



Chemical Composition and Biological Activity of the Essential Oil of *Campanula olympica* Boiss.

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The present work describes the chemical composition and antimicrobial activity of the essential oil of *Campanula olympica* Boiss. which was isolated from the all parts of the plant by hydrodistillation and analyzed by GC-FID and GC-MS. Nineteen components representing 94.0 % of the total oil were characterized and the main components of this specie were found to be 2E,6Z-farnesol (14.8 %), 3,3-dimethyl-2[5-methoxy-3-methyl-2-pentylidenen]-1-cyclohexanone (12.1 %), dehydro aromadendrane (11.6 %), tetracosane (9.0 %), pentacosane (7.9 %), epoxy alloaromadendrene (5.9 %) and cyclohexadecanolide (5.8 %). The antimicrobial activity of the isolated essential oil of the plant was also investigated and it showed moderate antimicrobial and antifungal activities against *Escherichia coli*, *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, *Mycobacterium smegmatis* and *Candida albicans*.

Key Words: *Campanula olympica*, Essential oil, GC-FID/MS, Antimicrobial activity.

INTRODUCTION

The genus *Campanula* L. (Campanulaceae) is represented with 113 native species, 61 of them is endemics, in Turkey^{1,2}. Some species such as *C. rapunculus* L is used in Anatolian folk medicines against constipation and wound^{3,4}. *C. olympica* Boiss. is an Transcaucasian elements and distributed mainly in north and inner Anatolia. It is a biennial or perennial herb grown specially in alpine meadows, rocky and grassy slopes of ca. 15-2700 m above sea level⁵. As a result of our literature search, no published record has been found for the volatile chemical composition and antimicrobial activity of the essential oil of *Campanula olympica*.

EXPERIMENTAL

Campanula olympica Boiss. was collected from Akçaabat, Trabzon-Turkey (at a height of ca. 450 m) in June 2009. The plant was authenticated by Coskunçelebi^{1,2,5}. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 16650), Karadeniz Technical University, Turkey. The fresh plant was air-dried at room temperature for later analysis.

Isolation of the essential oils: The air-dried whole plant (100 g) of *C. olympica* was hydrodistilled in a Clevenger-type apparatus using cooling bath (-15 °C) system (4 h) (yield: 0.08 (v/w)). The obtained oil was extracted into in HPLC grade

n-hexane (0.5 mL), dried over anhydrous sodium sulphate and stored at 4-6 °C in a sealed brown vial.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis: GC-FID and GC-MS analyses were done as described previously⁶.

Identification of components: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C₆-C₃₂) as standards. Identification of individual components was made by comparison of their retention times with those of available analytical standards (α -pinene, eicosane, heneicosane, docosane, tricosane, tetracosane and pentacosane) and by computer search, matching mass spectral data with those held in Nist and Wiley library of mass spectra and literature comparison⁷⁻¹⁵.

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *E. coli* ATCC35218, *Y. pseudotuberculosis* ATCC911, *P. aeruginosa* ATCC43288, *E. faecalis* ATCC29212, *S. aureus* ATCC25923, *B. cereus* 709 Roma, *M. smegmatis* ATCC607 and *C. albicans* ATCC60193. The essential oil was weighed and dissolved in dimethyl sulphoxide to prepare extract stock solution of 6100 µg/mL.

Agar well diffusion method: The antimicrobial effects of the essential oil from *C. olympica* was tested quantitatively in respective broth media by using double dilution and the

minimal inhibition concentration (MIC) values ($\mu\text{g/mL}$) were determined^{16,17}. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. The *M. smegmatis* was grown for 3-5 days at 35 °C. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 μg), streptomycin (10 μg) and fluconazole (5 μg) were used as standard antibacterial and antifungal drugs, respectively. Dimethyl sulphoxide with dilution of 1:10 was used as solvent control. The results are shown in Table-3.

RESULTS AND DISCUSSION

The general chemical profile of the essential oil, the percentage content and retention indices of the constituents of *C. olympica* are summarized in Table-1. A total of 19 compounds were identified in the essential oil of *C. olympica* on the basis of a typical library search (NIST, WILEY), reference compounds and literature data⁷⁻¹⁵ with selecting only components showed matches exceeding 85 %, which represented about 94.0 % of the essential oils in *C. olympica*. The main compo-

Compounds	Area* (%)	Exp. RI**	Lit. RI
α -Pinene***	0.2	943	939
Nonanol	0.9	1167	1169
2E,4E-Nonadienal	1.1	1210	1212
Dehydro aromadendrane	11.6	1466	1463
Chamigrene	0.8	1500	1503
E- β -Guaiene	1.1	1501	1503
β -Bisabolene	1.7	1504	1506
Zonarene	5.3	1533	1530
Spathulenol	4.3	1578	1578
Epoxy alloaromadendrene	5.9	1640	1641
2E,6Z-Farnesol	14.8	1745	1746
3,3-Dimethyl-2[5-methoxy-3-methyl-2-pentylidenen]-1-cyclohexanone	12.1	1864	MS
Cyclohexadecanolide	5.8	1933	1935
Eicosane***	1.4	1998	2000
Heneicosane***	1.0	2099	2100
Docosane***	6.2	2198	2200
Tricosane***	2.9	2297	2300
Tetracosane***	9.0	2398	2400
Pentacosane***	7.9	2499	2500

*Percentage Area obtained by FID peak-area normalization. **RI calculated from retention times relative to that of *n*-alkanes (C₆-C₃₂) on the non-polar HP-5 column. ***Identified by authentic samples.

nents of the oil were 2E,6Z-farnesol (14.8 %), 3,3-dimethyl-2[5-methoxy-3-methyl-2-pentylidenen]-1-cyclohexanone (12.1 %), dehydro aromadendrane (11.6 %), tetracosane (9.0 %), pentacosane (7.9 %), epoxy alloaromadendrene (5.9 %) and cyclohexadecanolide (5.8 %).

The chemical class distribution of the essential oil components are reported in Table-2. The compounds are classified into five classes, which are terpenoids (monoterpene hydrocarbon, sesquiterpene hydrocarbons and oxygenated sesquiterpenes), ester, aldehydes, hydrocarbons and others. As seen in Table-2, hydrocarbons (28.4 %), oxygenated sesquiterpenes (25.0 %) and sesquiterpene hydrocarbons (20.5 %) were the main constituents in the essential oil of *C. olympica*.

TABLE-2
CHEMICAL CLASS DISTRIBUTION OF
THE ESSENTIAL OIL FROM *C. olympica**

Constituents	Area (%)	NC*
Terpenoids		
Monoterpene hydrocarbon	0.2	1
Sesquiterpene hydrocarbons	20.5	5
Oxygenated sesquiterpenes	25.0	3
Ester	5.8	1
Aldehyde	1.1	1
Hydrocarbons	28.4	6
Others	13.0	2
Total	94.0	19

*NC: Number of compounds.

The antimicrobial activity for the essential oil of *C. olympica* was tested *in vitro* using the agar-well diffusion method^{16,17} with the microorganisms (Table-3). The essential oil showed moderate antimicrobial and antifungal activity against *E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *B. cereus*, *M. smegmatis* and *C. albicans*. The minimal inhibition concentration (MIC) values for bacterial strains for the essential oil of *C. olympica*, were from 152.2-305 $\mu\text{g/mL}$, respectively. Previously mentioned antimicrobial activities of the extract from the leaf of *Campanula betulifolia*¹⁸ showed no activity against the *E. coli*, *B. catarrhalis*, *S. aureus*, *B. subtilis*, *H. pylori*, *C. albicans* and *T. rubrum*, that could be due to the different extracts in the *Campanula* taxa.

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TABLE-3
SCREENING RESULT FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM *C. olympica* ($\mu\text{g}/100 \mu\text{L}$)

Sample	Stok sol. ($\mu\text{g}/0.8 \text{ mL}$)	Microorganisms and minimal inhibition concentration							
		<i>E. coli</i>	<i>Y. pseudotuberculosis</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>M. smegmatis</i>	<i>C. albicans</i>
<i>C. olympica</i>	6100	152.2	152.2	305	305	305	> 305	152.2	152.2
Ampicillin	10	10	> 18	> 18	10	35	15	-	-
Streptomycin	10	-	-	-	-	-	-	35	-
Fluconazole	5	-	-	-	-	-	-	-	25

(-): No activity at stock solution concentration. The values are the average of three determinations.

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