Chemical Composition and Antibacterial Activities of Goniothalamus marcanii Flower Essential Oil

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ABSTRACT

The essential oil of Goniothalamus marcanii flowers extracted by hydrodistillation was investigated in terms of its chemical composition by gas chromatography-mass spectrometry with their retention indices. The analyses revealed the presence of 116 compounds, representing 93.83% of the essential oil. The major compounds of the oil were caryophyllene oxide, E-caryophyllene, α-humulene, δ-cadinene and linalool. Essential oil of Goniothalamus marcanii flowers were tested in vitro antibacterial activities against six human pathogens including Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus agalactiae, Proteus mirabilis, Escherichia coli and Salmonella typhimurium by using agar paper disc diffusion assay. Flower oil of Goniothalamus marcanii exhibited broad spectrum antibacterial activities along with their respective minimum inhibitory concentration values ranging from 15.62 to 1000 µg/mL according to various terpenes and its derivatives.

INTRODUCTION

The genera Goniothalamus (Annonaceae) contains approximately 160 species (Saunders, 2003). These plants are ancient shrubs and treelets distributed in tropical and subtropical Asia such as Malaysia and Thailand (Saunders and Chalermglin, 2008). They have been broadly considered as sources for natural products with potential anticancer (Seyed et al., 2014), antiplasmodial, antimycobacterial, cytotoxic, antimicrobial and antibacterial activities (Calixto et al., 2012). The leaves of G. macrophyllus and G. giganteus are used to treat fever while the roots of G. macrophyllus and G. scortechinii are used to cure post-partum and abortion (Abdullah et al., 2013; Choo et al., 2014). The roots of G. tapis are used as abortifacient during pregnancy with a few months and treat typhoid fever (Colegate et al., 1990). The seeds of G. amuyon are used to treat scabies, rheumatism and tympanites (Ahmad et al., 1991; Macabeo et al., 2013; Pradupsri et al., 2009). Characterizations of the chemical constituents of Goniothalamus plants resulted in the isolation of bioactive compounds including styrllactones, alkaloids, annonaceous acetogenins, flavonoids, azaanthraquinones and naphthoquinones (Cao et al., 1998). Essential oils from Goniothalamus leaves and stem barks mainly consists of monoterpeneoid and sesquiterpenoids (Jantan et al., 2003). Stem bark oil of G. cardiopetalus and G. cylindrostigma exhibited strong inhibitory activity against Staphylococcus epidermidis and Candida albicans (Hisham et al., 2006). The essential oils from the twig and root of G. macrophyllus showed strong antimicrobial properties against Vancomycin intermediate-resistance S. aureus, S. epidermidis and C. albicans (Humeirah et al., 2010). Goniothalamus marcanii, known as Khao Lam in Thai, is planted widely in the northern, northeastern and southern regions of Thailand (Saunders and Chalermglin, 2008). It is a shrub tree with smooth, thin and fibrous trunk. The leaves are simple, alternate and exstipulate. Its flowers are axillary greenish yellow with specific aromatic fragrance.
In Thailand, *G. marcanii* are used treating for infectious diseases in early childhood under 5 years old (Maihiwan et al., 2013). Phytochemical investigation of *G. marcanii* leaf and twig demonstrated the presence of non-volatile compounds including 1-azaanthraquinone and naphthoquinone derivatives with cytotoxicity against human tumor cell lines including lung carcinoma, colon adenocarcinoma, breast carcinoma, melanoma and brain carcinoma (Soonthornchareonnon et al., 1999). Recently, three styryllactones including 5-hydroxygoniothalamin, 5-acetylgoniothalamin and gonioppyrone were isolated from *G. marcanii* leaves and twigs with potential anticancer activities in cell lines (Maihiwan et al., 2013).

Essential oils, also known as volatile oil, are aromatic oily viscous liquid derived from different parts of plants. They have been traditionally known to demonstrate pharmacological effects such as anti-inflammatory, antioxidant, cytotoxic properties (Sindhu et al., 2010; Baser and Buchbauer 2015). Essential oils are also used as naturalicides against broad range of bacterial (Rondón et al., 2016), fungal, viral (Ibrahim et al., 2015) and protozoal pathogens (Dai et al., 2010). According to less toxic and biodegradation properties comparing to synthetic antibiotics and preservatives, essential oils from plants possesses great potential for their antimicrobial activities in the medicine and food industry (Koul et al., 2008). To the best of our knowledge, the chemical composition and antibacterial activities of *G. marcanii* flower oil have not yet been explored. Therefore, this research aims to investigate the essential oil composition of *G. marcanii* flower as well as its antibacterial activities which will be tested against human pathogenic bacteria.

**MATERIAL AND METHODS**

**Plant materials**

The flowers of *G. marcanii* were collected in June 2016 at the health garden, Mae Fah Luang University, Chiang Rai, Thailand. A voucher specimen (MFL No. 10002) was deposited at Mae Fah Luang Botanical Garden, Mae Fah Luang University, Chiang Rai, Thailand.

**Essential oil isolation**

The *G. marcanii* flowers were subjected to hydrodistillation for 3 hours using a modified Clevenger-type apparatus. After extraction the distillate was collected in a conical flask which was then dried over anhydrous Na$_2$SO$_4$. The oil obtained was kept in a sealed vial until required with a yield of 0.62% w/w.

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

The chemical composition of *G. marcanii* flower oil obtained from hydrodistillation was analyzed using a Hewlett-Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS (5% phenylpolymethylsiloxane) capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, USA) interfaced to an HP model 5973 mass-selective detector. The oven temperature was initially held at 60 °C and then increased by 3 °C/min to 220 °C. The injector and detector temperatures were 250 and 280 °C, respectively. Purified helium was used as the carrier gas at a flow rate 1 ml/min. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 29–300. The ion multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230 and 150 °C, respectively. Identification of volatile components was performed by comparison of their Kovat retention indices, relative to C$_5$–C$_{30}$ n-alkanes, and using a comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 7N and NIST05 databases. The quantity of all identified components was investigated by using a percent relative peak area as shown in Table 1.

**Pathogenic bacteria**

The human pathogenic bacteria used in this study were Gram-positive bacterial strains i.e., *Staphylococcus epidermidis* DMST 15505, *S. aureus* DMST 8840 and *S. agalactiae* DMST 17129, Gram-negative bacterial strains i.e., *Proteus mirabilis* DMST 8212, *Escherichia coli* DMST 4212 and *Salmonella typhimurium* DMST 562. These bacteria were obtained from Department of Medical Science, Ministry of Health, Bangkok, Thailand. The bacterial strains were revived for bioassay by sub-culturing in tryptic soy broth medium for 24 hours before testing.

**Screening for antibacterial activity**

Antibacterial activity was done by modifying a method of Rajendran et al. (2014). The paper disc diffusion method was used to investigate for antibacterial activity of *G. marcanii* flower oil. Each human pathogenic bacterial strain was uniformly spread using sterile cotton swab on a sterile Petri dish Muller Hinton agar after adjusted to 0.5 McFarland standard. A sterilized 6-mm of paper disc (Whatman™, USA) was added with 30 μL of difference essential oil concentration and then placed on the infusion agar individually. The essential oils with different concentrations were prepared using dichloromethane as a solvent by two-fold dilution, (1000, 500, 250, 125, 62.50, 31.25, 15.62 7.81 and 3.91 μg/mL), then further dropped on to each paper disc (30 μL per paper disc). The treated plates were incubated at 37 °C for 24 hours. The antimicrobial activity was determined, using a ruler to measure the sizes in diameter of a paper disc including the inhibition clear zone. A negative control was also performed in the test, using a filter paper disc saturated with dichloromethane. Moreover, Chloramphenicol antibiotic dissolved in distilled water was also used as a reference control and each experiment was carried out in triplicate. Finally, the minimum inhibition concentration (MIC) defined as the lowest concentration of an essential oil extract that prevent bacterium growth was also done.

**RESULTS AND DISCUSSION**

The GC-MS chromatogram and all identified volatile compounds, the relative area percentages and their retention
indices are summarized in Fig. 1 and Table I, respectively. The *G. marcanii* flower oil contained 116 volatile components representing 93.83% of the oil were identified. The major components were caryophyllene oxide (19.28%), *E*-caryophyllene (14.58%), β-copaene (4.16%), α-humulene (3.64%), 1-epi-cubenol (3.37%), linalool (3.28%) and δ-cadinene (3.25%). *Cis*-cadin-4-en-7-ol (2.79%), 3E-cembrene A (2.77%), isolongifolan-7-α-ol (2.76%), trans-cadin-1(6),4-diene (2.61%), sesquithüferol (2.24%) and vulgarone B (2.21%) were also found to be minor components of the *G. marcanii* flower oil (Adams, 2007; König et al., 1999; Thang et al., 2013).

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The essential oil of *G. marcanii* flowers showed antibacterial activities against all tested bacterial pathogens (Table 2). A strong inhibitory effect against all tested pathogens was recorded as compared with chloramphenicol antibiotic. The flower oil of *G. marcanii* at the concentration of 1000 μg/ml diluted with dichloromethane (30 μL) exhibited the strong antibacterial properties against all tested human pathogenic bacteria ranging from 13.3 mm to 23.5 mm. A significant antibacterial activity of the *G. marcanii* flower oil was detected against *E. coli* and *S. aureus*. However, the *G. marcanii* flower oil revealed a moderate antibacterial activity against *S. epidermidis*, *S. agalactiae*, *S. typhimurium* and *P. mirabilis*.

**Table 2:** Inhibition zone diameter of bacterial pathogens by essential oil of *G. marcanii* flowers (30 μL corresponding to concentration of 1000 μg/mL).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition zone diameter (mm)</th>
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<tbody>
<tr>
<td>Gram-positive</td>
<td></td>
</tr>
<tr>
<td><em>S. agalactiae</em> DMST 17129</td>
<td>14.7 ± 1.4</td>
</tr>
<tr>
<td><em>S. aureus</em> DMST 8840</td>
<td>19.5 ± 2.7</td>
</tr>
<tr>
<td><em>S. epidermidis</em> DMST 15505</td>
<td>14.9 ± 1.8</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em> DMST 8212</td>
<td>13.3 ± 1.7</td>
</tr>
<tr>
<td><em>S. typhimurium</em> DMST 562</td>
<td>13.5 ± 2.1</td>
</tr>
<tr>
<td><em>E. coli</em> DMST 4212</td>
<td>23.5 ± 3.1</td>
</tr>
</tbody>
</table>

MIC values described as the lowest concentrations of *G. marcanii* flower oil that provided complete growth inhibition of all the test pathogens and inhibition zone diameter are also demonstrated in Table 3. The MIC value was against the most susceptible species of *E. coli* and *S. aureus* marked as 15.62 μg/mL and 31.25 μg/mL, followed by *S. epidermidis*, *S. agalactiae*, *S. typhimurium* and *P. mirabilis* with 125 μg/mL, 125 μg/mL, 125 μg/mL and 1000 μg/mL, respectively. As can be noticed, the antibacterial properties of the *G. marcanii* flower oil could be attributed to the occurrence of high proportions of terpenes and its derivatives in the oil as indicated by the work of (Abdelwahab et al., 2009) and (Zengin and Baysal, 2014) due to various functional groups among these volatile components. Antibacterial properties of *G. marcanii* flower oil might be related to compounds containing a high potential in strong inhibiting bacterial pathogens. These volatile components are considered to play an important role as antibacterial agents including caryophyllene oxide, E-caryophyllene, α-humulene, 1-epi-cubenol, linalool, sesquithuriferol and vulgarenone B corresponding to the high amount presented in the *G. marcanii* flower oil (Lago et al., 2011).

Conversely, the antibacterial activity was independant significantly of the amount of the volatile components. Therefore, some minor compounds such as acetylthione, dihydro-linalool, iso-isopulegol, β-cyclotricarvone and geraniol could be also correlated to its antibacterial properties. MIC values of *G. marcanii* flower oil ranged from 15.62 to 1000 μg/ml exhibited board range of antibacterial activity. The results indicated that the *G. marcanii* flower oil possessed bacteriostatic and bactericidal activities and demonstrated great potential as an antibacterial compound.

The obtained results for the antibacterial activity of the *G. marcanii* flower oil were correlated to accordance with Hisham et al. (2006), Wiert (2007) and Humeirah et al. (2010) who reported that *Goniothalamus* essential oils obtained from some species have potent antibacterial activities against the human pathogens such as *S. epidermidis* and *S. aureus*. The mode of action of the *G. marcanii* flower oil as antimicrobial agents may be due to inhibition of respiration and disrupting the permeability barriers of the cell membrane structures (Cox et al., 2000).

**Table 3:** Antibacterial activity expressed as inhibition zone diameter (mm) and MIC (μg/mL) of essential oils of *G. marcanii* flowers and chloramphenicol.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition zone diameter (mm)</th>
<th>MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>G. marcanii</em> oil</td>
<td><em>G. marcanii</em> oil</td>
</tr>
<tr>
<td><em>S. agalactiae</em> DMST 17129</td>
<td>0.84 ± 0.9</td>
<td>14.2 ± 1.1</td>
</tr>
<tr>
<td><em>S. aureus</em> DMST 8840</td>
<td>10.7 ± 1.8</td>
<td>12.4 ± 2.1</td>
</tr>
<tr>
<td><em>S. epidermidis</em> DMST 15505</td>
<td>9.8 ± 1.5</td>
<td>9.2 ± 1.4</td>
</tr>
<tr>
<td><em>P. mirabilis</em> DMST 8212</td>
<td>13.3 ± 1.7</td>
<td>–</td>
</tr>
<tr>
<td><em>S. typhimurium</em> DMST 562</td>
<td>13.5 ± 2.1</td>
<td>–</td>
</tr>
<tr>
<td><em>E. coli</em> DMST 4212</td>
<td>10.5 ± 2.1</td>
<td>10.3 ± 2.1</td>
</tr>
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~ not detected

**CONCLUSION**

The antibacterial activity of the essential oil extracted from *G. marcanii* flowers was due to the synergism between the various volatile components and mainly attributed to the presence of caryophyllene oxide, E-caryophyllene, α-copaene, α-humulene, 1-epi-cubenol, linalool and δ-cadinene as major constituents. The broad range antibacterial activities of the *G. marcanii* flower oil against variety of tested human pathogenic bacteria, can recommend its incorporation in different pharmaceutical applications.
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