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Antimicrobial and antioxidant activities of the essential oil of *Chaerophyllum libanoticum* Boiss. *et* Kotschy ☆

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Abstract

Chaerophyllum libanoticum Boiss. *et* Kotschy from Apiaceae, is collected and used as a food plant in Turkey. The essential oil obtained by hydrodistillation from the crushed fruits of *C. libanoticum* collected from Osmaniye, Southern Turkey, was simultaneously analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). As a result, a total of seventy three components were characterized, representing 98.3% of the total oil with monoterpenes as the major group. The principal constituents were identified as β-phellandrene (17.6%), limonene (15.9%), β-pinene (8.8%), and sabinene (8.5%), respectively. The essential oil was evaluated for its antimicrobial activity using a microdilution assay resulting in the inhibition of a number of common human pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeast *Candida albicans*. The minimum inhibitory concentrations (MIC) varied between 0.25 and 0.5 mg/ml which is within a moderate antimicrobial activity range. Furthermore, the antioxidant capacity of the essential oil was examined using an *in vitro* radical scavenging activity test. The *C. libanoticum* essential oil scavenged 1,1-diphenyl-2-picrylhydrazyl radical (DPPH˙), resulting in $IC_{50} > 30$ mg/ml. In addition, the effect on inhibition of lipid peroxidation of the essential oil was assayed using β-carotene bleaching and haemoglobin induced linoleic acid peroxidation methods resulting in 16% antioxidative activity.

Keywords: *Chaerophyllum libanoticum*; Apiaceae; Essential oil composition; Monoterpenes; Antimicrobial activity; Antioxidant activity

1. Introduction

The Apiaceae family comprising about 300 genera and 3000 species worldwide is also widespread in Turkey. The genus *Chaerophyllum* L. is represented in the Flora of Turkey by 15 species of which four are endemic according to Davis (1972) and Güner, Özhatay, Ekim, and Başer, 2000. *Chaerophyllum libanoticum* Boiss. *et* Kotschy is locally known as "Mentik" in Hatay region, Southern parts of Turkey, where its stems are consumed as food according to Baytop (1994). It was also reported that other *Chaerophyllum* species are used as food and in food preparations as aromatizer (Baytop, 1994). *C. macropodum* Boiss. is used along with other Apicaceae species in cheese production for flavoring (Durmaz, Sagun, Tarakçı, & Özgökçe, 2006) and in food preparations in Turkey (Çoruh, Sağdıçoğlu-Celep, & Özgökçe, 2007).

Essential oil compositions of various *Chaerophyllum* species from different origin have previously been investigated (Kubeczka, Bohn, Schultze, & Formacek, 1989; Letchamo, Korolyuk, & Tkachev, 2005; Sefidkon & Abdoli, 2005). Başer et al. (2000) reported on the essential oil chemistry of *C. aksekiense* and Kürkçüoğlu, Başer, İşcan, Malyer, and Kaynak (2006) recently reported the composition of *C. byzantinum* growing in Turkey.

Previous phytochemical investigations of *Chaerophyllum* species have revealed the presence of secondary plant

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metabolites like lignans, phenylpropanoids and polyacetylenes (Mikaya, Turabelidze, Kemertelidze, & Vul'fson, 1981; Rollinger, Zidorn, Dobner, Ellmerer, & Stuppner, 2003), phenolic acids and related compounds (Dall'Acqua, Viola, Piacente, Cappelletti, & Innocenti, 2004), flavonoid glycosides as reported by Gonnet (1983, 1986) and are rich in carbohydrates especially in their roots according to a report by Ayala Garay, Briard, and Péron (2003).

The biological activity of some *Chaerophyllum* species such as antimicrobial (Durmaz et al., 2006; Kürkçüoğlu et al., 2006; Rollinger et al., 2003), antioxidant (Dall'Acqua & Innocenti, 2004), glutathione-*S*-transferase inhibitory activity (Çoruh et al., 2007) and cytotoxic properties (Dall'Acqua et al., 2004) have been investigated.

The trend to use essential oils or essential oil containing plants in foods which may act as natural antimicrobials or antioxidant preservatives, may also influence health of consumers as well as prolong the shelf-life of relevant food products (Burt, 2004; Dorman, Figueiredo, Barroso, & Deans, 2000; Svoboda, Brooker, & Zrustova, 2006).

To the best of our knowledge, according to a literature survey there is no report on the phytochemical and essential oil constituents of *C. libanoticum*. In this work, we report for the first time the essential oil composition as well as the antimicrobial and antioxidant activities of *C. libanoticum*.

2. Materials and methods

2.1. Chemicals

All chemicals, standards, solvents, and culture media of high purity (>99%) were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany) or Merck KGaA (Darmstadt, Germany).

2.2. Plant material

Fruits were collected in Osmaniye in Southern Turkey by one of us (MD). A voucher specimen (M. Dinç 2485) was submitted and kept at the Herbarium of Selçuk University, Faculty of Education, Konya, Turkey.

2.3. Isolation of the essential oil

Air dried fruits were crushed using a mortar and immediately hydrodistilled for 3 h using a Clevenger apparatus to provide essential oil with a 1.5% yield on a dry weight basis. The distilled essential oil was dried over anhydrous sodium sulphate, filtered and stored at +4 °C until the time of analysis and bioassay.

2.4. Analysis of the essential oil

2.4.1. Gas chromatography–mass spectrometry (GC–MS)

The GC–MS analysis was carried out using an Agilent 5975 GC/MSD system (SEM Ltd. Istanbul, Turkey). One microliter of essential oil sample in *n*-hexane was withdrawn by the autosampling system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness from SEM Ltd. Istanbul, Turkey). was used with helium as the 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 at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV and mass range was from *m*/*z* 35 to 450.

2.4.2. Gas chromatography (GC)

The GC analysis was carried out using an Agilent 6890 N GC system (SEM Ltd. Istanbul, Turkey). FID detector temperature was set at 300 °C. To obtain the same elution order with GC/MS, simultaneous autoinjection was done on a duplicate on the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms as seen in Table 1.

2.4.3. Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) with a series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data, was used for the identification (Joulain, König, & Hochmuth, 2001; McLafferty & Stauffer, 1989).

2.5. Antimicrobial activity

2.5.1. Microorganisms

Microorganisms were obtained from ATCC, NRRL and clinical isolates (Faculty of Medicine, Eskisehir Osmangazi University, Turkey) and were stored in 10% glycerol containing micro-test tubes at −86 °C (strain numbers and origin of microorganisms were given in Table 2). The yeast *C. albicans* was inoculated on Sabouraud Dextrose Agar (SDA), whereas bacteria were inoculated using Mueller Hinton Agar (MHA) at 37 °C overnight, for purity check. All microorganisms were then transferred to double strength Mueller Hinton Broth (MHB) for further incubation at 37 °C for another 24 h.

2.5.2. Antimicrobial assay

Antimicrobial activity of the essential oil was evaluated using the microdilution broth method (İşcan, Kırımer, Kürkçüoğlu, Başer, & Demirci, 2002). The essential oil and the antimicrobial standards were first dissolved in a 25% dimethyl sulphoxide (DMSO) which was used to prepare dilution series from 2.0 to 0.0019 mg/ml in distilled

Table 1
Essential oil composition of *C. libanoticum*

RRI	Compound	%
1032	α-Pinene	2.9
1035	α-Thujene	0.2
1076	Camphene	0.1
1118	β-Pinene	8.8
1132	Sabinene	8.5
1159	δ-3-Carene	0.2
1174	Myrcene	1.5
1176	α-Phellandrene	0.2
1183	*p*-Mentha-1,7(8)-diene (=*Pseudolimonene*)	0.1
1188	α-Terpinene	0.2
1203	Limonene	15.9
1218	β-Phellandrene	17.6
1246	(*Z*)-β-Ocimene	1.1
1255	γ-Terpinene	9.9
1266	(*E*)-β-Ocimene	0.3
1280	*p*-Cymene	1.9
1290	Terpinolene	2.4
1296	Octanal	0.2
1468	*trans*-1,2-Limonene epoxide	0.1
1474	*trans*-Sabinene hydrate	0.1
1497	α-Copaene	0.1
1553	Linalool	0.1
1556	*cis*-Sabinene hydrate	0.1
1594	*trans*-β-Bergamotene	0.2
1597	β-Copaene	0.1
1600	β-Elemene	0.1
1611	Terpinen-4-ol	0.3
1612	β-Caryophyllene	0.1
1650	γ-Elemene	0.4
1668	(*Z*)-β-Farnesene	0.2
1687	α-Humulene	0.1
1690	Cryptone	0.1
1726	α-Zingiberene	2.7
1726	Germacrene D	4.3
1741	β-Bisabolene	1.4
1755	Bicyclogermacrene	0.6
1773	δ-Cadinene	0.5
1783	β-Sesquiphellandrene	7.9
1854	Germacrene-B	3.1
1864	*p*-Cymen-8-ol	0.3
2008	Caryophyllene oxide	0.1
2053	Germacrene-D-1,10-epoxide	0.1
2069	Germacrene D-4β-ol	1.5
2144	Spathulenol	0.2
2187	T-Cadinol	0.1
2209	T-Muurolol	0.2
2219	δ-Cadinol (=α-Muurolol)	0.1
2255	α-Cadinol	0.7
2296	Myristicine	0.1
2931	Hexadecanoic acid	0.3
Total		98.3

RRI: Relative retention indices calculated against *n*-alkanes on the HP Innowax column.
%: Percentages were calculated from Flame Ionization Detector (FID) data.

sterile water. The serial dilutions were then transferred into 96-well microtiter plates in 100 μl aliquots, where the last row was filled only with water. Microorganism suspensions grown overnight were first diluted in double strength MHB and standardized to 10^8 CFU/ml (using McFarland No: 0.5) under sterile conditions. Then each micoorganism suspension was pipetted into each well in an equal volume and incubated at 37 °C for 24 h. Chloramphenicol and ampicillin were used as standard antibacterial agents whereas ketoconazole was used as a standard antifungal agent against *C. albicans*. Sterile distilled water and medium served as a positive growth control. The first well without turbidity was assigned as the minimum inhibitory concentration (MIC, in mg/ml). Average results of separately performed three experiments are given in Table 2.

2.6. Antioxidant activity

Two different methods were applied for the determination of antioxidant activity of the test materials as given below

2.6.1. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH·) scavenging assay

The ability of the fractions to scavenge DPPH radicals was determined by the method of Gyamfi, Yonamine, and Aniya (1999). A 50 μl aliquot of each fraction, in 50 mM tris-HCl buffer at pH 7.4, was mixed with 450 μl of tris-HCl buffer and 1.0 ml of 0.1 mM DPPH· diluted in MeOH. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated by the difference of absorbance of the control and the sample versus the absorbance of control multiplied by a factor of 100. The resulting IC_{50} values are presented as mean value of quadruplicate analyses in Table 3. BHT (butylated hydroxytoluene) and ascorbic acid were used as standard substances for comparison.

2.6.2. β-Carotene–linoleic acid co-oxidation inhibition assay

Antioxidant activity of essential oil of *C. libanoticum* was determined using β-carotene bleaching method (Oomah & Mazza, 1996; Velioğlu, Mazza, & Oomah, 1998). Briefly, 1 ml of β-carotene (0.2 mg/ml dissolved in chloroform) was added to the flask containing linoleic acid (40 mg) and Tween 80 (400 mg). Chloroform was evaporated using nitrogen, and 50 ml of distilled water was added and shaken vigorously. A control was prepared without sample or standards using the same procedure. Blanks of control and sample were also prepared without β-carotene. Absorbances were measured on a spectrophotometer at 470 nm. The samples were then subjected to thermal autoxidation by keeping them in a constant temperature water bath at 50 °C for 2 h. The rate of bleaching of β-carotene was monitored by recording the resulting absorbances at 15 min intervals (Abs^0, Abs^{120}; absorbance at 0 and 120 min, respectively). BHT and ascorbic acid were used as standards. Antioxidative activity (AA%) in percentages was calculated according to Oomah and Mazza (1996) using the following equation:

Table 2
Minimum inhibitory concentration (MIC, mg/ml) values for *C. libanoticum* essential oil

Pathogen	Source	CL	St1	St2	St3
Escherichia coli, (G−)	NRRL B-3008	0.5	0.0039	0.0156	nt
Pseudomonas aeruginosa, (G−)	NRRL B-23	0.25	0.0078	0.0156	nt
Enterobacter aerogenes, (G−)	NRRL 3567	0.5	0.0019	0.0078	nt
Salmonella typhimurium, (G−)	NRRL B-4420	0.5	0.0078	–	nt
Staphylococcus epidermidis, (G+)	ATCC 12228	0.25	0.0009	–	nt
Staphylococcus aureus (MR), (G+)	OGU	0.25	0.0312	0.25	nt
Candida albicans, yeast	OGU	0.5	nt	nt	0.0625

CL: *C. libanoticum* essential oil.
St1: Chloramphenicol (antibacterial).
St2: Ampicillin (antibacterial).
St3: Ketoconazole (antifungal).
MR: Methycillin Resistant *Staphylococcus aureus* (MRSA), clinical isolate.
OGU: Eskişehir Osmangazi University, Faculty of Medicine, clinical isolate.
nt: Not tested; – : Inactive.

Table 3
The antioxidant activity results of *C. libanoticum* essential oil

Sample	DPPH· radical scavenging activity [IC_{50}, mg/ml]	Inhibition of β-carotene/linoleic acid oxidation [AA%]
CL	>30	16.73 ± 0.12
BHT	0.078 ± 0.001[a]	93.16 ± 1.59
Ascorbic acid	0.102 ± 0.001	9.55 ± 1.51

[a] Mean ± SD ($n = 5$).

$$AA\% = [1 - (Abs^{0}_{sample} - Abs^{120}_{sample}) / (Abs^{0}_{control} - Abs^{120}_{control})] \times 100$$

3. Results and discussion

3.1. Chemical composition of the essential oil

The dried and crushed fruits of *C. libanoticum* yielded 1.5% of essential oil. The analysis of the oil was simultaneously performed using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The detected components of the essential oil of *C. libanoticum* and their relative percentages according to their relative retention indices (RRI) are given in Table 1. Seventy three components were characterized representing 98.3% of the oil. β-Phellandrene (17.6%), limonene (15.9%), β-pinene (8.8%), and sabinene (8.5%) were the major constituents of the oil. The oil of *C. libanoticum* was characterized by a high content of monoterpene hydrocarbons (71.8%). Sesquiterpene hydrocarbons comprised 21.8% of the essential oil of *C. libanoticum*. Oxygenated sesquiterpenes (3%), and oxygenated monoterpenes (1%) were present in relatively low amounts. Trace components (<0.1%) such as isoterpinolene, *cis*-alloocimene, α,*p*-dimethylstyrene, *cis*-1,2-limonene epoxide, 4,8-epoxyterpinolene, cyclosativene, β-cubebene, *trans*-*p*-menth-2-en-1-ol, β-ylangene, *cis*-*p*-menth-2-en-1-ol, (*E*)-2-decenal, *trans*-pinocarveol, *p*-mentha-1,8-dien-4-ol (=limonen-4-ol), γ-muurolene, α-terpineol, *cis*-carveol, *epi*-cubebol, cubebol, salvial-4(14)-en-1-one, (*E*)-nerolidol, cubenol, 1-*epi*-cubenol, and elemicine were also detected.

3.2. Antimicrobial activity

The essential oil rich in monoterpenes was tested for its *in vitro* antimicrobial activity using a broth microdilution assay (İşcan et al., 2002). A battery of common Gram (+) and Gram (−) human pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeast *C. albicans* were challenged against the essential oil with antimicrobial standard agents for comparison. The assay results showed that pathogens like *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and MRSA were more sensitive towards the essential oil, with an average inhibitory concentration of 0.25 mg/ml, than the remaining bacteria and the yeast *C. albicans*, as seen in Table 2. The overall MIC values in comparison with antimicrobial standard agents, however, suggest that the oil is moderate to weak in antibacterial and anticandidal activities. To the best of our knowledge, this is the first report on the antimicrobial properties of *C. libanoticum*.

Rollinger et al. (2003) demonstrated the antibacterial activity of falcarindiol of *C. aureum* and some of its constituents. The essential oil of *C. byzantinum* was effective against *Candida* species with a good inhibitory activity (Kürkçüoğlu et al., 2006). In agreement with Kalemba and Kunicka (2003), our results for monoterpene hydrocarbons displayed the lowest inhibitory activity against bacteria and yeast.

3.3. Antioxidant activity

The antioxidant capacity of the essential oil was evaluated using an *in vitro* radical scavenging activity test. The essential oil obtained from *C. libanoticum* was effective in scavenging of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·). In addition, the effect on inhibition of lipid peroxidation of

the essential oil was assayed using β-carotene bleaching and haemoglobin induced linoleic acid peroxidation methods.

Free radicals involved in the process of lipid peroxidation play a primary role in numerous chronic diseases and are implicated in the aging process. Phytochemicals recognized as possessing potent antioxidant activity are also strong scavengers of DPPH· (Depkevicius et al., 2002). Substances capable of donating electrons/hydrogen atoms are able to convert DPPH· into their non-radical form 1,1-diphenyl-2-picrylhydrazine, a reaction which can be followed spectrophotometrically. On the other hand, the cell walls contain unsaturated fatty acids such as linoleic and arachidonic acids. The cell permeability is changed after the oxidation of these fatty acids then chronic diseases may occur. The extension of shelf-life and control of deterioration of fatty foods can be achieved *via* the protection of these acids (Braca et al., 2003; Liyana-Pathirana & Shahidi, 2006). For this reason, the free radical scavenging activity and β-carotene/linoleic acid co-oxidation activity of the essential oil were investigated and the results are given in Table 3. BHT and ascorbic acid were used as positive controls in both test systems. The essential oil did not show any activity in the DPPH· scavenging test while it showed some activity in the β-carotene/linoleic acid emulsion system. The positive control BHT showed the highest activity in both activity tests, but essential oil was not as active as BHT. As the essential oil contained mainly monoterpene hydrocarbons such as β-phellandrene, limonene, β-pinene, and sabinene among others (Table 1), it is not surprising that a strong activity was not found in the essential oil of *C. libanoticum*.

In conclusion, essential oils and their components generally displayed strong antimicrobial and antioxidant properties, which are useful in daily life in foods and as preventive agents from various diseases. In the case of *C. libanoticum*, although there is no striking antimicrobial and antioxidant activity in the fruit essential oil, it is still worthwhile to investigate the other parts of the plant as a natural source for essential oil composition or for non-volatile fractions and their biological activities.

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