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Essential Oil Composition and Antimicrobial Activity of *Inula thapsoides* subsp. thapsoides from Turkey

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The composition of the essential oil isolated from the air-dried *Inula thapsoides* subsp. thapsoides was analyzed by GC-MS. 51 Components were identified in the essential oil and the main components of this species were dihydro edulan (12.4 %), β-selinene (9.9 %), caryophyllene oxide (9.0 %), pentacosane (8.3 %) and epi-α-cadinol (5.2 %). The isolated essential oil of *I. thapsoides* subsp. thapsoides was also tested for antimicrobial activity against the bacteria *E. coli, Y. pseudotuberculosis, K. pneumoniae, S. marcescens, E. faecalis, S. aureus, B. subtilis* and the fungus *C. albicans* and *C. tropicalis* at maximum essential oil concentration in acetone of 1000 μg/mL and they showed moderate antibacterial activity against Gram-positive and Gram-negative bacteria.

Key Words: *Inula thapsoides* subsp. thapsoides, Asteraceae, Essential oil, Antimicrobial activity, GC-MS.

INTRODUCTION

Inula (asteraceae) represented with 27 species in Turkey and 6 of them are endemic[1,2]. *Inula thapsoides* (Bieb. ex Willd.) Sprengel subsp. thapsoides is a bush like plant and grows in east, northeast and west of Anatolia in the rocky and rare woodland place. As part of a phytochemical investigation of *I. thapsoides* subsp. thapsoides, guaianolides, eudesmanolides, sclareol, 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol, β-sitosterol, stigmasterol and stigmasterol 3β-D-glucoside were isolated[3]. The objective of this study was to investigate major volatiles and antimicrobial activity of the essential oil from *I. thapsoides* subsp. thapsoides by GC-MS.

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EXPERIMENTAL

I. thapsoides subsp. thapsoides was collected from Haneke plateaue, Kelkit-Ünlüpinar, Gümüshane: (at a height of *ca.* 2000 m) in the North-Eastern part of Turkey in July 2004. The plant was authenticated immediately after collection and air-dried at room temperature for later analysis[1,2]. Voucher specimens (No. Coskunçelebi 491-2004 KTUB) were deposited in the Herbarium of the Department of Biology, Karadeniz Technical University, Turkey.

Isolation of the essential oil: Crude essential oil of *I. thapsoides* subsp. thapsoides was obtained from the air-dried crushed material (*ca.* 69 g) by hydrodistillation in a Clevenger-type apparatus with cooling bath (-15 ºC) system (3 h) (yield: 0.10 % (v/w)). The oils were taken by HPLC grade *n*-hexane (0.5 mL) and dried over Na_2SO_4 kept at 4 ºC in a sealed brown vial. 1 mL of the extracts was directly injected into the GC-MS instruments.

Gas chromatography/mass spectrometry (GC-MS): GC-MS analyses were as described previously[4].

Identification of components: The identity of the components was achieved from their retention indices, calculated by linear interpolation relative to retention times of series of *n*-alkanes (C_6-C_{32}) and their mass spectra, which were compared with those from literature library (NIST and Willey) and data[5-8].

Antimicrobial activity assessment: All test microorganisms were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows: Ec: *Escherichia coli* ATCC 35218, Yp: *Yersinia pseudotuberculosis* ATCC 911, Kp: *Klebsiella pneumoniae* ATCC 13883, Sm: *Serratia marcescens* ATCC 13880, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bs: *Bacillus subtilis* ATCC 6633, Ca: *Candida albicans* ATCC 60193, Ct: *Candida tropicalis* ATCC 13803.

Agar dilution MIC assay: Using a modification of the assay described in the literature[9,10], the samples were added to molten Mueller-hinton agar (MHA) and potato dextrose agar (PDA)/Tween 20 medium at 48 ºC, to give concentrations ranging from 50 to 1000 µg/mL. The antibacterial assay was performed in Mueller-Hinton broth (MHB) (Difco, Detroit, MI) at pH.7.3 containing 1 % agar and 0.25 % Tween 20. The antifungal assay was performed in PDA (Difco, Detroit, MI) at pH 6.2 containing 0.25 % Tween 20. Plates prepared in triplicate were spot inoculated with 3 µL aliquots of culture in MHB adjusted to yield a density within McFarland 0.5 turbidity. Plates were incubated at 35 ºC for 18 h and the minimal inhibition concentration (MIC) was determined as the lowest concentration of the samples to result in no growth of the inoculum on two of three plates. The essential oils were dissolved in hexane to prepare the stock solutions. Acetone was used as control. Ceftazidime and triflucan were standard drugs.

RESULTS AND DISCUSSION

The composition of essential oil of *I. thapsoides* subsp. thapsoides was analyzed by GC-MS with HP-5 column. A total of 51 components were characterized on the basis of a typical library search and literature data with selecting only the components showing matches exceeding 80 %, which represented about 81.9 % of total composition of the essential oils in *I. thapsoides* subsp. thapsoides[5-8]. The general chemical profile of the essential oil, the percentage content and the retention indices of the constituents are summarized in Table-1.

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OIL OF *I. thapsoides* subsp. *thapsoides*[a,b]

No	Compounds	Exp. RI	Lit. RI	Q (%)	Area (%)
1	α-Pinene	940	939	94	0.6
2	2-Pentyl furan	998	998	91	0.7
3	α-Terpinene	1017	1017	97	0.1
4	*o*-Cymene	1028	1026	93	0.2
5	γ-Terpinene	1060	1060	97	0.2
6	Terpinolene	1086	1089	96	0.1
7	*cis*-Linalool oxide	1088	1087	91	0.3
8	Camphor	1144	1146	96	0.2
9	Safranal	1198	1197	95	0.1
10	Decanal	1205	1202	91	0.1
11	β-Cyclositral	1221	1218	89	0.1
12	*cis*-Chrysanthenyl acetate	1265	1265	80	0.7
13	Dihydro edulan	1290	1289	90	12.4
14	Theaspirane A	1299	1298	91	0.4
15	(2E,4E)-Decadienal	1317	1317	83	0.5
16	(E)-β-Damascenone	1387	1385	97	1.2
17	Cyperene	1400	1399	99	0.7
18	Isocaryophyllene	1409	1409	98	0.3
19	*trans*-Caryophyllene	1421	1419	97	2.3
20	2,5-Dimetoksi-*p*-Cymene	1426	1427	95	0.3
21	*cis*-Thujopsene	1429	1431	83	0.5
22	*endo*-Arbozol	1434	1435	83	0.5
23	α-Humulene	1455	1455	83	0.5
24	*allo*-Aromadendrene	1463	1460	98	0.6
25	β-Selinene	1487	1490	83	9.9
26	Nerylisobutanoate	1492	1491	91	0.3
27	α-Selinene	1496	1498	97	0.7
28	Pentadecane	1500	1500	99	0.3
29	Geranyl isobutanoate	1515	1515	98	3.1

No	Compounds	Exp. RI	Lit. RI	Q (%)	Area (%)
30	δ-Cadinene	1526	1523	80	0.9
31	α-Calacorene	1546	1546	80	0.4
32	Nerolidol	1566	1563	90	0.4
33	Caryophyllene oxide	1586	1583	98	9.0
34	Humulene epoxide II	1610	1608	93	0.5
35	Silphiperfol-6-en-5-one	1626	1626	82	0.3
36	Caryophylla-4(14),8(15)-dien-5-β-ol	1638	1641	98	3.2
37	*epi*-α-Cadinol	1645	1640	91	5.2
38	α-Cadinol	1657	1654	99	1.8
39	Selin-11-en-4α-ol	1661	1660	87	0.5
40	Benzyl benzoate	1763	1760	98	0.8
41	Hexahydrofarnesylacetone	1847	1845	99	2.5
42	Nonadecane	1900	1900	97	0.5
43	Farnecyl acetone	1917	1914	80	0.7
44	Heneicosane	2101	2100	89	0.5
45	(Z)-Phytol	2113	2114	87	2.9
46	Docosane	2200	2200	96	0.3
47	Tricosane	2300	2300	98	2.9
48	Tetracosane	2400	2400	98	0.7
49	Pentacosane	2500	2500	98	8.3
50	Heptacosane	2700	2700	98	1.3
51	Nonacosane	2900	2900	96	0.4
	Total				81.9

Unknown	RI	m/z (%)	Q (%)
Un-1	1395	192(2), 174(8), 134(58), 119(100), 91(38), 79(16), 55(8)	1.8
Un-2	1405	207(1), 176(18), 121(76), 107(100), 91(78), 71(44), 55(24)	1.1
Un-3	1557	204(5), 189(52), 119(60), 105(82), 91(100), 77(54), 55(56)	4.1
Un-4	1561	253(4), 204(18), 189(64), 161(62), 105(82), 91(100), 55(64)	4.1
Un-5	1579	250(8), 207(2), 180(100), 165(78), 91(10), 77(6), 55(4)	1.0
Total unknown			12.1
Total isolate			81.9
Total			94.0

[a]RI, retention index; LRI, literature retention index; Q: Quality; [b]Compounds are listed in order of elution. RI (retention index) values are calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the non-polar HP-5 column.

The main constituents of the essential oil of *I. thapsoides* subsp. thapsoides were dihydro edulan (12.4 %), β-selinene (9.9 %), caryophyllene oxide (9.0 %), pentacosane (8.3 %) and epi-α-cadinol (5.2 %). The compounds were separated into six classes, which were monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenoid and others. The chemical class distribution and the main components in each class of the essential oil of *I. thapsoides* subsp. thapsoides are reported in Table-2.

TABLE-2
CHEMICAL CLASSIFICATION AND THE MAIN COMPONENTS IN EACH CLASS OF THE ESSENTIAL OIL OF *I. thapsoides* subsp. *thapsoides*

Compound class	Area (%)	Number of compounds	Major component	Exp. RI
Monoterpens	1.2	5	α-Pinene	940
Monoterpenoids	19.6	12	Dihydro edulan	1290
Sesquiterpens	16.8	10	β-Selinene	1487
Sesquiterpenoids	24.1	10	Caryophyllene oxide	1586
Diterpenoid	2.9	1	(Z)-Phytol	2113
Others	17.3	13	Pentacosane	2500

TABLE-3
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL COMPONENT OF *I. thapsoides* subsp. *thapsoides* (MIC µg/mL)

Sample	Stock (µg/ML)	Microorganisms and inhibition zone (mm)								
		Ec	Yp	Kp	Sm	Ef	Sa	Bs	Ca	Ct
I. thapsoides subsp. *thapsoides*	1000	-	+	-	-	+	++	-	-	-
Ceftazidime	10	*	*	*	*	*	*	*		
Triflucan	5								*	*

Results were interpreted in terms of the diameter of the inhibition zones: (-): < 5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (*): ≥ 16 mm.
Ec: *Escherichia coli* ATCC 35218, Yp: *Yersinia pseudotuberculosis* ATCC 911, Kp: *Klebsiella pneumoniae* ATCC 13883, Sm: *Serratia marcescens* ATCC 13880, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bs: *Bacillus subtilis* ATCC 6633, Ca: *Candida albicans* ATCC 60193, Ct: *Candida tropicalis* ATCC 13803.

The antimicrobial activity of the essential oil from *I. thapsoides* subsp. thapsoides was tested against the bacteria *E. coli, Y. pseudotuberculosis, K. pneumoniae, S. marcescens, E. faecalis, S. aureus, B. subtilis* and the fungus *C. albicans* and *C. tropicalis* at maximum essential oil concentration

in acetone of 1000 µg/mL, by using ceftazidime and triflucan as standard antibacterial and antifungal agents[9,10]. The test extracts showed better antimicrobial activity against Gram-positive bacteria in comparison to the Gram-negative bacteria. The essential oil extracts of *I. thapsoides* subsp. thapsoides showed antimicrobial activity against *Y. pseudotuberculosis, E. faecalis* and *S. aureus*, but no antimicrobial activity was observed against the bacteria *E. coli, K. pneumoniae, S. marcescens, B. subtilis* and the fungus *C. albicans* and *C. tropicalis*. The results are shown in Table-3.

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