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Volatile profile and sensory property of *Gardenia jasminoides* aroma extracts

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Synopsis

The volatile profiles of aroma extracts prepared from the flower of *Gardenia jasminoides* by different methods were investigated using gas chromatography-mass spectrometry. The enfleurage extraction using spermaceti wax and palm oil afforded the best aroma extract with a preference that was significantly ($p < 0.05$) better than those from solvent extractions, as sensorially evaluated in 43 volunteers. The odor quality of the absolute de enfleurage was similar to the floral scent of fresh gardenia, as confirmed in 152 volunteers. Although female volunteers were insignificantly ($p > 0.05$) better sensed than male volunteers, age was significant ($p < 0.05$). The nuance gardenia floral scent was contributed by farnesene, *Z*-3-hexenyl tiglate, *Z*-3-hexenyl benzoate, and indole. The relaxing and refreshing sensations of the gardenia odor suggest its applications in body care, cleansing products, and perfume. This study addresses the increasing interest in floral fragrances. The aroma profile and sensory property of this sweet and elegant scent flower will strengthen and expand the applications of gardenia from traditional medicine to those of perfumery and the field of phytochemistry.

INTRODUCTION

Gardenia jasminoides Ellis (Rubiaceae) or gardenia is a fragrant flowering plant that has long been used in traditional Chinese medicines. Its fruit is used for hepatic pain caused by cirrhosis, abdominal pain caused by dysentery, and it is considered to have antiphlogistic, diuretic, laxative, choleretic, and homeostatic effects when applied topically. In addition, the fruit also shows antiangiogenic activity. Accordingly, the fruit has been studied extensively to identify the active components, which are mainly iridoids (1,2). In addition, use of the fruit extract for treatment and/or protection of gastritis has been reported (3).

This herb with white fragrant flowers is largely cultivated in warm temperate and subtropical climates and is abundant in Thailand. However, the volatile components of the *G. jasminoides* flower have received sparse attention. We consider that aroma profile analysis of this sweet and elegant scent flower will strengthen and expand the usefulness

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of *G. jasminoides* from its use in traditional medicine into perfumery and the field of phytochemistry (4–6).

Aroma extracts of the *G. jasminoides* flower were prepared by means of enfleurage and solvent extraction suitable for flower fragrance preparation (7–9). The aroma profiles of each extract were comparatively analyzed and sensory evaluation was conducted by volunteers.

MATERIALS AND METHODS

PLANT MATERIALS

Gardenia jasminoides flowers were cultivated at Mae Fah Luang University, Chiang Rai, Thailand. The flowers were harvested in the morning at the beginning of its blossoming stage from March to May.

ENFLEURAGE BASE PREPARATION

Spermaceti wax (Namsiang Trading, Thailand) was mixed with palm oil (Tanakorn Vegetable Oil Products, Samutprakarn, Thailand). The warm melt base was poured into aluminum trays (4.5 × 17.0 cm).

FLOWER OIL PREPARATION

All the solvents used were of analytical grade unless otherwise stated.

SOLVENT EXTRACTION

Petals were macerated in an Erlenmeyer flask in 700 ml of absolute ethanol, *n*-hexane, or petroleum ether (Merck, Darmstadt, Germany) for 24 h. The maceration vessels were sealed from light and air at ambient temperature. The mixture was filtered and the solvent fraction was concentrated to give a concrete (10).

ENFLEURAGE

Fresh petals were strewn by hand on the top of the enfleurage bases in a single layer. The trays were wrapped with aluminum foil and plastic wrap to prevent exposure to light and air. The enfleurage trays were kept at room temperature for 24 h. The defleurage flowers were replaced daily with fresh flowers each morning for 14 days. The resulting pomade was removed and extracted with denatured alcohol (Merck). The temperature of the ethanolic fragrant solution was kept under 20°C before filtration. These processes were repeated until a clear solution was obtained to give an absolute de pomade. The absolute de pomade was further concentrated under reduced pressure at 35°C to yield an absolute de enfleurage (10).

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS)

The aroma extract (50 mg) was diluted in CH_2Cl_2 (Fisher Scientific, Leicestershire, United Kingdom). An aliquot (5 μl) was injected into a gas chromatograph (GC; Agilent 6890N, Santa Clara, CA) equipped with an HP-5MS column (Agilent 19091S-433, 30 m × 250 μm, 0.25 μm film thickness) and connected to a mass spectrometer (MS; Agilent 5973N). The samples were separated under the following analytical conditions. The oven program started at 80°C, rising to 300°C at a rate of 7°C/min. Helium was used as carrier gas at a flow rate of 1.0 ml/min at a pressure of 64 kPa. Injection was performed in splitless mode with the injector temperature set at 220°C. Reference mass spectra were obtained from the MS-Wiley 7n.1 database. The enfleurage bases and maceration solvents were also injected to determine background cutoffs. Aroma compounds were identified on the basis of the mass spectra showing more than 95% similarity. Content was reported based on the peak area of the identified compounds.

PREFERENCE TEST

Nonsmoking, healthy Thai male (21) and female (22) volunteers aged more than 16 years without olfactory disorders and fragrance and pollen allergies participated in the preliminary preference test. The absolute de enfleurage and concretes (50 mg) were prepared in amber bottles with a sniff port diameter of 1 mm. The volunteers were assigned to sniff each sample for 1 min with a 2-min rest period before the next test. This sensory evaluation was conducted in a controlled-environment room without interference factors. The volunteers were denied access to fragrance and scent products for 3 h before the test and during the test.

The Likert scale was used for odor intensity (1–5) and preference (1–5) evaluations. The score interval was [(maximum means – minimum means)/5]. The data were analyzed and presented as means ± standard deviation.

ODOR QUALITY OF ABSOLUTE DE ENFLEURAGE

One hundred and fifty-two healthy male (76) and female (76) Thai volunteers were classified into four age groups: 16–25, 26–35, 36–45, and >45 years. All of the volunteers refrained from smoking and drinking of liquor, and they did not have respiratory disorders such as head colds, asthma, or allergies. The most preferred *G. jasminoides* aroma extract from 2.5 was diluted in mineral oil (Namsiang) at a concentration of 50 mg/ml. The aroma sample (20 μl) was adsorbed on filter paper (Whatman no. 1, Sigma-Aldrich, Singapore) cut into squares (1 × 1 cm) and placed inside the amber bottle with a sniff port as above, and was sensorially evaluated. A Likert scale was used for odor preference (1–5) and difference (1–5) evaluations. The difference was compared with fresh *G. jasminoides*. The odor quality was statistically analyzed using SPSS V.11.5 (IBM, Thailand) and the significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Spermaceti wax was selected to prepare the enfleurage base because of its optimal physical properties and no rancidity that could alter the extracted flower scent (11). The wax was mixed with palm oil, which is a versatile product in Thailand with significant applications in cosmetics. The oil and wax ratio were varied to optimize the base for gardenia odor extraction. A high-consistency, odorless, colorless, semihard base that allowed easy removal of the flower was obtained (10) with a spermaceti wax to palm oil ratio of 3:2. Thereafter, the enfleurage base was used for the fragrant oil extraction in addition to the maceration extraction.

Table I
Appearances and Extractive Yields of *G. jasminoids* flower oil

Sample			
No.	Name		Appearance
1	Concrete	Absolute ethanol	Yellow viscous semisolid
2		Petroleum ether	Deep yellow viscous semisolid
3		*n*-Hexane	Greenish yellow viscous semisolid
4	Absolute de enfleurage		Pale viscous liquid with fresh gardenia odor

Maceration of fresh gardenia using absolute ethanol, petroleum ether, and *n*-hexane gave fragrant oil extracts with different appearances. Although all of the concretes were viscous semisolids, absolute ethanol concrete was the least colored. In contrast, the concretes from extractions with nonpolar solvents were colored (Table I). The aroma sample of enfleurage yielded a slightly colored absolute de enfleurage with an obvious scent of gardenia.

All of the gardenia aroma extracts were comparatively analyzed, as shown in Table II. The volatile profiles of concretes from different solvents differed, possibly because of bioconversion that can occur during the picking and extraction processes (10). The gardenia flower waxes of palmitic acid, *E*-5,10-secocholest-1(10)-en-3,5-dione, stigmasta-5,22-dien-3-ol, and γ-sitosterol were extracted using absolute ethanol, along with the flavoring substance 2-methoxy-4-vinylphenol. In contrast, the concrete from petroleum ether and *n*-hexane gave linalool, *Z*-3-hexenyl tiglate, and guaiol as the main aroma contributions, while ethyl linoleate and bicyclo[4.3.1]dec-1(9)-ene were detected in the

Table II
Volatile Profiles (%) of Gardenia Aroma Extracts

		Sample			
Compound	RT	1	2	3	4
E-3,7-Dimetyl-1,3,6-octatriene	6.81	-	0.39	-	0.28
L-Linalool	8.21	-	2.34	2.17	-
1*H*-Indole	12.98	-	-	-	0.15
2-Methoxy-4-vinylphenol	13.35	0.66	-	-	-
Z-3-Hexenyl tiglate	13.52	-	1.64	1.01	0.42
6-(Pent-2'-enyl)-tetrahydropyran-2-one	16.90	-	0.76	-	-
E,E-α-Farnesene	17.09	-	-	-	0.60
2,4-di-*t*-Butylphenol	17.22	-	0.52	-	-
Z-3-Hexenyl benzoate	18.25	-	-	-	0.22
Guaiol	18.78	-	1.73	0.83	-
Ethyl linoleate	23.19	-	-	1.05	-
Bicyclo[4.3.1]dec-1(9)-ene	23.29	-	-	2.78	-
Palmitic acid	24.17	1.18	-	-	-
E-9-Octadecene	25.11	-	-	-	0.12
Linoleic acid	46.44	-	0.78	-	-
E-5,10-Secocholest-1(10)-en-3,5-dione	47.52	1.75	-	-	-
Stigmasta-5,22-dien-3-ol	48.35	1.75	-	-	-
γ-Sitosterol	49.97	2.45	-	-	-

Table III
Sensory Evaluation of Each Gardenia Extracts in 43 Volunteers

Sample		Likert score	
		Satisfaction	Intensity
Concrete	Absolute ethanol	2.53 ± 0.15	2.79 ± 0.20
	Petroleum ether	2.33 ± 0.16	4.28 ± 0.13
	n-Hexane	2.23 ± 0.18	4.12 ± 0.13
Absolute de enfleurage		3.72 ± 0.15	2.74 ± 0.13

n-hexane concrete. In the absolute de enfleurage, farnesene was the most prominent, followed by *Z*-3-hexenyl tiglate, *E*-3,7-dimethyl-1,3,6-octatriene, *Z*-3-hexenyl benzoate, indole, and *E*-9-octadecene. These aroma extracts were thereafter submitted for preliminary evaluation for sensory response by a small group of volunteers.

The preliminary preference tests of the gardenia flower oil extracts were conducted with 43 Thai volunteers using the Likert scale (Table III). All of the concretes received lesser approval from the volunteers because of their high intensities. Although the absolute ethanol concrete was best among the solvent-extracted fragrant oils with the intensity close to the absolute de enfleurage, the preference was significantly ($p < 0.05$) lower. In contrast, the approval for the absolute de enfleurage was obvious. The nuance of the aromatic floral odor of the enfleurage may come from other aroma compounds that were not detected in the concretes. Therefore, the odor similarity of the absolute de enfleurage was compared with fresh gardenia in a larger group of 152 volunteers that included a variety of age groups (Table IV).

Absolute de enfleurage using spermaceti wax mixed with palm oil was evaluated for odor quality in terms of preference and difference compared with the fresh gardenia flower. Thai volunteers (male and female, 76 each) with the different age ranges (Table IV) were included in the study. Fragrant sample 4 (absolute de enfleurage from palm oil) gained high preference in male and female volunteers, and this preference was consistent across all ages. While female volunteers liked sample 4 more ($p = 0.01$) than the male volunteers, no significant difference in preference was observed for different age ranges. The aroma extract was also compared with fresh gardenia odor, but volunteers insignificantly

Table IV
Odor Quality of Fardenia Aroma Extracts

Demography		Preference		Difference	
Gender	*n*	Means	*p*	Means	*p*
Male	76	3.74 ± 0.07	0.01	3.12 ± 0.09	0.23
Female	76	4.01 ± 0.06		3.26 ± 0.08	
Age (years)					
16–25	38	3.87 ± 0.13	0.61	3.55 ± 0.09	0.00
26–35	37	4.00 ± 0.13		3.46 ± 0.11	
36–45	39	3.79 ± 0.10		3.00 ± 0.12	
>45	38	3.84 ± 0.10		2.76 ± 0.11	

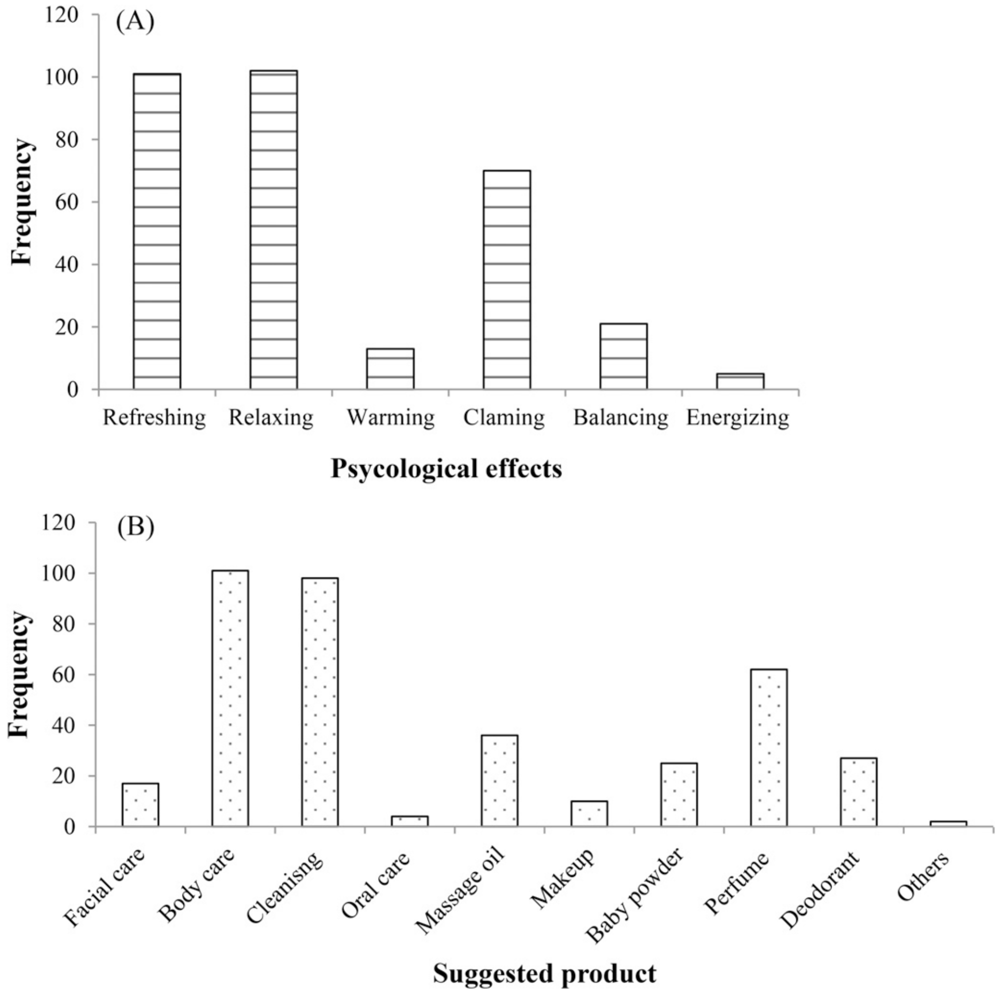

Figure 1. Psychological characters of (A) the gardenia absolute de enfleurage and (B) optimized products incorporated with the aroma extract.

($p > 0.05$) noticed the differences between the extract and the fresh flower. However, volunteer age clearly influenced the odor differences, with the younger volunteers rating higher scores than older volunteers.

Volunteers were also asked about the psychological effects of the aroma extract preparation. Relaxing and refreshing sensations were characteristic traits of the gardenia odor, followed by calming, as shown in Figure 1. The most notable personal care products that incorporate the gardenia aroma extract are body care and cleansing products, which is in accordance with the relaxing and refreshing characters of the extract. Surprisingly, only 62 volunteers recommended using this fragrant extract for perfume creation. Although the extract provides a relaxing sensation, massage oil was not strongly suggested. It is possible that the mild scents with floral note are less preferred than the top note citrus scents in massage oil (12).

CONCLUSIONS

Preparation of gardenia aroma by enfleurage gave the best-quality gardenia scent, which was contributed by farnesene, *Z*-3-hexenyl tiglate, *Z*-3-hexenyl benzoate, and indole. The contributions of these aromas directly governed the sensorial response to the absolute de enfleurage, which was similar to the sensory evaluation of fresh gardenia. This study addresses the increased interest in floral fragrances, particularly toward those used in herbal medicine (6). Application of this medicinal herb is expected to widen into the perfumery business based on the aroma compounds identified in this study.

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REFERENCES

(1) S.-C. Wang, T.-Y. Tseng, C.-M. Huang, and T.-H. Tsai, Gardenia herbal active Constituents: applicable separation procedures. *J. Chromatogr. B,* **812**, 193–202 (2004).
(2) M. C. Bergonzi, C. Righeschi, B. Isacchi, and A. R. Bilia, Identification and quantification of constituents of *Gardenia jasminoides* Ellis (Zhizi) by HPLC-DAD- ESI-MS. *Food Chem.,* **134**, 1199–1204 (2012).
(3) J.-H. Lee, D.-U. Lee, and C.-S. Jeong, *Gardenia jasminoids* Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. *Food Chem. Toxicol.,* **47**, 1127–1131 (2008).
(4) N. Yagi, H. Nakahashi, T. Kobayashi, and M. Miyazawa, Characteristic chemical components of the essential oil from white kwao krua (*Pueraria mirifica*). *J. Oleo Sci.,* **62**, 175–179 (2013).
(5) A. Usami, T. Ono, S. Marumoto, and M. Miyazawa, Comparison of volatile compounds with characteristic odor in flowers and leaves of Nojigiku (*Crysanthemum japonense*). *J. Oleo Sci.,* **62**, 631–636 (2013).
(6) Y. Kashima, S. Nakaya, and M. Miyazawa, Volatile composition and sensory properties of Indian herbal medicine—*Pavonia odirata*—used in Ayurveda. *J. Oleo Sci.,* **63**, 149–158 (2014).
(7) A. K. Singh, "Extraction Technologies for Medicinal and Aromatic Plants," in *Extraction Technologies for Medicinal and Aromatic Plants*, S. S. Handa, S. P. S. Khanuja, G. Longo, and D. D. Rakesh. Eds. (International centre for science and high technology, Trieste, 2008), pp. 88–91.
(8) M. Lis-Balchin, "Aromatherapy with Essential Oil," in *Handbook of Essential oils: Science, Technology, and Applications*, K. H. C. Başer and G. Buchbauer. Eds. (CRC Press, New York, 2010), pp. 555.
(9) D. Pybus and C. Sell, The Chemistry of Fragrances (Royal society of chemistry, Wiltshire, 1999), pp. 36.
(10) M. Kanlayavattanakul, S. Kitsiripaisarn, and N. Lourith, Aroma profiles and preferences of *Jasminum sambac* L. flowers grown in Thailand. *J. Cosmet. Sci.,* **64**, 483–493 (2013).
(11) A. M. R. Alvarez and M. L. G. Rodríquez, Lipids in pharmaceutical and cosmetic preparations. *Grasas y Aceites.,* **51**, 74–96 (2000).
(12) R. Patin, M. Kanlayavattanakul, and N. Lourith, Aromatherapy and essential oils in Thai spa business. *IJPS.,* **5**, 161–166 (2009).