VOLATILE CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *GARDENIA JASMINOIDES*

Jarubol Chaichana[1,2], Wirat Niwatananun[3], Suwanna Vejabhikul[1] Sudsawad Somna[2] and Sunee Chansakaow[1,*]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand [2]Regional Medical Science Center Chiang Mai, Maerim, Chiang Mai 50180, Thailand [3]Department of Pharmaceutical Care, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

ABSTRACT: The purposes of this research were to investigate volatile constituents and biological activities of *Gardenia jasminoides* J. Ellis flowers. Hydro-distillation and solvent extraction were used as the extraction methods to obtained essential oil and absolute, respectively. The chemical constituents of essential oils were characterized by GC-MS with KI. Absolute and fresh flowers were identified by Headspace-SPME-GC-MS. Major chemical constituents of the essential oil, absolute and fresh flowers of *G. jasminoides* were linalool, *alpha*-farnesene, *z*-3-hexenyl tiglate and *trans-beta*-ocimene. The essential oil and absolute possessed antimicrobial activities against *Staphylococcus aureus* and *Staphylococcus. epidermidis*, the activity towards *Escherichia coli* presented for only essential oil and the activity against *Candida albicans* appeared for only absolute. The essential oil exhibited TEAC 2.56 mg Trolox/1 ml essential oil and FRAP 7.27 mg Fe^{2+}/1 ml essential oil. The absolute presented TEAC 29.71 mg Trolox/1 g absolute and FRAP 77.96 mg Fe^{2+}/1 g absolute.

Keywords: *Gardenia jasminoides*, antimicrobial activities, antioxidant activities, chemical constituents, Kovats index, Headspace-SPME-GC-MS

INTRODUCTION: *Gardenia jasminoides* J. Ellis (Rubiaceae), commonly known as Pudson in Thailand, is a popular ornamental plant with white and sweet fragrant flowers[1,2]. According to a report on an ethnobotanical survey, the people in Northern Thailand used this flower in various occasions such as to worship a deity, to pin women's bun, to treat skin diseases in Thai traditional medicine. Nowadays, the Gardenia flowers are accepted popularly in Thai Lanna Spa because of their intensely sweet fragrant, beautiful conform.

Prone (1903) reported that benzyl acetate, terpineol and linalool were found in the essential oil[3]. Previous phytochemical investigations of fresh Gardenia flowers described the presence of farnesene, *cis*-ocimene, linalool, *cis-3*-hexenyl tiglate and methyl benzoate[4] Ishikawa (2004) reported that the fresh flowers possessed linalool and (*E,E*)-*α*-farnesene as the highest ratio with Aqua-space® technique[5]. The essential oil exhibited antimicrobial activity against *Campylobacter jejuni* and *Listeria monocytogenes*[6]. The purposes of this research were to investigate antibacterial activities against the pathogenic organisms of public health significance, anti-oxidant activities and chemical constituents of the essential oil, absolute and fresh living flowers.

MATERIALS AND METHODS:

Plant materials

Fresh *Gardenia jasminoides* flowers were collected from Amphur Sankampang, Chiang Mai in March-June 2008. The sample was identified by comparison with the voucher specimen deposited at The Queen Sirikit Botanic Garden Herbarium, Chiang Mai.

Extraction of essential oil

The essential oil was obtained by hydro-distillation using cleavenger - type apparatus for 5 hours. The essential oil was dried over anhydrous sodium sulphate and stores in sealed vial protected from light at 4°C before analyses.

Extraction of absolute

The petals of Gardenia flowers were cut into small pieces and then macerated with petroleum ether for 24 hours and evaporated to dryness by rotary evaporator. The concrete was treated with

*To whom correspondence should be addressed.
E-mail: pmischns@chiangmai.ac.th
Tel. +66 5394 4342, Fax. +66 5322 2741

denature alcohol to obtain absolute. Denature alcohol was eliminated from an absolute by rotary evaporator before testing of biological activities.

Chemical analysis of essential oil

A Shimadzu GCMS-QP 2010 Plus system was used, with a mass-selective detector with electron impact ionization. The samples were separated using a DB-5 MS capillary column. (5% phenyl-methylpolysiloxane, 30 m, 0.25 mm i.d., 0.25 μm film thickness) The temperature program used for analysis was as follows: the initial temperature was 80 °C, ramp to 106 °C with the rate of 5 °C/min for 1 min, ramp to 140 °C with the rate of 10°C/min and then ramp to 250 °C/min with the rate 8 °C/min for 6 min. Helium (99.999 %) was the carrier gas maintained at a flow rate 1 ml/min. The split flow ratio was 1: 50.

The identification of volatile components was based on computer matching with WILLEY 7 library, as well as by comparison of the mass spectra and Kovats retention indices (KI) with those reported in the literature.

Chemical analysis of absolute and fresh flowers

Absolute and fresh flowers were performed using headspace-SPME-GC-MS with 65 μm PDMS/DVB fiber coating. The condition of Headspace-SPME used for analysis was as follows; pre incubation time: 10 min, incubation temperature 40 °C, extraction time 30 min, desorption time 2 min. GC-MS instrumentation and column temperature program were performed with the same conditions as the analysis of essential oil.

Agar Disc Plate method[7]

The microorganisms including *S. aureus* ATCC 6538, *S. epidermidis*, *E. coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhi* DMST 22842, the fungi *C. albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 on tryptone soy agar slants were obtained from the culture collections of the NIH.

Then each microbe was incubated in tryptic soy broth (TSB) at 37 °C for 4-6 hrs. TSB were diluted with sterile water until the turbidity equal to McFarland No 5. Bacteria strains were cultivated on Mueller-Hinton Agar while fungi were cultivated on Saboraud Dextrose Agar. The surface of each plate was inoculated equally and entirely with a cotton swab moistened with a broth culture of microbes.

Sterile paper discs (6 mm diameter) were applied with essential oil or absolute and then placed on the inoculated agar surface with aseptic technique. The plates were incubated at 37 °C for 24 hr to 7 days, depending on the microorganism tested in the upright position. The zone of inhibition was observed, and measured the diameter (mm). 5% mg/ml Chloramphenichol was used as a positive control.

Determination of antioxidant activity

ABTS method [8]

$ABTS^{\bullet+}$ (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) stock solution was prepared, 7 mM ABTS : 2.45 mM $K_2S_2O_8$; ratio 1 : 0.5 mole/mole, and then the mixtures were allowed to stand in dark at 4-5 °C, 12 hours for complete reaction. The working solution of $ABTS^{\bullet+}$ was prepared by diluted with absolute alcohol, measured the absorbance at 734 nm approximately. Standard Trolox stock solution (2.5 mM) was prepared, and diluted to concentration of 2.0, 1.5, 1.0, 0.5 and 0.1 mM. Relative activities were calculated from the standard curve of standard solution under the same experiment condition and expressed as mg standard per gram weight materials. All measurements were done in triplicate.

FRAP method [9,10]

The FRAP Working solution was freshly prepared, 300 mM acetate buffer pH 3.6 : 10 mM TPTZ : 20 mM $FeCl_3.6H_2O$; ratio 10 : 1 : 1. The test sample and reagent blank were incubated in dark at room temperature for 4 min. At the end of incubation, the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. Aqueous solution of known Fe^{2+} concentration, ranging from 50 to 1000 μM $FeSO_4.7H_2O$, was used for the preparation of standard solution. The relative activities of samples were assessed by comparing their activities with that of Fe^{2+}.

RESULTS AND DISCUSSION: The percentage yield of the essential oil of *G. jasminoides* flowers was 0.02 % v/w (fresh weight). The comparative chemical constituents of the essential oil, absolute and fresh flowers were presented. (Table 1 and Figure 1)

Conditions of Headspace-SPME for extraction volatile compounds released from fresh flowers were setting mimic to the daytime temperature in natural environment at 40 °C. The headspace-SPME is rapid, solvent-free extraction technique and very suitable for field extraction of volatile compounds emitted from fresh flowers[11]. The major volatiles of the essential oil, absolute and fresh flowers were linalool, *alpha*-farnesene, *z*-3-hexenyl tiglate and *trans-beta*-ocimene. Noteworthy is the different extraction methods gave distinct minor compositions.

The essential oil of *G. jasminoides* possessed antimicrobial activities against *S. aureus*, *S. epidermidis* (gram positive bacteria) and *E. coli* (gram negative bacteria). The absolute exhibited antimicrobial activities against *S. aureus*, *S. epidermidis* and *C. albicans* as presented in Table 2. In the previous investigation, linalool and α-terpineol reported as the active antimicrobial compounds on standard organism such as *S. aureus*, *E. coli* and *C. albicans* supported the antimicrobial activities in this plant [12].

ABTS assay is a single electron transfer reaction, operationally simple, applied for both lipophilic and hydrophilic compounds. The mechanism is based on the ability of antioxidant molecules to quench the long-lived ABTS radical, a blue-green chromophore with absorption at 734 nm, compared with the ability of Trolox (Trolox

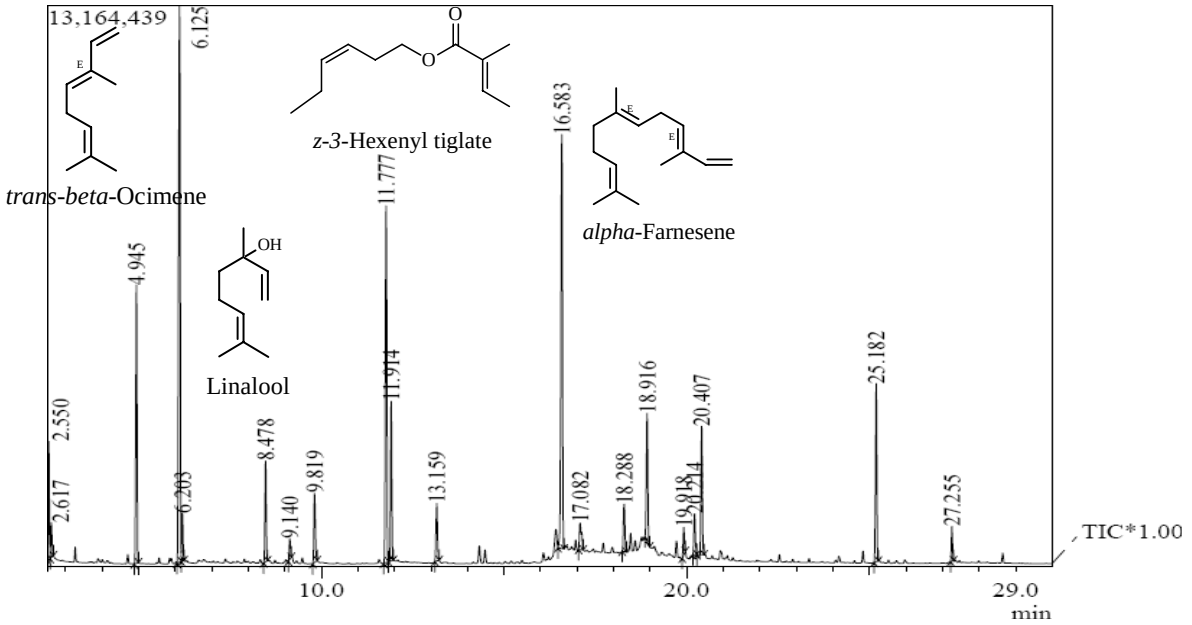

Figure 1 TIC of the essential oil of *G. jasminoides*

Table 2 Antimicrobial activities of the essential oil and absolute of *G. jasminoides*

Test microorganism	Inhibition zone (mm)		
	Essential oil	Absolute	5% mg/ml chloramphenicol
Staphylococcus aureus	17.3±1.5	19.0±1.0	30.3±1.5
Staphylococcus epidermidis	20.7±1.5	12.3±0.6	38.0±0.0
Salmonella typhi	-	-	8.3±1.2
Escherichia coli	15.0±0.0	-	27.7±0.6
Pseudomonas aeruginosa	-	-	14.0±0.0
Candida albicans	-	14.0±1.0	NA
Aspergillus niger	-	-	NA

- no inhibition zone, NA: not analysis

Table 1 Identification of volatile constituents of *G. jasminoides* flowers

No.	RT (min)	Compounds	KI[a]	Groups	Formular	Mw	Relative content (%)		
							Essential oil	Absolute	Fresh flowers
1	2.550	*cis-3*-Hexenol	-[b]	alcohols	$C_6H_{12}O$	100	2.63	-	0.76
2	2.586	Methyl tiglate	-[b]	esters	$C_6H_{10}O_2$	114	-	-	4.30
3	3.281	*alpha*-Thujene	935	monoterpenes	$C_{10}H_{16}$	136	-	-	0.06
4	3.786	1-Octen-3-ol	979	alcohols	$C_8H_{16}O$	128	-	0.22	-
5	4.125	*Z-3*-Hexenyl acetate	1005	esters	$C_8H_{14}O_2$	142	-	-	0.06
6	4.708	*cis*-Ocimene	1037	monoterpenes	$C_{10}H_{16}$	136	0.24	-	0.03
7	**4.945**	***trans-beta*-Ocimene**	**1050**	**monoterpenes**	**$C_{10}H_{16}$**	**136**	**8.64**	**2.61**	**9.50**
8	5.984	*z-3*-Hexenyl propionate	1098	esters	$C_9H_{16}O_2$	156	-	-	0.05
9	**6.125**	**Linalool**	**1104**	**Monoterpene alcohols**	**$C_{10}H_{18}O$**	**154**	**21.49**	**22.46**	**38.23**
10	6.203	Ho-trienol	1109	monoterpene alcohols	$C_{10}H_{16}O$	152	1.33	-	-
11	6.534	Phenethyl alcohol	1121	benzyl alcohols	$C_8H_{10}O$	122	-	2.31	-
12	7.866	Ethyl benzoate	1173	benzyl esters	$C_9H_{10}O_2$	150	-	2.46	-
13	7.988	Borneol	1182	monoterpene alcohols	$C_{10}H_{18}O$	154	-	0.94	-
14	8.067	*z-3*-hexenyl butyrate	1186	monoterpene esters	$C_{10}H_{18}O_2$	170	-	-	0.02
15	8.416	Methyl salicylate	1199	phenolic esters	$C_8H_8O_3$	152	-	1.04	0.15
16	8.478	*alpha*-Terpineol	1202	monoterpene alcohols	$C_{10}H_{18}O$	154	3.05	-	-
17	9.140	Nerol	1230	monoterpene alcohols	$C_{10}H_{18}O$	154	0.75	-	-
18	9.148	*z-3*-hexenyl2-methylbutanoate	1227	esters	$C_{11}H_{20}O_2$	184	-	-	0.25
19	9.600	Ethyl phenylacetate		phenyl esters	$C_{10}H_{12}O_2$	164	-	0.24	-
20	9.819	Geraniol	1253	monoterpenes alcohols	$C_{10}H_{18}O$	154	2.49	0.21	-
21	11.162	Indole	1301	miscellaneous	C_8H_7N	117	-	-	0.29
22	**11.777**	***z-3*-hexenyl tiglate**	**1324**	**esters**	**$C_{11}H_{18}O_2$**	**182**	**12.67**	**15.21**	**16.27**
23	11.914	Hexyl tiglate	1333	esters	$C_{11}H_{20}O_2$	184	4.84	3.48	3.94
24	12.520	Eugenol	1359	phenols	$C_{10}H_{12}O_2$	164	-	0.36	-
25	13.018	*alpha*-Copaene	1377	sesquiterpenes	$C_{15}H_{24}$	204	-	-	0.03
26	13.159	Calarene	1384	sesquiterpenes	$C_{15}H_{24}$	204	2.22	1.51	-
27	13.543	n-Tetradecane	1397	miscelaneous	$C_{14}H_{30}$	198	-	0.23	-
28	14.325	*trans*-Caryophyllene	1422	sesquiterpenes	$C_{15}H_{24}$	204	0.66	1.28	2.84
29	14.482	unknown	1428				0.48	0.35	0.16
30	15.163	isoeugenol	1454	phenols	$C_{10}H_{12}O_2$	164	-	1.28	0.31
31	15.989	Germacrene D	1485	sesquiterpenes	$C_{15}H_{24}$	204	-	-	0.20
32	16.180	*alpha*-Berganotene	1490	sesquiterpenes	$C_{15}H_{24}$	204	-	1.62	-
33	16.368	Bicyclogermacrene	1499	sesquiterpenes	$C_{15}H_{24}$	204	-	1.53	0.65
34	**16.583**	***alpha*-Farnesene**	**1506**	**sesquiterpenes**	**$C_{15}H_{24}$**	**204**	**17.54**	**23.35**	**21.40**
35	16.817	*beta*-Guaiene	1515	sesquiterpenes	$C_{15}H_{24}$	204	-	0.38	-
36	16.936	*delta*-Cadinene	1521	sesquiterpenes	$C_{15}H_{24}$	204	-	0.38	0.10
37	18.288	*cis-3*-Hexenyl benzoate	1574	benzyl esters	$C_{13}H_{16}O_2$	204	1.75	0.80	0.08
38	18.833	Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester	1600	esters	$C_{11}H_{10}O_3$	190	-	8.38	-
39	18.916	Guaiol	1601	sesquiterpene alcohols	$C_{15}H_{26}O$	222	6.36	1.42	-
40	19.885	*delta*-Cadinol	1649	sesquiterpene alcohols	$C_{15}H_{26}O$	222	-	0.24	-
41	20.214	Unknown	1666				1.68	-	-
42	20.407	Bulnesol	1673	sesquiterpene alcohols	$C_{15}H_{26}O$	222	4.26	1.28	-
43	24.551	Methyl palmitate	1923	esters	$C_{17}H_{34}O_2$	270	-	0.17	-
44	25.182	unknown	1961				5.86	1.77	-
45	27.210	n-Tricosane	-[b]	miscelaneous	$C_{23}H_{48}$	324	-	0.97	-
46	27.255	Tetracosane	-[b]	miscelaneous	$C_{24}H_{50}$	338	1.01	-	-

- not identified, [a] Kovats retention index relative to C_8-C_{20} *n*-alkanes on DB-5 column.
[b] Retention times is outside of retention times of homologous series of C_8-C_{20} *n*-alkanes

equivalent antioxidant capacity: TEAC) and the essential oil of *Zingiber cassumunar*. Dose-response curve presented linearity between percent inhibition and quantity of Trolox (R^2 = 0.9999). Antioxidant activities of essential oil and absolute of *G. jasminoides* exhibited TEAC 2.56 mg Trolox/1 ml essential oil and 29.71 mg Trolox/1 g absolute, respectively. While the essential oil of *Z. cassumunar* presented TEAC 8.58 mg Trolox/1 ml essential oil.

The reducing power of FRAP method measure reduction of Ferric-TPTZ to an intense blue colored product. Dose-response were linearity between absorbance changes at 593 nm 4 min reaction time and quantity of standard Fe^{2+} with R^2 = 0.9999. Ferric reducing antioxidant power of the essential oil and absolute of *G. jasminoides* were 7.27 mg Fe^{2+}/1 ml essential oil and 77.96 mg Fe^{2+}/1 g absolute, respectively. Whereas reducing power of the essential oil of *Z. cassumunar* was 15.37 mg Fe^{2+}/1 ml essential oil.

According to their biological activities, the essential oil and absolute of *G. jasminoides* may be one of the alternative natural extracts for antibacterial activity used in sanitary products to assist the consumer hygiene, to preserve food, and as anti-aging for cosmetic industries, spa products and aromatherapy.

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