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Chemical Composition and Antimicrobial Activities of the Essential Oils of *Viburnum opulus*, *Viburnum lantana* and *Viburnum orientala*

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The composition of the essential oils obtained from the air-dried *Viburnum opulus* and *Viburnum lantana* were analyzed by GC-MS. 40 and 53 Components were identified in the essential oils and the main component of these taxons were phytol and occidenol in the ratios 7.8 and 6.3 % from *V. opulus* and *V. lantana*, respectively. The isolated essential oils of *V. opulus*, *V. lantana* and *V. orientala* were also tested for antimicrobial activity against the bacteria *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *B. cereus* and the fungus *C. tropicalis*, at maximum essential oil concentrations in hexane of 250, 500 and 1000 µg/mL, respectively, though no activity was observed against all the test microorganisms for *V. lantana* and *V. opulus*. However, the essential oil of the *V. orientale* showed weak antibacterial activity against Gram-positive bacteria.

Key Words: *Viburnum opulus*, *V. lantana*, *V. orientala*, Essential oil, Antimicrobial activity, GC-MS.

INTRODUCTION

Viburnum L. (Caprifoliaceae) represented with 4 deciduous shrubs species in Turkey¹. *Viburnum opulus* L. are grown as ornamental plant in many countries and the dried fruits consisting of phenolic glucoside, tannes and some organic acids were used for complaints of uterine cramps, colicky pains in pelvic organs². The stem bark and fresh shots of *Viburnum lantana* L and *V. opulus* are also used in Anatolia folk medicine as a pain killer^{3,4}.

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Previous phytochemical studies on *V. lantana* and *V. opulus* have shown the presence of different natural compounds including iridoids, iridoid glucosides, lantanoside, flavonoids, saponins, tannins, arbutin, ursolic acid, flavones and anthocyanins⁵⁻¹². The essential oil composition and chemical constituents of *V. orientale* are reported¹³⁻¹⁵. But antimicrobial activity is not mentioned in the literature.

To our knowledge, there is no published report on the essential oil analysis and antimicrobial activity of *V. opulus* and *V. lantana*. As part of this systematic research, the essential oil constituents of the plants were obtained by the widely used hydrodistillation method in a Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC-MS technique. Identification of the compounds was made by a typical library search (NIST, WILLEY) and literature comparison¹⁶⁻²⁰.

EXPERIMENTAL

V. opulus and *V. orientale* were collected from Düzköy-Trabzon (at a height of ca. 1300 m) in the northeastern part of Turkey in September 2004. *V. lantana* was collected around Karaca cave, Torul-Gümüşhane (at a height of ca. 1500 m) in the northeastern part of Turkey in September 2004. The plants were authenticated immediately after collection and air-dried at room temperature for later analysis¹⁶. Voucher specimens (No. Coskunçelebi 546, 547 and 548-2004 KTUB) were deposited in the Herbarium of the Department of Biology, Karadeniz Technical University, Turkey.

Isolation of the essential oils: Crude essential oils of *V. opulus*, *V. lantana* and *V. orientale* were obtained from the air-dried whole plants (ca. 35 g, each) by hydrodistillation in a Clevenger-type apparatus with cooling bath (-15 °C) system (3 h) (yields: 0.10, 0.15, 0.43 % and (v/w), respectively). The oils were taken by HPLC grade *n*-hexane (0.5 mL) and dried over Na₂SO₄ kept at 4 °C in a sealed brown vial. One µL of the extracts was directly injected into the GC-MS instrument.

Gas chromatography: GC-MS analyses were done as described previously²¹.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Willey) and confirmed by comparison of their retention indices with data published in the literature¹⁶⁻²⁰.

Antimicrobial activity assessment: All test microorganisms were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows: Ec: *Escherichia coli* ATCC 25922, Kp: *Klebsiella pneumoniae* ATCC 13883, Pa: *Pseudomonas aeruginosa* ATCC 10145, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bc: *Bacillus cereus* 709 Roma, Ct: *Candida tropicalis* ATCC 13803.

Agar dilution MIC assay: Using a modification of the assay described by Southwell *et al.*²² and Mann *et al.*²³ the samples were added to molten Mueller-Hinton agar (MHA) and Potato Dextrose agar (PDA)/Tween 20 medium at 48 °C, 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 37 °C 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. Hexane was used as control. Ampicillin and fluconazole were standard drugs. The results are shown in Table-3.

RESULTS AND DISCUSSION

The compositions of essential oils of *V. opulus* and *V. lantana* were analyzed by GC-MS with HP-5 column. A total of 40 and 53 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 85.3 and 82.3 % of total composition of the essential oils in *V. opulus* and *V. lantana*, respectively¹⁶⁻²⁰. The general chemical profile of the essential oils, the percentage content and the retention indices of the constituents are summarized in Table-1.

Phytol (7.8%), *trans*-β-damascenone (4.9 %), α-cadinol (4.8%), γ-cadinene (4.7 %), Δ-cadinene (4.5 %) and methyl pentanoate (4.1 %) were the main constituents of the essential oil of *V. opulus*, whereas occidenedol (6.3 %), α-cadinol (5.6 %), γ-cadinene (4.6 %), 2E,4E-decadienal (4.5%), *n*-heptanal (3.9 %) and Δ-cadinene (3.4 %) were the main components of the essential oil of *V. lantana*.

The chemical class distribution and the main components in each class of the essential oils of *V. opulus* and *V. lantana* are reported in Table-2. The compounds were separated into six classes, which were monoterpene, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenoids and others (Table-2). 23 Compounds were common to all two species with the total ratio of 46.7 and 43.0 % in *V. opulus* and *V. lantana*, respectively. Some chemical differences on the composition of the essential oils of *V. opulus* and *V. lantana* were found and probably related to the different subspecies and/ or to the geographical origin of the plants.

The antimicrobial activity of the essential oils from *V. opulus*, *V. lantana* and *V. orientale* were tested against the bacteria *E. coli*, *K. pneumoniae*,

TABLE-1
 IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF
Viburnum opulus AND *Viburnum lantana*^{a, b}

No.	Compounds	<i>V. opulus</i>		<i>V. lantana</i>		Exp. RI	Lit. RI
		Q (%)	Area (%)	Q (%)	Area (%)		
1	Methyl pentanoate	89	4.1	-	-	826	828
2	3Z-Hexen-1-ol	86	1.7	-	-	858	859
4	2-Heptanone	-	-	82	0.3	887	889
5	<i>n</i> -Heptanal	88	1.9	91	3.9	901	902
6	Benzaldehyde	-	-	94	1.9	957	960
7	1-Octen-3-ol	-	-	87	0.8	979	978
8	6-Methyl-5-hepten-2-one	-	-	87	0.1	990	992
9	2-Pentylfuran	91	2.0	94	2.5	995	998
10	2, 4 Heptadienal	-	-	91	1.1	998	1003
11	<i>n</i> -Octanal	-	-	90	1.1	999	999
12	Limonene	-	-	94	0.9	1026	1029
13	Phenyl acetaldehyde	91	1.9	-	-	1043	1042
14	<i>n</i> -Octanol	-	-	84	0.6	1069	1068
15	Linalool oxide	64	1.6	-	-	1075	1078
16	<i>cis</i> -Linalool oxide	-	-	88	0.5	1086	1087
17	Terpinolene	88	0.5	-	-	1088	1089
18	L-Linalool	81	1.4	88	0.5	1096	1097
19	<i>n</i> -Nonanal	87	0.9	91	2.9	1105	1102
20	2 <i>E</i> , 6 <i>Z</i> -Nonadienal	91	0.3	-	-	1153	1155
21	4-Terpineol	91	1.9	-	-	1175	1177
22	α -Terpineol	90	1.7	83	0.3	1187	1189
23	Methyl salicylate	97	1.8	93	0.3	1190	1192
24	Myrtenol	86	0.9	-	-	1194	1196
25	<i>n</i> -Decanal	85	0.8	91	0.6	1204	1202
26	2 <i>E</i> , 4 <i>E</i> -Nonadienal	-	-	80	0.6	1213	1212
27	<i>trans</i> -Carveol	28	0.6	-	-	1219	1217
28	Geraniol	82	1.7	-	-	1255	1253
29	2 <i>E</i> -Decanal	86	1.3	82	3.3	1263	1264
30	Cinnamaldehyde	-	-	95	0.5	1271	1270
31	2 <i>E</i> , 4 <i>Z</i> -Decadienal	80	1.7	90	1.6	1293	1293
32	2 <i>E</i> , 4 <i>E</i> -Decadienal	81	1.8	93	4.5	1316	1317
33	α -Cubebene	-	-	97	0.8	1352	1351
34	α -Copaene	87	0.7	98	3.5	1377	1377
35	<i>trans</i> - β -Damascenone	95	4.9	-	-	1384	1385
36	β -Bourbonene	-	-	96	1.0	1388	1388
37	<i>trans</i> - α -Ambrinol	86	1.8	-	-	1415	1417
38	<i>E</i> -Caryophyllene	-	-	88	0.8	1420	1419
39	β -Copaene	-	-	93	0.5	1430	1432
40	Geranyl acetone	-	-	89	0.3	1454	1455
41	γ -Murolene	-	-	95	1.6	1479	1480
42	α -Amorphene	86	1.6	97	0.8	1481	1485
43	Germacrene D	93	0.7	98	1.7	1482	1485
44	β -Ionone	89	1.9	93	0.4	1487	1489

45	α -Muurolene	-	-	96	1.2	1500	1500
46	γ -Cadinene	95	4.7	96	4.6	1515	1514
47	Δ -Cadinene	95	4.5	95	3.4	1525	1523
48	<i>trans</i> -Cadina-1, 4-diene	93	1.8	89	0.2	1535	1535
49	α -Calacorene	80	2.4	95	0.7	1545	1546
50	Occidentalol	-	-	99	0.9	1552	1552
51	<i>E</i> -Nerolidol	-	-	74	0.6	1566	1563
52	Ledol	93	0.8	-	-	1573	1569
53	Spathulenol	-	-	99	1.5	1580	1578
54	Caryophyllene oxide	-	-	98	1.4	1584	1583
55	Salvial-4(14)-en-1-one	-	-	98	1.3	1595	1595
56	Tetradecanal	91	3.7	-	-	1612	1613
57	γ -Eudesmol	-	-	93	0.9	1634	1632
58	α -Muurolol	81	3.5	93	2.6	1645	1646
59	β -Eudesmol	-	-	99	2.7	1652	1651
60	α -Cadinol	99	4.8	94	5.6	1655	1654
61	Occidenol	-	-	90	6.3	1677	1678
62	Eudesma-4(15, 7-dien-1- β -ol	-	-	85	0.9	1685	1688
63	Pentadecanal	94	3.8	91	1.4	1715	1717
64	6, 10, 14-trimethyl-2-pentadecanone	-	-	97	1.6	1847	1846
65	Nonadecane	-	-	98	0.4	1900	1900
66	Manool	64	0.9	-	-	2057	2057
67	<i>n</i> -Heneicosane	95	1.9	98	1.8	2100	2100
68	Phytol	74	7.8	-	-	2114	2113
69	<i>n</i> -Docosane	87	1.2	89	1.3	2200	2200
70	<i>n</i> -Tricosane	80	1.4	91	0.8	2300	2300
Total isolate		85.3		82.3			
Unknown	RI	m/z (%)		<i>V. opulus</i>	<i>V. lantana</i>		
Un-1	828	101(5), 87(42), 74(100), 69(20), 57(63)		2.6	-		
Un-2	899	124(8), 104(100), 103(52), 78(46), 51(28)		4.2	-		
Un-3	973	124(5), 106(3), 85(100), 69(12), 57(96), 53(15)		1.9	-		
Un-4	979	112(8), 97(20), 83(100), 70(58), 57(63), 55(86)		-	2.7		
Un-5	1056	125(4), 108(12), 97(23), 83(72), 70(100), 55(96)		-	2.3		
Un-6	1287	194(23), 179(100), 138(22), 107(39), 69(50), 55(21)		1.7	-		
Un-7	1591	220(46), 177(43), 159(100), 91(98), 55(587)		-	1.3		
Total unknown				10.4	6.3		
Total isolate				85.3	82.3		
Total				95.7	88.6		

^aRI, retention index; LRI, literature retention index; Q: Quality;

^bCompounds are listed in order of elution. RI (retention index) values are calculated from retention times relative to that of *n*-alkanes (C₆-C₃₂) on the non-polar HP-5 column.

TABLE-2
CHEMICAL CLASS DISTRIBUTION AND THE MAIN COMPONENTS
IN EACH CLASS OF THE ESSENTIAL OILS OF *Viburnum opulus*
AND *Viburnum lantana*

Compound class	<i>Viburnum opulus</i>			<i>Viburnum lantana</i>		
	Area (%)	N ^a	Major component	Area (%)	N ^a	Major component
Monoterpene	0.5	1	Terpinolene	0.9	1	Limonene
Monoterpenoids	18.4	10	<i>t</i> -β-Damascenone	2.1	5	L-Linalool
Sesquiterpenes	16.4	7	γ-Cadinene	20.8	13	γ-Cadinene
Sesquiterpenoids	9.1	3	α-Cadinol	26.3	12	Occidenol
Diterpenoids	8.7	2	Phytol	–	–	–
Others	32.2	17	Methyl pentanoate	32.3	22	2 <i>E</i> ,4 <i>E</i> -Decadienal

^aN = Number of compounds.

P. aeruginosa, *E. faecalis*, *S. aureus*, *B. cereus* and the fungus *C. tropicalis* at maximum essential oil concentrations in hexane of 250, 500 and 1000 µg/mL, respectively, by using ampicillin and fluconazole as standard antibacterial and antifungal agents^{22,23}. However, no antimicrobial activity was observed against all the test microorganisms for the essential oils from *V. opulus*, *V. lantana*. But, the essential oil of the *V. orientale* showed weak antibacterial activity against Gram-positive bacteria *E. faecalis*, *S. aureus* and *B. cereus*. The results are shown in Table-3.

TABLE-3
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY OF THE
ESSENTIAL OIL COMPONENTS OF *Viburnum orientale*, *Viburnum opulus*
AND *Viburnum lantana* (MIC 250-1000 µg/mL)

Compounds	Stock (µg/mL)	Microorganisms and MIC value						
		Ec	Kp	Pa	Ef	Sa	Bc	Ct
<i>V. orientale</i> ^a	4700	–	–	–	1000	1000	1000	–
<i>V. opulus</i> ^a	900	–	–	–	–	–	–	–
<i>V. lantana</i> ^a	1800	–	–	–	–	–	–	–
Hegzan	–	–	–	–	–	–	–	–
Ampicillin	–	8	32	>128	2	2	2	–
Fluconazole	–	–	–	–	–	–	–	8

^aThe highest concentrations of *V. orientale*, *V. opulus* and *V. lantana* are >1000, >250 and >500, respectively.

Ec = *Escherichia coli* ATCC 25922, Kp = *Klebsiella pneumoniae* ATCC 13883, Pa = *Pseudomonas aeruginosa* ATCC 10145, Ef = *Enterococcus faecalis* ATCC 29212, Sa = *Staphylococcus aureus* ATCC 25923, Bc = *Bacillus cereus* 709 Roma, Ct = *Candida tropicalis* ATCC 13803; (–) = No activity at stock solution concentration.

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