

Research Article

Chemical Constituents of Medicinal Plants, *Gardenia elata*, *G. gjellerupii*, and *G. volkensii*Kittiya S Suwannakud^{1,2}, Arunrat Chaveerach^{2,3}, Runglawan Sudmoon^{2,4}, Tawatchai Tanee^{2,5*}¹Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand.²Genetics and Environmental Toxicology Research Group, Khon Kaen University, Khon Kaen 40002, Thailand.³Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.⁴Faculty of Law, Khon Kaen University, Khon Kaen 40002, Thailand.⁵Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand.Received: 7th Nov, 16; Revised 6th March, 17, Accepted: 10th March, 17; Available Online: 25th March, 2017**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 monoterpene, α -pinene; terpinenes, β -pinene, palmitic acid; diterpene, neophytadiene; stearic acid; (6E,10E,14E,18E)-2,6,10,14,18-pentamethylcosa-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 cycloartanetriterpenoids in *G. sootepensis*. The compounds exhibited interesting biological activities, including cytotoxic, anti-implantation, and abortifacient effects, as well as antiulcer, 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, coronalolide, 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 antimetabolic 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, secaubryenol, secaubrytriol, and secaubryolide from above ground plant part exudates of *G. aubryi*, which showed *in vitro* cytotoxic capacity to reduce four human solid tumor cell lines⁸. Methanol extracts from fresh *G. sootepensis* apical buds were identified as cycloartanes, sootepins and triterpenes. 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, z-3-hexenyl tiglate, and trans- β -ocimene. The oils exhibited antimicrobial properties against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *S. epidermidis*⁹. 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 gardenoin A through D, and the known compound secaubryenol 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

characterized two new dammrane triterpenes along with a known compound (20R,24R)-ocotillone from *G. collinsae* and revealed cytotoxic against cancer cell lines. Nuanyai et al.¹⁴ characterized four new cycloartanes, termed gardenoins 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, secoabryenol, sootepin E, and coronalioic acid were isolated from *G. thailandica* and it was reported that sootepin E showed cytotoxicity against cancer cell lines¹⁵. Kongkum et al.¹⁶ isolated flavones, syringaldehyde, vanillic acid, and scopoletin from *G. carinata* leaves and twigs and reported cytotoxic, DNA topoisomerase II α 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. gjellerupii* 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. gjellerupii* and *G. volkensii* species were collected from Lampang province, northern Thailand. They were identified followed the literatures¹⁸⁻²². 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 \times 250 μ m \times 0.25 μ m). 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 μ L 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. gjellerupii* 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 monoterpenes, 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 (terpinenes), stearic acid, and (6E,10E,14E,18E)-2,6,10,14,18-pentamethylcosa-2,6,10,14,18-pentaene.

Compounds shared in *G. gjellerupii* 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 lines¹⁴; the important flavonoid substances extracted from *G. carinata* leaves and twigs exhibited anti-HIV-1 property and showed considerable effectiveness against HIV-1 reverse transcriptase¹⁶.

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 monoterpenes and terpinenes, which were classified as triterpenes, as their major constituents. The introduced ornamental species, *G. gjellerupii* 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,

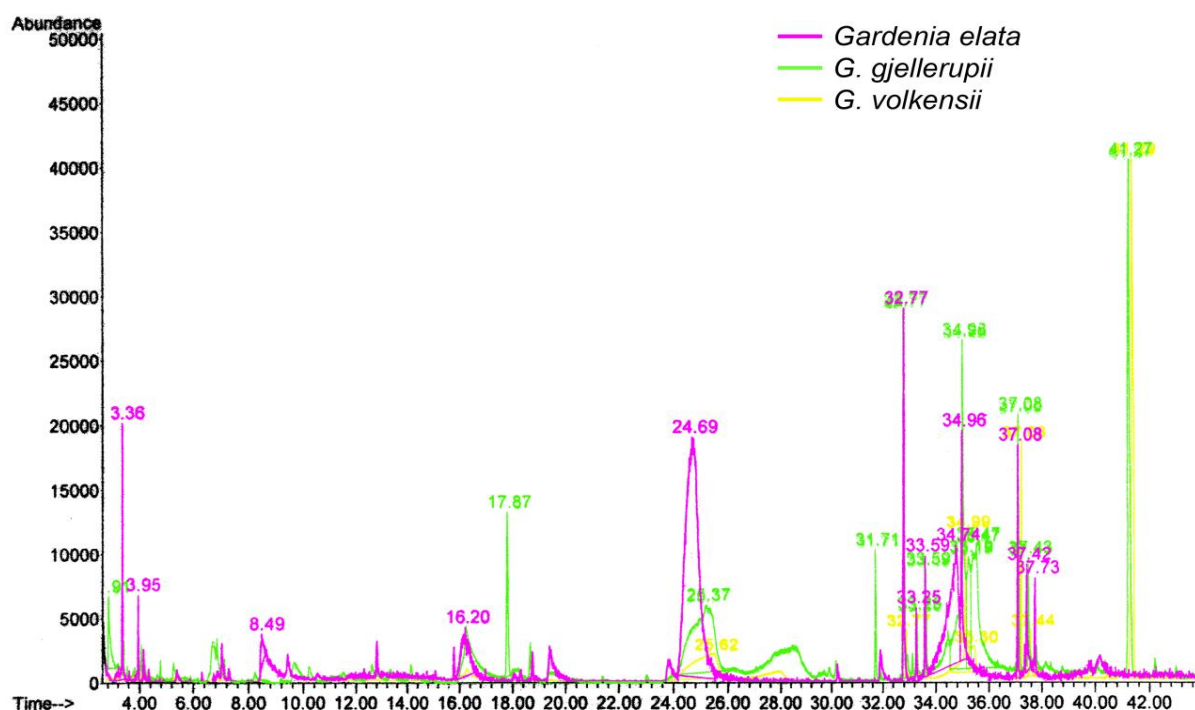


Figure 1: GC-MS chromatograms of hexane crude extracts from the leaves of the examined *Gardenia* species, *G. elata*, *G. gjellerupii*, and *G. volkensii*.

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

Compounds	Formula	MW	Relative content (%)		
			<i>G. elata</i>	<i>G. gjellerupii</i>	<i>G. volkensii</i>
alpha-Pinene	C ₁₀ H ₁₆	136	2.33	-	-
beta-Pinene	C ₁₀ H ₁₆	136	1.07	-	-
Neophytadiene	C ₂₀ H ₃₈	278	1.70	11.63	9.90
Palmitic acid	C ₁₆ H ₃₂ O ₂	256	27.62	15.89	12.36
Stearic acid	C ₁₈ H ₃₆ O ₂	284	2.14	-	-
(6E,10E,14E,18E)-2,6,10,14,18-pentamethylcosa-2,6,10,14,18-pentaene	C ₃₀ H ₅₀	410	43.93	53.09	31.08
Linolenyl alcohol	C ₁₈ H ₃₂ O	264	-	3.27	2.14
Squalene	C ₃₀ H ₅₀	410	-	16.13	44.52
unknown*	-	-	43.93	53.09	31.08

*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-pentamethylcosa-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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