

# 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,  $\alpha$ -humulene,  $\delta$ -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  $\mu$ g/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 tree lets distributing 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 styryllactones, alkaloids, annonaceous acetogenins, flavonoids, azaanthraquinones and naphthoquinones (Cao *et al.*, 1998). Essential oils from *Goniothalamus* leaves and stem barks mainly consists of monoterpenoid 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.

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In Thailand, *G. marcanii* are used treating for infectious diseases in early childhood under 5 years old (Mahiwan *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 goniopyrone were isolated from *G. marcanii* leaves and twigs with potential anticancer activities in cell lines (Mahiwan *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<sub>2</sub>SO<sub>4</sub>. 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 × 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 electron 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<sub>8</sub>-C<sub>23</sub> 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 1, 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%),  $\beta$ -copaene (4.16%),  $\alpha$ -humulene (3.64%), 1-epi-cubenol (3.37%), linalool (3.28%) and  $\delta$ -cadinene (3.25%). *Cis*-cadin-4-en-7-ol (2.79%), 3*E*-cembrene A (2.77%), isolongifolan-7- $\alpha$ -ol (2.76%), *trans*-cadin-1(6),4-diene (2.61%), sesquithuriferol (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).

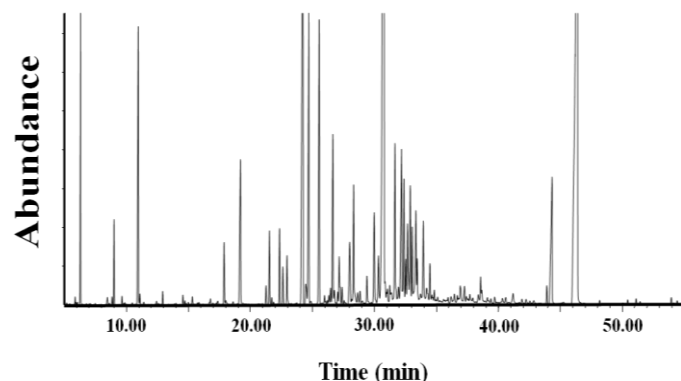


Fig. 1: GC-MS chromatogram of essential oil of *G. marcanii* flowers.

Table 1: Volatile constituents of essential oil of *G. marcanii* flowers.

No.	Retention time (min)	Compound	RI	%Peak area
1	5.86	3-methyl cyclohexanol	935	0.10
2	5.94	$\beta$ -citronellene	942	0.11
3	6.05	$\alpha$ -fenchene	945	0.12
4	6.28	benzaldehyde	952	2.54
5	6.78	sabinene	969	0.09
6	6.99	$\beta$ -pinene	979	0.07
7	7.11	<i>cis</i> -meta-mentha-2,8-diene	983	0.08
8	7.51	yomogi alcohol	999	0.09
9	8.48	benzyl alcohol	1025	0.11
10	8.64	sylvestrene	1026	0.14
11	8.85	benzene acetaldehyde	1032	0.07
12	9.01	<i>E</i> - $\beta$ -ocimene	1044	0.71
13	9.40	$\gamma$ -terpinene	1054	0.06
14	9.64	acetophenone	1059	0.12
15	9.77	<i>trans</i> -arbusculone	1066	0.06
16	9.88	<i>cis</i> -linalool oxide (furanoid)	1067	0.06
17	10.47	<i>trans</i> -linalool oxide (furanoid)	1084	0.09
18	10.71	methyl benzoate	1088	0.08
19	10.92	linalool	1095	3.28
20	11.08	$\alpha$ -pinene oxide	1099	0.09
21	11.39	phenyl ethyl alcohol	1106	0.08
22	12.32	dihydro-linalool	1131	0.07
23	12.42	benzeneacetonitrile	1134	0.06
24	12.57	<i>E</i> -epoxy-ocimene	1137	0.17
25	12.66	<i>trans</i> -limonene oxide	1137	0.16
26	12.92	<i>trans</i> -verbenol	1140	0.12
27	13.65	$\beta$ -pinene oxide	1154	0.05
28	13.99	iso-isopulegol	1155	0.07
29	14.53	$\alpha$ -terpineol	1186	0.17
30	14.69	methyl salicylate	1190	0.06
31	14.81	<i>cis</i> -dihydro carvone	1191	0.07
32	14.98	<i>trans</i> -4-caranone	1196	0.08
33	15.29	<i>cis</i> -4-caranone	1200	0.09
34	15.71	<i>trans</i> -carveol	1215	0.06

35	15.85	$\beta$ -cyclocitral	1217	0.07
36	16.65	pulegone	1233	t
37	16.76	cumin aldehyde	1238	0.08
38	16.95	carvone	1239	0.09
39	17.23	geraniol	1249	0.09
40	17.36	<i>cis</i> -myrtanol	1250	0.07
41	17.86	<i>E</i> -cinnamaldehyde	1267	0.75
42	18.00	tetrahydro-lavandulol acetate	1268	0.09
43	18.58	neryl formate	1280	0.05
44	18.94	$\gamma$ -terpinen-7-ol	1290	0.06
45	19.13	1-nitro-2-phenyl ethane	1294	2.11
46	21.25	$\alpha$ -cubebene	1345	0.19
47	21.52	$\alpha$ -longipinene	1350	0.91
48	21.71	neoisodihydro carveol acetate	1356	0.11
49	21.86	<i>Z</i> - $\beta$ -damascenone	1361	0.06
50	22.34	$\alpha$ -copaene	1374	0.83
51	22.61	<i>E</i> -methyl cinnamate	1376	0.41
52	22.93	$\beta$ -cubebene	1387	0.59
53	23.17	sativene	1390	0.08
54	23.30	$\alpha$ -chamipinene	1396	0.05
55	23.61	$\alpha$ -funebrene	1402	0.07
56	23.73	italicene	1405	0.06
57	24.19	<i>E</i> -caryophyllene	1417	14.58
58	24.46	<i>cis</i> -thujopsene	1429	0.05
59	24.69	$\beta$ -copaene	1430	4.16
60	24.87	isoamyl benzoate	1433	0.06
61	25.55	$\alpha$ -humulene	1452	3.64
62	25.76	$\alpha$ -patchoulene	1454	0.06
63	25.98	dehydro-aromadendrene	1460	0.07
64	26.08	$\gamma$ -decalactone	1465	0.06
65	26.22	<i>cis</i> -thujopsadiene	1465	0.07
66	26.33	dauca-5,8-diene	1471	0.08
67	26.48	$\alpha$ -neocallitropsene	1474	0.09
68	26.62	<i>trans</i> -cadin-1(6),4-diene	1475	2.61
69	26.77	amorpho-4,7(11)-diene	1479	0.07
70	27.05	$\alpha$ -amorphene	1483	0.08
71	27.17	germacrene D	1484	1.13
72	27.40	<i>cis</i> - $\beta$ -guaiene	1492	0.08
73	27.56	viridiflorene	1496	0.07
74	28.01	$\delta$ -amorphene	1511	0.09
75	28.19	eugenol acetate	1521	0.08
76	28.33	$\delta$ -cadinene	1522	3.25
77	28.65	zonarene	1528	0.05
78	28.85	$\gamma$ -cuprenene	1532	0.05
79	29.03	furopergane A	1538	0.06
80	29.40	italicene epoxide	1547	0.43
81	29.98	<i>E</i> -nerolidol	1561	1.49
82	30.34	dendrolasin	1570	0.06
83	30.69	caryophyllene oxide	1582	19.28
84	31.22	viridiflorol	1592	0.55
85	31.63	sesquithuriferol	1604	2.24
86	31.91	epi-cedrol	1618	0.09
87	32.15	isolongifolan-7- $\alpha$ -ol	1618	2.76
88	32.34	1-epi-cubenol	1627	3.37
89	32.51	5-cedranone	1628	0.05
90	32.64	$\beta$ -cedren-9-one	1631	0.06
91	32.86	<i>cis</i> -cadin-4-en-7-ol	1635	2.79
92	33.01	hinesol	1640	0.08
93	33.31	vulgarone B	1649	2.21
94	33.42	pogostol	1651	0.05
95	33.93	<i>E</i> -amyl cinnamaldehyde	1667	1.44
96	34.18	5-iso-cedranol	1672	0.46
97	34.44	khusinol	1679	1.38
98	34.79	<i>Z</i> -apritone	1689	0.05
99	35.91	sedanenolide	1719	0.17
100	36.16	guaial acetate	1725	0.19
101	36.42	curcumenol	1733	0.23
102	36.89	2 <i>E</i> ,6 <i>E</i> -farnesol	1745	0.68
103	37.24	$\beta$ - <i>Z</i> -curcumen-12-ol	1754	1.05
104	38.35	$\gamma$ -eudesmol acetate	1783	0.09

105	38.53	$\beta$ -eudesmol acetate	1792	1.21
106	38.64	$\alpha$ -eudesmol acetate	1794	0.09
107	41.16	Z,Z-farnesyl acetone	1860	0.31
108	43.89	cembrene	1938	0.28
109	44.29	3E-cembrene A	1947	2.77
110	48.14	canellal	2045	0.15
111	50.41	laurenan-2-one	2115	0.17
112	51.10	nezukol	2132	0.14
113	51.40	osthole	2140	0.19
114	53.99	catalpalactone	2199	0.18
115	54.10	$\alpha$ -santonine	2203	0.17
116	54.38	phyllocladanol	2209	0.18
<b>Total (%)</b>			93.83	
<b>Essential oil yield (%)</b>			0.62	

RI: retention index on HP-5MS column; t: trace amount <0.05

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  $\mu$ g/ml diluted with dichloromethane (30  $\mu$ L) 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  $\mu$ L corresponding to concentration of 1000  $\mu$ g/mL).

Bacteria	inhibition zone diameter (mm)
Gram-positive	
<i>S. agalactiae</i> DMST 17129	14.7 $\pm$ 1.4
<i>S. aureus</i> DMST 8840	19.5 $\pm$ 2.7
<i>S. epidermidis</i> DMST 15505	14.9 $\pm$ 1.8
Gram-negative	
<i>P. mirabilis</i> DMST 8212	13.3 $\pm$ 1.7
<i>S. typhimurium</i> DMST 562	13.5 $\pm$ 2.1
<i>E. coli</i> DMST 4212	23.5 $\pm$ 3.1

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  $\mu$ g/mL and 31.25  $\mu$ g/mL, followed by *S. epidermidis*, *S. agalactiae*, *S. typhimurium* and *P. mirabilis* with 125  $\mu$ g/mL, 125  $\mu$ g/mL, 125  $\mu$ g/mL and 1000  $\mu$ g/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,  $\alpha$ -humulene, 1-epi-cubenol, linalool, sesquithuriferol and vulgarone B corresponding to the high amount presented in the *G. marcanii* flower oil (Lago *et al.*, 2011).

Conversely, the antibacterial activity was independent significantly of the amount of the volatile components. Therefore, some minor compounds such as acetophenone, dihydro-linalool, iso-isopulegol,  $\beta$ -cyclocitral, carvone and geraniol could be also correlated to its antibacterial properties. MIC values of *G. marcanii* flower oil ranged from 15.62 to 1000  $\mu$ g/ml exhibited broad 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), Wiart (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 ( $\mu$ g/mL) of essential oils of *G. marcanii* flowers and chloramphenicol.

Bacteria	inhibition zone diameter (mm)		MIC ( $\mu$ g/mL)	
	<i>G. marcanii</i> oil	chloramphenicol	<i>G. marcanii</i> oil	chloramphenicol
Gram-positive				
<i>S. agalactiae</i> DMST 17129	0.84 $\pm$ 0.9	14.2 $\pm$ 1.1	500	15.62
<i>S. aureus</i> DMST 8840	10.7 $\pm$ 1.8	12.4 $\pm$ 2.1	31.25	15.62
<i>S. epidermidis</i> DMST 15505	9.8 $\pm$ 1.5	9.2 $\pm$ 1.4	125	15.62
Gram-negative				
<i>P. mirabilis</i> DMST 8212	13.3 $\pm$ 1.7	–	1000	–
<i>S. typhimurium</i> DMST 562	13.5 $\pm$ 2.1	–	1000	–
<i>E. coli</i> DMST 4212	10.5 $\pm$ 2.1	10.3 $\pm$ 2.1	15.62	7.81

–: 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,  $\beta$ -copaene,  $\alpha$ -humulene, 1-epi-cubenol, linalool and  $\delta$ -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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**Conflict of Interests:** There are no conflicts of interest.

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