



## Antimicrobial Activity and Volatile Constituents of *Omphalodes cappadocica* (Willd.) DC.

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The composition of the essential oil isolated by hydrodistillation from *Omphalodes cappadocica* (Willd.) DC. (Boraginaceae) collected from 'Of', a town in Trabzon, was determined by GC-FID and GC-MS. Sixty components were identified corresponding 90.8 % of the oil. The major components were benzaldehyde (18.1 %), nonanal (10.0 %), 2E-nonenal (8.1 %), tricosane (4.4 %), octanal (3.9 %), 2-pentyl furan (3.8 %), 2E,6Z-nonadienal (3.7 %) and 2E,4E-decadienal (2.7 %). In addition, antimicrobial activity of the essential oil of *O. cappadocica* was investigated against *Escherichia coli*, *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Mycobacterium smegmatis* and *Candida albicans* and *Saccharomyces cerevisiae* at maximum essential oil concentration in hexane of 1000 µg/mL. They showed only antibacterial activity against bacteria *E. coli*, *S. aureus*, *B. cereus*, *M. smegmatis* and the fungicidal activity against *C. albicans* and *S. cerevisiae*.

**Key Words:** *Omphalodes cappadocica*, Essential oil, Antimicrobial activity, Gas chromatography, GC-MS.

### INTRODUCTION

Family *Boraginaceae* to which *Omphalodes* belong, has been represented by more than 30 annual, perennial or dwarf shrubby genera in Turkish flora<sup>1</sup>. The genus has 7 taxa in Turkey, 4 of which are endemic to Turkey<sup>1</sup>. *Omphalodes cappadocica* (Willd.) DC. (Boraginaceae) contains an euxine element that occur only in Caucasian Biodiversity Hotspot area of Georgia and Turkish Caucasus<sup>1</sup>. It grows under the forest, shady banks and damp rock ledges and nearly 20-1000 m a.s.l in Turkey. Ethnobotanical uses of the species have not been recorded yet in Turkey.

Previous phytochemical investigation of the genus of *Omphalodes* revealed the presence of alkaloids and phenolic compounds<sup>2-5</sup>. However, to our knowledge no report has been mentioned concerning the composition and antimicrobial activity of the essential oil of this plant. The essential oil constituents of *O. cappadocica* were obtained by the widely used hydrodistillation method in a Clevenger-type apparatus and analyzed by GC-FID and GC-MS technique<sup>6-16</sup>. Identification of the compounds was made by a typical library search (Nist, Wiley), authentic compounds and literature comparison<sup>6-16</sup>.

### EXPERIMENTAL

*O. cappadocica* was collected in May 2009 from Of, a town in Trabzon, (at heights of ca. 120 m) in the northeastern

part of Turkey. The plant was authenticated by Terzioğlu<sup>1</sup>. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 12471), Karadeniz Technical University, Turkey.

**Isolation of the essential oils:** Crude essential oil of *O. cappadocica* was obtained from the air-dried whole plant (ca. 40 g) by hydrodistillation in a Clevenger-type apparatus with cooling bath (-15 °C) system (4 h) (yields: 0.12 % and (v/w), respectively). The obtained oil was extracted with HPLC grade *n*-hexane (0.5 mL) and 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 previously<sup>7</sup>.

**Identification of compounds:** Retention indices of all the components were determined by Kovats method using *n*-alkanes (C<sub>6</sub>-C<sub>32</sub>) as standards. The identification of the GC peaks corresponding to the components of the essential oil was based on direct comparison of the retention times and mass spectral data with those for standard compounds ( $\alpha$ -pinene, limonene, undecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, eicosane, heneicosane, docosane, tricosane, tetracosane and pentacosane), computer matching with the Nist and Wiley library and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature<sup>6-16</sup>.

**Antimicrobial activity assessment:** All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus* 702 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193 and *Saccharomyces cerevisiae* RSKK 251. The sample was dissolved in hexane to prepare extracts stock solution (1000 µg/mL).

**Agar well diffusion method:** Simple susceptibility screening test using agar-well diffusion method<sup>17</sup> as adapted earlier<sup>18</sup> was used. Each bacterium was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth. The yeast like fungi was suspended in Yeast extracts broth. Then the microorganisms were diluted approximately 10<sup>6</sup> colony forming unit (cfu) per mL. For yeast like fungi, Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) were used. They were "flood-inoculated" onto the surface of Mueller Hinton agar and Sabouraud Dextrose agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 50 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. The *Mycobacterium smegmatis* was grown for 3-5 days on Mueller Hinton agar plates at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg), streptomycin (10 µg) and fluconazole (5 µg) were standard drugs.

## RESULTS AND DISCUSSION

The results obtained by GC and GC-MS analysis of the essential oil of *O. cappadocica* are presented in Table-1. The yield was 0.12 % (v/w). Sixty components which constitute 90.8 %, were identified in oil. The major compounds of the essential oil were benzaldehyde (18.1 %), nonanal (10.0 %), 2E-nonenal (8.1 %), tricosane (4.4 %), octanal (3.9 %), 2-pentyl furan (3.8 %), 2E,6Z-nonadienal (3.7 %) and 2E,4E-decadienal (2.7 %). The chemical class distributions of the essential oils of the constituents are summarized in Table-2. The compounds were separated into four classes, which were terpenoids (monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, diterpenes, terpene related compounds), aldehydes, hydrocarbons and others (Table-2). Aldehydes were shown to be the main group of constituents of the plant in the ratio of 56.6 %. Seventeen terpenoids (11.6 %) were identified in the oil of *O. cappadocica*. As a result, the essential oil of *O. cappadocica* was rich in aldehydes. The mass spectral data of all these compounds were identical with published data for the respective compounds<sup>6</sup>, only differing occasionally in the relative intensity of some minor fragments.

The antimicrobial activity for the essential oil of *O. cappadocica* was tested *in vitro* using the agar-well diffusion method<sup>17,18</sup> with the microorganisms as seen in Table-3 at maximum essential oil concentration in hexane of 1000 µg/mL, by using ampicillin and fluconazole as standard antibacterial and antifungal agents. The test extracts showed antimicrobial activity against bacteria *E. coli*, *S. aureus*, *B. cereus*, *M. smegmatis*

TABLE-1  
IDENTIFIED COMPONENTS IN THE  
ESSENTIAL OILS OF *O. cappadocica*

Compound	Exp. RI	Lit. RI	Area (%)
2E-Hexenal	858	855	0.3
α-Pinene	930	933	0.5
2E-Heptenal	952	954	0.4
Benzaldehyde	959	960	0.9
1-Octen-3-one	979	980	0.4
1-Octen-3-ol	980	979	1.4
3-Octanone	982	984	2.6
2-Pentyl furan	989	991	3.8
2E,4Z-Heptadienal	997	998	0.2
Octanal	1004	999	3.9
2E,4E-Heptadienal	1013	1014	1.5
Limonene	1029	1029	0.4
2,2,6-Trimethyl-cyclohexanone	1036	1035	0.2
Benzene acetaldehyde	1045	1042	18.1
2-Octenal	1062	1061	1.0
3E,5Z-Octadien-2-one	1073	1072	2.3
3E,5E-Octadien-2-one	1094	1092	0.3
Undecane	1100	1100	1.1
Nonanal	1106	1101	10.0
2E,6Z-Nonadienal	1158	1155	3.7
2E-Nonenal	1165	1162	8.1
Menthol	1172	1172	0.9
Safranal	1197	1197	0.9
Decanal	1206	1202	1.2
2E,4E-Nonadienal	1215	1212	0.5
β-Cyclocitral	1221	1223	1.1
2,6,6-Trimethyl 1-cyclohexene acetaldehyde	1258	1261	0.2
2E-Decenal	1266	1264	0.5
2E,4Z-Decadienal	1294	1293	0.8
Tridecane	1300	1300	0.8
Dehydro elsholtzia ketone	1303	1303	0.2
Undecanal	1309	1307	0.2
2E,4E-decadienal	1320	1317	2.7
Tetradecane	1400	1400	0.4
(Z) β-Farnesene	1442	1443	0.2
Geranyl acetone	1456	1455	0.4
Farnesane	1462	1462	0.9
(E) β-Ionone	1486	1489	2.2
Pentadecane	1500	1500	0.5
β-Bisabolene	1506	1506	0.3
Tridecanal	1512	1510	0.2
Caryophyllene oxide	1580	1583	0.4
Hexadecane	1600	1600	0.3
Tetradecanal	1613	1613	1.4
(Z) Asarone	1677	1676	0.4
Heptadecane	1698	1700	0.2
Pentadecanal	1713	1714	0.6
Octadecane	1799	1800	0.2
Hexadecanal	1814	1817	0.4
Hexahydrofarnesyl acetone	1846	1847	1.9
Diisobutyl phthalate	1866	1867	1.4
Farnesyl acetone	1918	1919	0.8
3E-Cembrene A	1951	1949	0.3
Eiocosane	1997	2000	0.2
Heneicosane	2095	2100	0.2
Docosane	2196	2200	0.4
Tricosane	2297	2300	4.4
Tetracosane	2398	2400	0.6
Pentacosane	2495	2500	0.5
Total isolate			90.8

<sup>a</sup>RI calculated from retention times relative to that of *n*-alkanes (C<sub>6</sub>-C<sub>32</sub>) on the non-polar HP-5 column. <sup>b</sup>Percentages obtained by FID peak-area normalization. <sup>c</sup>Identified by authentic samples.

TABLE-3  
SCREENING FOR ANTIMICROBIAL ACTIVITY IN THE ESSENTIAL OIL OF *O. cappadocica* (50 µL)

Sample	Conc. (µg/mL)	Microorganisms and inhibition zone (mm)									
		Ec	Yp	Pa	Sa	Ef	Li	Bc	Ms	Ca	Sc
<i>O. cappadocica</i>	1000	6	–	–	6	–	–	6	7	7	10
Ampicillin	10	10	18	18	35	10	10	15	–	–	–
Streptomycin	10	–	–	–	–	–	–	–	35	–	–
Fluconazole	5	–	–	–	–	–	–	–	–	25	>25

Ec: *Escherichia coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Li: *Listeria monocytogenes*, Bc: *Bacillus cereus*, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, Sc: *Saccharomyces cerevisiae*, (–): no activity.

TABLE-2  
CHEMICAL CLASS DISTRIBUTION IN  
THE ESSENTIAL OILS OF *O. cappadocica*

Compound class	Area (%)	NC*
Terpenoids		
Monoterpenes	0.9	3
Oxygenated monoterpenes	3.3	5
Sesquiterpenes	1.4	3
Oxygenated sesquiterpenes	0.4	1
Diterpenes	0.3	1
Terpene related compounds	5.3	4
Aldehydes	56.6	21
Hydrocarbons	9.8	13
Others	12.8	9

\*NC: Number of compounds.

and the fungus *C. albicans* and *S. cerevisiae*, but no antimicrobial activity was observed against the bacteria *Y. pseudotuberculosis*, *P. aeruginosa*, *E. faecalis* and *L. monocytogenes*.

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