSION

According to our findings, the essential oil isolated from the fruits of *C. libanoticum* was rich in monoterpene hydrocarbons and oxygenated monoterpenes. The essential oil possesses moderate antioxidant and antityrosinase activity. The effects were weak when compared with positive controls. activity.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Table 3. Activity results of the essential oil of *C. libanoticum*

	DPPH• scavenging activity %[a]	TEAC[b] (mmol/L)	Tyrosinase inhibition %[b]
C. libanoticum EO	31.3 ± 1.1	0.027 ± 0.008	17.7 ± 1.6
Gallic acid[c]	1.93 ± 0.02	-	-
Ascorbic acid[c]	9.3 ± 0.01	-	-
Kojic acid[c]	-	-	3.6 ± 0.01

Data was given as mean ± SD ($n = 3$) [a] ; Samples tested at 5 mg/mL concentration, [b] ; Samples tested at 1 mg/mL concentration; [c] ; Positive controls, values represented IC_{50}, µg/mL), EO: essential oil

Author contribution

Concept: HGA, BT, MK; Design: HGA, BT, MK; Supervision: KHCB; Materials: AD; Data Collection and/or Processing: MK, BT; Analysis and/or Interpretation: MK, BT; Literature Search: MK, HGA, BT; Writing: BT, MK, HGA, KHCB; Critical Reviews: KHCB.

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Conflict of interest

The authors declared that there is no conflict of interest.

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