Chemical Constituents of Medicinal Plants, *Gardenia elata*, *G. gjellerupii*, and *G. volkensii*

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ABSTRACT

Many native Thailand *Gardenia* species have been reported to possess chemicals with properties effective in treating a variety of human illnesses. The present study examined chemical constituents in three species including one native *G. elata*, and two introduced species *G. gjellerupii* and *G. volkensii*. Crude hexane leaf extracts of the species were evaluated by gas chromatography-mass spectrometry. *Gardenia elata* contained monoterpane, α-pinene; terpinenes, β-pinene, palmitic acid; diterpene, neophytadiene; stearic acid; (6E,10E,14E,18E)-pentamethylicosa-2,6,10,14,18-pentaene. *Gardenia gjellerupii* and *G. volkensii* composed of identical compounds, however in different amount, including diterpene, neophytadiene; terpinene, palmitic acid; squalene; linolenyl alcohol. These chemicals have been reported for their valuable medicinal properties. In addition to traditional knowledge that these plants are edible, this finding ensures that the plants are useful and should be consumed as healthy foods.

Keywords: Phytochemicals, *Gardenia elata*, *Gardenia gjellerupii*, *Gardenia volkensii*, human illness, healthy food.

INTRODUCTION

*Gardenia* is a large genus in Rubiaceae family, with approximately 60 species centered in the old world tropics. Many species are native to Thailand and are distributed in diverse vegetation types. Because of their large, showy, and fragrant flowers, *Gardenia* species are often cultivated1. In addition, their chemical contents and medicinal properties have been reported. Nuanyai et al.2 reported a rich source of cycloartenetriterpenoids in *G. sootepensis*. The compounds exhibited interesting biological activities, including cytotoxic, anti-implementation, and abortifacient effects, as well as antilucre, antibacterial, diuretic, analgesic, hypertensive, and larvicidal activities. *Gardenia jasminoides* fruit, used as a pigment source, contains glycosidal type carotenoid pigments which can be obtained in larger quantities than obtaining from saffron (*Crocus sativus* L.) and without the accompanying flavors and high costs3. Grougnet et al.4 isolated coronalolide methyl ester, coronisolide, and coronalolic acid from *G. coronaria* leaves and/or stems, and *G. sootepensis* leaves, and demonstrated broad cytotoxic effects against a panel of human cancer cell lines. Certain chemicals and modified compounds extracted from *G. obtusifolia* leaves and twigs showed significant cytotoxic activities in several mammalian cell lines and exhibited potent cytotoxicities, and antimitotic and anti-HIV properties5. Suksamran et al.6 reported 10 triterpenes isolated from *G. saxatilis* twigs with antiplasmodial property against *Plasmodium falciparum*. Several anti-HIV cycloartanes, which are classified as triterpenes, were extracted from *G. thailandica* leaves and twigs7. Grougnet et al.8 extracted 3,4-seco-cycloartanes, secabuyrenol, secabuyryltriol, and secabuyrolide from above ground plant part exudates of *G. aubryi*, which showed in vitro cytotoxic capacity to reduce four human solid tumor cell lines9. Methanol extracts from fresh *G. sootepensis* apical buds were identified as cycloartanes, sootepins and triterpines. The extracts were tested for in vitro cytotoxic effects against human breast, lung, liver, gastric, and colon cancer cell lines2. *Gardenia jasminoides* oils and fresh flowers contain linalool, α-farnesene, β-3-hexenyl tiglate, and trans-β-ocimene. The oils exhibited antimicrobial properties against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *S. epidermidis*. Canamares et al.10 identified the carotenoid colorant components crocetin and crocin, the same pigmentation identified in saffron from *Crocus sativus*. Four new 3,4-seco-cycloartane triterpenes, termed gardenoine A through D, and the known compound secabuyrenol were resolved from exudates of *G. tubifera* aboveground plant parts11. *Gardenia obtusifolia*, a species traditionally used in Thailand for a variety of health problems, contains 5,3-dihydroxy-3,6,7,8,4-pentamethoxyflavone (DH-PMF) which exhibit anticancer property12. Additional research

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characterized two new dammarane triterpenes along with a known compound (20R,24R)-ocotillone from *G. collinsae* and revealed cytotoxic against cancer cell lines. Nuanyai et al.\(^4\) characterized four new cycloartanes, termed gardenoin E through H, together with five known cycloartanes isolated from *G. obtusifolia* apical buds. Gardenoin E was the only cycloartane that displayed cytotoxicity in reducing colon, hepatic, and lung cancer cell lines. Whereas, gardenoins I and J, secabryenol, sootepin E, and coronaloic acid were isolated from *G. thailandica* and it was reported that sootepin E showed cytotoxicity against cancer cell lines\(^3\). Kongkum et al.\(^16\) isolated flavones, syringaldehyde, vanillic acid, and scopoletin from *G. carinata* leaves and twigs and reported cytotoxic, DNA topoisomerase I/II inhibitory, and anti-HIV-1 activities. These reports clearly established the value of various chemical constituents characterizing *Gardenia* species in anti-HIV and anti-cancer treatment. Based on the important medicinal properties inherent in *Gardenia* species, it is vital to chemically characterize all species. The present study investigated a suite of chemical extracts from the three well-known species namely, *Gardenia* species, *G. elata*, *G. glleruppii* and *G. volkensii* to examine their potential medicinal properties. Getting to know in-depth chemical contents and scientifically properties will lead to further uses and applications of the plants as well as consumer confidence of consuming the plants.

**MATERIALS AND METHODS**

**Plant materials**

*Gardenia elata* was collected from Pattani province, northern Thailand, whereas *G. glleruppii* and *G. volkensii* species were collected from Lampang province, northern Thailand. They were identified followed the literatures\(^16\)\(^-\)\(^22\). Young leaves were collected for molecular study and mature leaves were collected for chemical analyses.

**Preparation of chemical extracts**

The mature leaves were rinsed with tap water and air-dried at room temperature for three days, then were finely ground. A 25-g of the leaf powder was soaked in 120 ml of hexane (analytical grade) in a glass bottle wrapped with aluminum foil and left at room temperature for three days. The mixture was filtered through a Whatman at room temperature. Ninety ml filtrates were obtained and kept at -20°C until analysis.

**Gas chromatography-mass spectrometry (GC-MS) analysis**

The GC-MS analysis of the crude extracts was performed using Agilent Technologies GC 6890N/5973 inert MSD fused with capillary column (30.0 m × 250 μm × 0.25 μm). Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injection and mass transferred line temperature were set at 280°C. The oven temperature was programmed from 70°C to 120°C at 3°C/min, then held isothermally for 2 min and finally raised to 270°C at 5°C /min. The crude extract of 1 μL was injected in the split mode. The relative percentage of crude constituents was expressed as a percentage by peak area normalization. The identity of the components of the crude was assigned by comparison of their GC-MS spectra from the Wiley 7N.1.

**RESULTS AND DISCUSSION**

*Gardenia elata* is native to Thailand, which was brought into cultivation for ornamental purpose, whereas *G. glleruppii* and *G. volkensii* were imported to Thailand and successfully used for domestic gardens.

Chemical identification of crude hexane extracts using GC-MS for the three species (Figure 1 and Table 1) detected the presence of different chemicals, including monoterpennes, diterpenes, and terpinenes, which were all classified as terpene groups. *Gardenia elata* contained the following compounds, not shared by the introduced ornamental species: α-pinene (monoterpene), β-pinene (terpenenes), stearic acid, and (6E,10E,14E,18E)-2,6,10,14,18-pentamethylcicosa-2,6,10,14,18-pentaene. Compounds shared in *G. glleruppii* and *G. volkensii* were squalene and linolenyl alcohol. Compounds shared in all the three species were palmitic acid (terpinenes) and neophytadiene (diterpene).

Our literature reviews indicated *Gardenia* possesses many chemicals with specific anticancer and anti-HIV treatment properties. In addition, different plant structures result in distinct chemical types, i.e. stem, flower, leaf, and twig extracts are often chemically distinct and ultimately used in different applications. For example, gardenoin E, a triterpene was isolated from *G. obtusifolia* apical buds and displayed cytotoxicity against colon, hepatic, and lung cancer cell lines\(^1\); the important flavonoid substances extracted from *G. carinata* leaves and twigs exhibited anti-HIV-1 property and showed considerable effectiveness against HIV-1 reverse transcriptase\(^16\).

The majority of chemical compounds reported in the genus *Gardenia* from distinct plant parts and extraction buffers were triterpenes and flavonoids, which exhibited variable illness treatment characteristics. Additional previous studies on native Thailand species and our results from *G. elata* revealed monoterpennes and terpinenes, which were classified as triterpenes, as their major constituents. The introduced ornamental species, *G. glleruppii* and *G. volkensii* showed diterpenes and terpinenes, which are also triterpenes, and two other chemical components, including linolenyl alcohol and squalene. Therefore, based on chemical composition, these species are viable candidates to treat HIV and cancer. Triterpenes and flavonoids extracted from various *Gardenia* species plant parts have been demonstrated to elicit anti-cancer cell and anti-HIV activity. In addition to illness treatment, these two chemical groups should be markers of the genus *Gardenia*, applied in systematics.

**CONCLUSIONS**

Many native Thailand *Gardenia* species have been reported to possess chemicals with medicinal properties. Most of the species have been used for treating a variety of human illnesses. Out of 13 species expected to exist in Thailand, these tree species have been reported for their chemical constituents for the first time. *Gardenia elata* contained monoterpene, α-pinene; terpinenes, β-pinene,
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Figure 1: GC-MS chromatograms of hexane crude extracts from the leaves of the examined Gardenia species, G. elata, G. gjellerupii, and G. volkensii.

Table 1: Chemical contents of crude hexane extracts from the three species determined using GC-MS.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Formula</th>
<th>MW</th>
<th>Relative content (%)</th>
<th>G. elata</th>
<th>G. gjellerupii</th>
<th>G. volkensii</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha-Pinene</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>2.33</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>beta-Pinene</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>1.07</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neophytadiene</td>
<td>C_{20}H_{38}</td>
<td>278</td>
<td>1.70</td>
<td>11.63</td>
<td>9.90</td>
<td>-</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>C_{16}H_{32}O_2</td>
<td>256</td>
<td>27.62</td>
<td>15.89</td>
<td>12.36</td>
<td>-</td>
</tr>
<tr>
<td>Steric acid</td>
<td>C_{12}H_{24}</td>
<td>284</td>
<td>2.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(6E,10E,14E,18E)-2,6,10,14,18-pentamethylicosa-2,6,10,14,18-pentaene</td>
<td>C_{15}H_{34}</td>
<td>342</td>
<td>21.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linolenyl alcohol</td>
<td>C_{18}H_{32}O</td>
<td>264</td>
<td>-</td>
<td>3.27</td>
<td>2.14</td>
<td>-</td>
</tr>
<tr>
<td>Squalene</td>
<td>C_{20}H_{30}</td>
<td>410</td>
<td>-</td>
<td>16.13</td>
<td>44.52</td>
<td>-</td>
</tr>
<tr>
<td>unknown*</td>
<td>-</td>
<td>-</td>
<td>43.93</td>
<td>53.09</td>
<td>31.08</td>
<td>-</td>
</tr>
</tbody>
</table>

*unknown from each species were not the only one unknown and may be not the same unknown.

palmitic acid; diterpene, neophytadiene; stearic acid; (6E,10E,14E,18E)-2,6,10,14,18-pentamethylicosa-2,6,10,14,18-pentaene. Gardenia gjellerupii and G. volkensii composed of identical compounds, however in different amount, including diterpene, neophytadiene; terpinene, palmitic acid; squalene; linolenyl alcohol. All of these compounds were reported for their valuable medicinal properties.

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REFERENCES


