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 Turkey. *Viburnum opulus* L. are grown as ornamental plant in many countries and the dried fruits consisting of phenolic glucoside, tannins and some organic acids were used for complaints of uterine cramps, colicky pains in pelvic organs. The stem bark and fresh shots of *Viburnum lantana* L and *V. opulus* are also used in Anatolia folk medicine as a pain killer.
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 anthocyanins\(^5\)-\(^{12}\). The essential oil composition and chemical constituents of *V. orientale* are reported\(^{13}\)-\(^{15}\). 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 comparison\(^{16}\)-\(^{20}\).

**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 analysis\(^{16}\). 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 Na\(_2\)SO\(_4\) 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 previously\(^{21}\).

**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 literature\(^{16}\)-\(^{20}\).

**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.\textsuperscript{22} and Mann et al.\textsuperscript{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 \textit{V. opulus} and \textit{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 \textit{V. opulus} and \textit{V. lantana}, respectively\textsuperscript{16-20}. 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%), \textit{trans}-\textit{β}-damascenone (4.9 %), \textit{α}-cadinol (4.8%), \textit{γ}-cadinene (4.7 %), \textit{Δ}-cadinene (4.5 %) and methyl pentanoate (4.1 %) were the main constituents of the essential oil of \textit{V. opulus}, whereas occisedenol (6.3 %), \textit{α}-cadinol (5.6 %), \textit{γ}-cadinene (4.6 %), 2E,4E-decadienal (4.5 %), \textit{n}-heptanal (3.9 %) and \textit{Δ}-cadinene (3.4 %) were the main components of the essential oil of \textit{V. lantana}.

The chemical class distribution and the main components in each class of the essential oils of \textit{V. opulus} and \textit{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 \textit{V. opulus} and \textit{V. lantana}, respectively. Some chemical differences on the composition of the essential oils of \textit{V. opulus} and \textit{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 \textit{V. opulus}, \textit{V. lantana} and \textit{V. orientale} were tested against the bacteria \textit{E. coli}, \textit{K. pneumoniae}, and
## TABLE-1

IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF Viburnum opulus AND Viburnum lantana

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<td>1.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>Phytol</td>
<td>74</td>
<td>7.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>n-Docosane</td>
<td>87</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>n-Tricosane</td>
<td>80</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total isolate: 85.3  82.3

<table>
<thead>
<tr>
<th>Unknown</th>
<th>RI</th>
<th>m/z (%)</th>
<th>V. opulus</th>
<th>V. lantana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-1</td>
<td>828</td>
<td>101(5), 87(42), 74(100), 69(20), 57(63)</td>
<td>2.6</td>
<td>-</td>
</tr>
<tr>
<td>Un-2</td>
<td>899</td>
<td>124(8), 104(100), 103(52), 78(46), 51(28)</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>Un-3</td>
<td>973</td>
<td>124(5), 106(3), 85(100), 69(12), 57(96), 53(15)</td>
<td>1.9</td>
<td>-</td>
</tr>
<tr>
<td>Un-4</td>
<td>979</td>
<td>112(8), 97(20), 83(100), 70(58), 57(63), 55(86)</td>
<td>-</td>
<td>2.7</td>
</tr>
<tr>
<td>Un-5</td>
<td>1056</td>
<td>125(4), 108(12), 97(23), 83(72), 70(100), 55(96)</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>Un-6</td>
<td>1287</td>
<td>194(23), 179(100), 138(22), 107(39), 69(50), 55(21)</td>
<td>1.7</td>
<td>-</td>
</tr>
<tr>
<td>Un-7</td>
<td>1591</td>
<td>220(46), 177(43), 159(100), 91(98), 55(587)</td>
<td>-</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Total unknown: 10.4  6.3
Total isolate: 85.3  82.3
Total: 95.7  88.6

*RI, retention index; LRI, literature retention index; Q: Quality; Compounds 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

<table>
<thead>
<tr>
<th>Compound class</th>
<th>Viburnum opulus</th>
<th>Viburnum lantana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (%)</td>
<td>N</td>
</tr>
<tr>
<td>Monoterpene</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Monoterpenoids</td>
<td>18.4</td>
<td>10</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>16.4</td>
<td>7</td>
</tr>
<tr>
<td>Sesquiterpenoids</td>
<td>9.1</td>
<td>3</td>
</tr>
<tr>
<td>Diterpenoids</td>
<td>8.7</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>32.2</td>
<td>17</td>
</tr>
</tbody>
</table>

*N = 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. 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)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Stock (µg/mL)</th>
<th>Ec</th>
<th>Kp</th>
<th>Pa</th>
<th>Ef</th>
<th>Sa</th>
<th>Bc</th>
<th>Ct</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. orientale</td>
<td>4700</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>–</td>
</tr>
<tr>
<td>V. opulus</td>
<td>900</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>–</td>
</tr>
<tr>
<td>V. lantana</td>
<td>1800</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>–</td>
</tr>
<tr>
<td>Hegzan</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>–</td>
<td>8</td>
<td>32</td>
<td>&gt;128</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>–</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The 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.
ACKNOWLEDGEMENTS

This study was supported by grants from Karadeniz Technical University Research Fund and State Planning Agency (DPT) of Turkey.

REFERENCES


(Received: 12 March 2007; Accepted: 25 January 2008) AJC-6256