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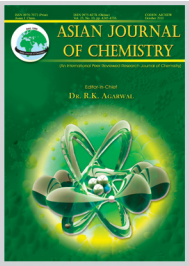

Antimicrobial Activity and Chemical Composition of the Essential Oil from *Campanula glomerata* L. Subsp. *Hispida* (Witasek) Hayek

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The volatile components of the essential oil from *Campanula glomerata* L. subsp. *hispida* (Witasek) Hayek was analyzed by GC and GC-MS. Forty-eight compounds representing 89.0 % of the total oil were characterized and the main constituents of this specie were found to be hexadecanoic acid (24.51 %), docosane (15.9 %), isocitronellene (12.6 %), heneicosane (4.6 %), hexahydrofarnesyl acetone (3.2 %), 9-tricosene (1.6 %), octadecanol (1.4 %), caryophyllene oxide (1.3 %), α-funebrene (1.2 %), β-thujaplicinol (1.1 %), pentadecanoic acid (1.1 %), tricosane (1.1 %), (2*E*,4*E*)-decadienal (1.0 %), (E)-β-damascenone (1.0 %) and (*E*)-caryophyllene (1.0 %). The antimicrobial activity of the isolated essential oil of the plant was also investigated and it showed moderate antimicrobial and antifungal activities against *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Mycobacterium smegmatis, Candida albicans* and *Saccharomyces cerevisiae*.

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

INTRODUCTION

The genus *Campanula* L. belongs to the Campanulacea family. There are more than 300 species in the temperate and subtropical region of the northern hemisphere[1]. The genus *Campanula* is represented with 113 native species, 61 of them is endemics, in Turkey[1-3]. Some species of *Campanula* were sued as folk medicines against cough, constipation and wound[4,5]. Phytochemical investigation of the genus of *Campanula glomerata* revealed the presence of anthocyanin, flavonoid, polyacetylenes, triterpene saponins, quercetin diglycoside and flavonolic biosides compounds[6-14]. Previous works on the chemical composition of the essential oils of some *Campanula* included *Campanula olympica* Boiss[14], *Campanula punctuate* Lam[15], *Campanula rhomboidalis* and *Campanula barbata*[16].

The main components of *C. olympica* were found to be 2*E*,6Z-farnesol (14.8 %), 3,3-dimethyl-2[5-methoxy-3-methyl-2-pentylidenen]-1-cyclohexanone (12.1 %), dehydro aroma-dendrane (11.6 %), tetracosane (9.0 %), pentacosane (7.9 %), epoxy alloaromadendrene (5.9 %) and cyclohexadecanolide (5.8 %)[14]. Forty-seven volatile compounds has been identified for the analysis of solvent free extraction (SFE) compositions in *C. punctuate*[15]. Mono and sesquiterpenoids were identified from *Campanula rhomboidalis* Gorter and *Campanula barbata* L.[16]. Furthermore, biological activities (antimicrobial and antioxidant) of *C. olympica*[14], *C. punctuate*[15] and *C. betulifolia*[17] have been reported. However, to the best of our knowledge, no report has been mentioned concerning the composition and antimicrobial activity of the essential oil of this plant.

EXPERIMENTAL

Campanula glomerata subsp. *hispida* was collected from Koyulhisar, Sivas-Turkey (at a height of *ca.* 1650 m) in August 2009. The plant was authenticated by Prof. S. Terzioglu[1-3]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 16626), Karadeniz Technical University, Turkey. The fresh plant was air-dried at room temperature for later analysis.

Isolation of the essential oil: The air-dried whole plant (74 g) of *C. glomerata* subsp. *hispida* was hydrodistilled in a Clevenger-type apparatus using cooling bath (*ca.* 15 ºC) system (4 h) (yield: 0.07 (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 ºC in a sealed brown vial.

Gas chromatography and Gas chromatography-mass spectrometry analysis: GC-FID and GC-MS analyses were done as described previously[18,19].

Identification of components: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds (limonene, linalool, α-terpineol, heneicosane, docosane, tricosane, tetracosane and pentacosane) and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature[20-33].

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 auroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607 and *Candida albicans* ATCC 60193. The essential oil was dissolved in hexane to prepare chemicals stock solution of 16.200 µg /40 µL.

Agar well diffusion method: Simple susceptibility screening test using agar-well diffusion method[34] as adapted earlier[35] 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 10^6 colony forming unit (cfu) per mL. For yeast like fungi, Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) were used. Brain Heart Infusion Agar (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*. They were flood-inoculated onto the surface of Mueller Hinton and Sabouraud Dextrose agars and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 40 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 ºC. The *M. smegmatis* was grown for 3 days on Brain Heart Infusion agar plates[36] at 35 ºC. 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. Hexane was used as solved control.

RESULTS AND DISCUSSION

The essential oil of *C. glomerata* subsp. *hispida* was obtained by the widely used hydrodistillation method in a Clevenger-type apparatus and analyzed by GC-FID and GC-MS[20-33]. The general chemical profile of the essential oil, the percentage content and retention indices of the constituents of *C. glomerata* subsp. *hispida* are summarized in Table-1. A total of 48 compounds were identified in the essential oil of *C. glomerata* subsp. *hispida* on the basis of a typical library search (Nist, Wiley), authentic compounds and literature comparison[20-33] with selecting only components showed matches exceeding 85 %, which represented about 89 % of the essential oils in *C. glomerata* subsp. *hispida*. Five components were not characterized (5.4 %). The chemical class distribution of the essential oil components are reported (Table-1). The compounds are classified into seven classes, which are terpenoids (28.5 %, monoterpene hydrocarbon, oxygenated monoterpenes, oxygenated sesquiterpene, sesquiterpene hydrocarbons and terpene related compunds) aldehydes (4.6 %), hydrocarbons (26.3 %), alcohols (1.9 %), esters (3.5 %), carboxylic acids (25.6 %) and other (0.7 %). Monoterpene hydrocarbons (13.7 %) were the major constituents in the terpenoid constituents of *C. glomerata* subsp. *hispida*. The main components of the oil were hexadecanoic acid (24.51 %), docosane (15.9 %), isocitronellene (12.6 %), heneicosane (4.6 %), hexahydrofarnesyl acetone (3.2 %), 9-tricosene (1.6 %), octadecanol (1.4 %), caryophyllene oxide (1.3 %), α-funebrene (1.2 %), β-thujaplicinol (1.1 %), pentadecanoic acid (1.1 %), tricosane (1.1 %), (2*E*,4*E*)-decadienal (1.0 %), (*E*)-β-damascenone (1.0 %) and (*E*)-caryophyllene (1.0 %), which were quite different form the main compounds of *C. olympica*[14] (2*E*,6*Z*-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 %)), *C. rhomboidalis* and *C. barbata*[16] (mono and sesquiterpenoids).

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OIL OF *C. glomerata* subsp. *hispida.*

Compounds	Area[a] (%)	Exp. RI[b]	Lit. RI
Santolina triene	0.7	891	909
Isocitronellene	12.6	920	924
Allyl isovalerate	0.8	935	938
2-Pentyl furan	0.7	990	991
4,4-Dimethyl-2-pentene	0.6	990	972
p-Cymene	0.2	1023	1025
Limonene[c]	0.2	1027	1029
2-Phenyl acetaldehyde	0.3	1044	1042
(2*E*)-Octenal	0.3	1058	1058
Linalool[c]	0.4	1097	1097
Nonanal	0.7	1102	1101
trans-Pinocarveol	0.3	1137	1139
α-Terpineol[c]	0.3	1189	1189
Myrtenal	0.3	1193	1196
α-Ionene	0.3	1251	1255
1-Decanol	0.3	1269	1272
(2*E*,4*E*)-Decadienal	1.0	1314	1317
Undecanol	0.2	1370	1370
2-Butyl-2-octenal	0.3	1371	1367
(*E*)-β-Damascenone	1.0	1383	1385
α-Funebrene	1.2	1389	1403
(*E*)-Caryophyllene	1.0	1417	1419
β-Duprezianene	0.3	1422	1423
Geranyl acetone	0.4	1455	1455
2,6-Di(*t*-Butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	0.6	1472	1475
(*E*)-β-Ionene	0.5	1485	1489
α-Muurolene	0.4	1499	1500
γ-Cadinene	0.4	1510	1514
δ-Cadinene	0.6	1519	1523
β-Thujaplicinol	1.1	1532	1538
α-Calacorene	0.4	1541	1546
Spathulenol	0.4	1575	1578
Caryophyllene oxide	1.3	1583	1583
Amiyl cinnamaldehyde	0.2	1646	1649
Selin-11-en-4α-ol	0.4	1660	1660
Myristaldehyde	0.9	1731	1737
(*Z*)-2-Hexyl-cinnamaldehyde	0.9	1748	1750
Hexahydrofarnesyl acetone	3.2	1847	1848
Pentadecanoic acid	1.1	1862	1866
Methyl hexadecanoate	0.6	1920	1922
Hexadecanoic acid	24.5	1983	1983

TABLE-2
SCREENING RESULT FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM *C. glomerata* subsp. *hispida.*

Sample	Stok Sol. µg/40 µL	Microorganisms and inhibition zone (mm)							
		Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Bc*	*Ms*	*Ca*
C. glomerata subsp. *hispida.*	16.200	-	-	6	10	8	10	25	25
Ampicillin	10	10	10	18	35	10	15		
Streptomycin	10							35	
Fluconazole	5								25

Ec*: Escherichi coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, *Saccharomyces cerevisiae*, (-): no activity

Compounds	Area[a] (%)	Exp. RI[b]	Lit. RI
1-Octadecanol	1.4	2078	2078
Heneicosane[c]	4.6	2099	2100
Docosane[c]	15.9	2200	2200
9-Tricosene	1.6	2280	2281
Tricosane[c]	1.1	2300	2300
Tetracosane[c]	0.4	2400	2400
Pentacosane[c]	2.1	2500	2500
Isolate	89.0		
Un-1	2.0	856	MS1
Un-2	1.2	882	MS2
Un-3	0.8	914	MS3
Un-4	0.7	931	MS4
Un-5	0.7	1160	MS5
Total unknown	5.4		
Total isolate	94.4		
Terpenoids		Number of compounds	
Monoterpene hydrocarbons	13.7	4	
Oxygenated monoterpenes	1.3	4	
Oxygenated sesquiterpene	2.1	3	
Sesquiterpene hydrocarbons	4.3	7	
Terpene related compounds	7.1	7	
Aldehydes	4.6	8	
Hydrocarbons	26.3	7	
Alcohols	1.9	3	
Esters	1.4	2	
Carboxylic acids	25.6	2	
Other	0.7	1	

MS1: 85(100), 75(54), 71(10), 58(30); MS2: 89(60), 85(100), 72(56), 57(70), 53(5); MS3: 136(8), 121(20), 93(32), 85(100), 75(24), 57(92); MS4: 85(100), 69(14), 61(28), 57(66), 51(8); MS5: 150(8), 134(22), 120(76), 91(62), 70(74), 55(100). [a]% Area obtained by FID peak-area normalization. [b]RI calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the non-polar HP-5 column. [c]Identified by authentic samples

The antimicrobial activity for the essential oil of *C. glomerata* subsp. *hispida* was tested *in vitro* using the agar-well diffusion method[34-36] with the microorganisms (Table-2). The essential oil showed moderate antimicrobial and antifungal activity against *P. aeruginosa*, *S. aureus, E. faecalis, B. cereus, M. smegmatis, C. albicans* and *S. cerevisiae*. The minimal inhibition zone values for bacterial strains for the essential oil of *C. glomerata* subsp. *hispida*, were from 6 to 25 mm, respectively. Previously mentioned antimicrobial activities of the extract from the leaf of *C. betulifolia*[17] showed no activity against the *E. coli, B. catarrhalis, S. aureus, B. subtilis, H. pylori, C. albicans* and *T. rubrum*. But, the essential oil of *C. olympica* showed moderate antimicrobial and antifungal activity[14] against *E. coli, Y. pseudotuberculosis, P. aeruginosa, E. faecalis, S. aureus, B. cereus, M. smegmatis* and *C. albicans* with the minimal inhibition concentration values for bacterial strains from 152.2 µg/mL to 305 µg/mL, respectively. That could be due to the different extracts in the *Campanula* taxa.

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