Determination of Volatile Constituents of Thai Fragrant Orchids by Gas Chromatography-Mass Spectrometry with Solid-Phase Microextraction

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ABSTRACT

Volatile constituents of four Thai fragrant orchid species, Rhynchostylis gigantea Ridl., Rhynchostylis gigantea var. harrisonianum Holtt., Vanda coerulea and Dendrobium parishii Rchb. f., were examined by Gas Chromatography-Mass Spectrometry (GC-MS). Three parts of each plant sample (the flowers, leaves and roots) were analyzed for volatile compounds using the Headspace – Solid-Phase Microextraction method (HS-SPME). Alcohols, aldehydes, alkanes, esters, ethers, ketones, monoterpenes and sesquiterpenes were identified quantitatively from the volatile compounds isolated from the flower parts. The aromatic compounds isolated differed among the orchid species. The major aromatic compounds of the flowers of R. gigantea, R. gigantea var. harrisonianum, V. coerulea and D. parishii. were nerol (25.42%), 2,3-dihydrofarnesol (34.30%), nonanal (34.69%) and 2-pentadecanone (43.47%), respectively.

Keywords: *Rhynchostylis gigantea, Rhynchostylis gigantea var. harrisonianum Holtt., Vanda coerulea, Dendrobium parishii,* Volatile compounds, HS-SPME, GC-MS

INTRODUCTION

Many volatile compounds from plant flowers are pleasant to humans and have potential applications as components of perfumes. Thus, there is demand to characterize and synthesize new aromatic compounds to fulfill these purposes. Toward this end, many studies have identified the substances responsible for the characteristic aromas and flavors of many flowers.

Thailand is located in the tropical zone of Southeast Asia, with many endemic orchid species distributed across the country. Some important groups of orchids include: Dendrobium, Bulbophyllum, Eraia and Vanda. Because of their beautiful flowers and attractive scent, orchids are used predominantly as ornamental plants. Orchid plants are the largest and most evolved family of flowering plants. Numerous orchid species have been used in many parts of the world for their therapeutic properties and medicinal value, including as a bronchodilator, cooling agents and snakebite remedies (Arditti, 1992; Hossain, 2011). Several studies have reported isolating chemical compounds from orchids and shown that some of their constituents are valuable for their medicinal value (immunomodulatory activity, antioxidant activity and antitumor activity) as well as for their aromatic products (Ye et al., 2002; Lo et al., 2004; Cai et al., 2004; Wong et al.; 2006; Luo et al., 2009; Wang et al., 2010).

Four species of orchid plants were selected for study: *Rhynchostylis gigantea, Rhynchostylis gigantea* var. *harrisonianum Holtt., Vanda coerulea* and *Dendrobium parishii* Rchb. f. These species were selected because they are endemic to Thailand, easily grown, widely available and popular. These aspects have also made them high-valued agricultural exports, particularly *Dendrobium*.

Solid-phase microextraction (SPME) was developed by Arthur and Pawliszyn in 1990 (Arthur and Pawliszyn, 1990). The technique is increasingly useful in organic analytical chemistry because it is a fast, simple and solventless extraction procedure with the capacity to produce high extract concentrations. SPME has been used to analyze the volatile compounds in many plants and some products such as lavender flower (An et al., 2001), pesticides in water (Cortada et al., 2009), olive oil (Kandylis et al., 2011), honey (Plutowska et al., 2011), papaya (Fuggate et al., 2010), fermented soy product (Dajanta et al., 2011), red wine (Zhang et al., 2011) and biological and environmental water samples (Aresta et al., 2010). SPME is also advantageous because it diminishes the decomposition of the plant compounds, minimizes enzyme activity and decreases the loss of constituents.

The aim of this study is to characterize the volatile compounds derived from the flowers, leaves and roots of four Thai fragrant orchid species, *Rhynchostylis gigantea* Ridl., *Rhynchostylis gigantea* var. *harrisonianum* Holtt., *Vanda coerulea* and *Dendrobium parishii* Rchb. f. using the HS-SPME technique coupled with GC-MS. This study is the first report on volatile compound identification of four Thai fragrant orchids, including their plant parts. The identified constituents may provide useful ingredients for cosmetics or medicinal applications. Our findings will be applied to other studies of fragrant orchids.

MATERIALS AND METHODS

Plant materials

Four Thai fragrant orchids species (*R. gigantea* Ridl., *R. gigantea* var. *harrisonianum* Holtt., *V. coerulea* and *D. parishii* Rchb. f.) were collected from a cultivated farm in Chiang Mai Province, Thailand during February-March 2010. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Chiang Mai University, Thailand, with code number 02109-02112. Plant materials for analysis were taken from the flowers, leaves and roots of each sample.

HS-SPME extraction

An AOC 5000 Combi PAL SPME holder (CTC Analytics AG., Switzerland) was used with a 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber assembly (Supelco, Bellefonte, PA, USA). Before using, the fiber was conditioned as recommended by the manufacturer. The samples were placed in 20 ml screw-capped glass vials (National Sciencetific, Rockwood, TN, USA). First, the fiber was suspended in the headspace and equilibrated for 30 min. in the agitator (500 rpm, 80°C). Then, the fiber was introduced into the GC injector. The desorption time was 2 min.

Instrumentation

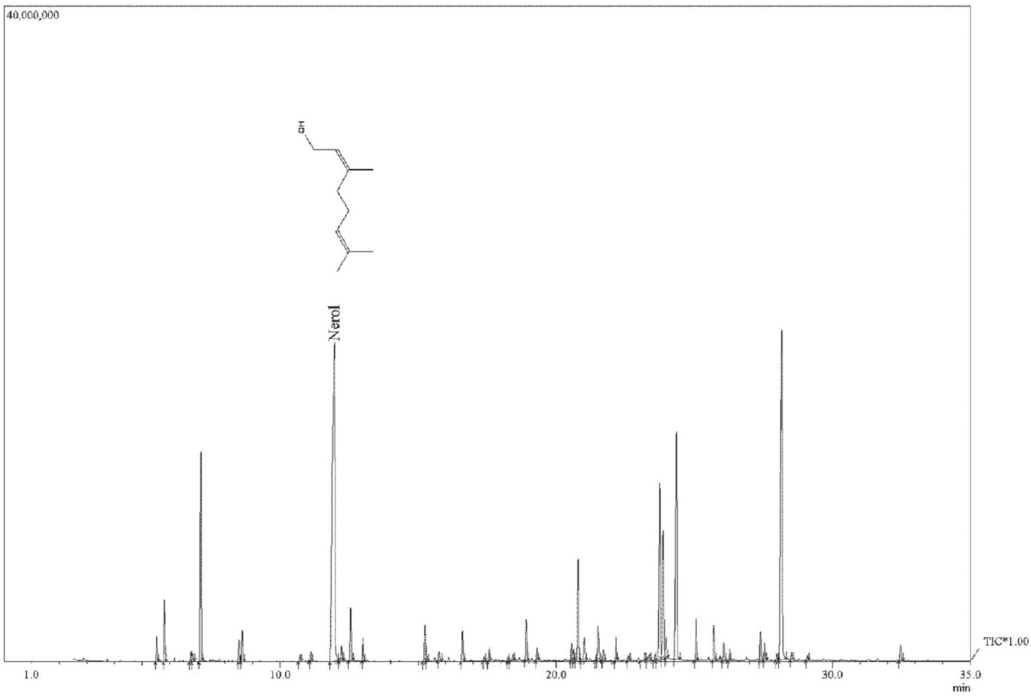
A Shimudzu GCMS-QP 2010 Plus system (Shimudzu, Kyoto, Japan) was used, with a mass-selective detector with electron impact ionization. Analytes were separated by using a DB-5 MS capillary column of 5% phenylmethylpolysiloxane, 30 m × 0.25 mm with a phase thickness of 0.25 µm from J. & W. Scientific, USA. During analysis, the initial temperature was 60°C, then increased to 200°C at the rate of 5°C/min. The final temperature was held for 10 min. The injector temperature was 180°C. Helium (99.999%) was the carrier gas, maintained at a flow rate of 1 ml/min. Split injection with a ratio of 1:5 was used.

The mass spectrometer was fitted with an electron impact ionization source operated at 70 eV with an ion source temperature of 200°C and mass spectra were recorded in the range m/z 40-400 amu in the full-scan acquisition mode. Volatile compounds were identified by comparing the obtained mass spectra of relevant chromatographic peaks with spectra of the WILEY 7 library and Kovats retention indices with other published mass spectra (Adam, 2007).

RESULTS

Figure 1 shows the total ion GC-MS patterns performed by HS-SPME of the volatile compounds of four Thai fragrant orchid species flowers, *R. gigantea, R. gigantea* var. *harrisonianum*, *V. coerulea* and *D. parishii*. Sixty-one volatile compounds were detected from R. gigantea. The main volatile compounds of the flowers, leaves and roots were nerol (25.42%), hexahydrofarnesyl acetone (26.03%) and heptadecane (14.93%), respectively (Table 1). Fifty-eight volatile compounds were obtained from *R. gigantea* var. harrisonianum. The main volatile compounds of the flowers, leaves and roots were 2,3-dihydrofarnesol (34.30%), hexadyhydrofarnesyl acetone (19.53%) and 1-methoxy naphthalene (26.32%), respectively (Table 2). In *V. coerulea*, fifty-four volatile compounds were detected. The main volatile compounds of the flowers, leaves and roots were nonanal (34.69%), dihydroedulan (15.45%) and heptadecane (25.33%), respectively (Table 3). Fifty-five volatile compounds were isolated from *D. parishii*. The main compound obtained from the flowers and roots was 2-pentadecanone 43.47% and 20.51%, respectively. The main compound obtained from the leaves was nonanal (18.02%) (Table 4).

a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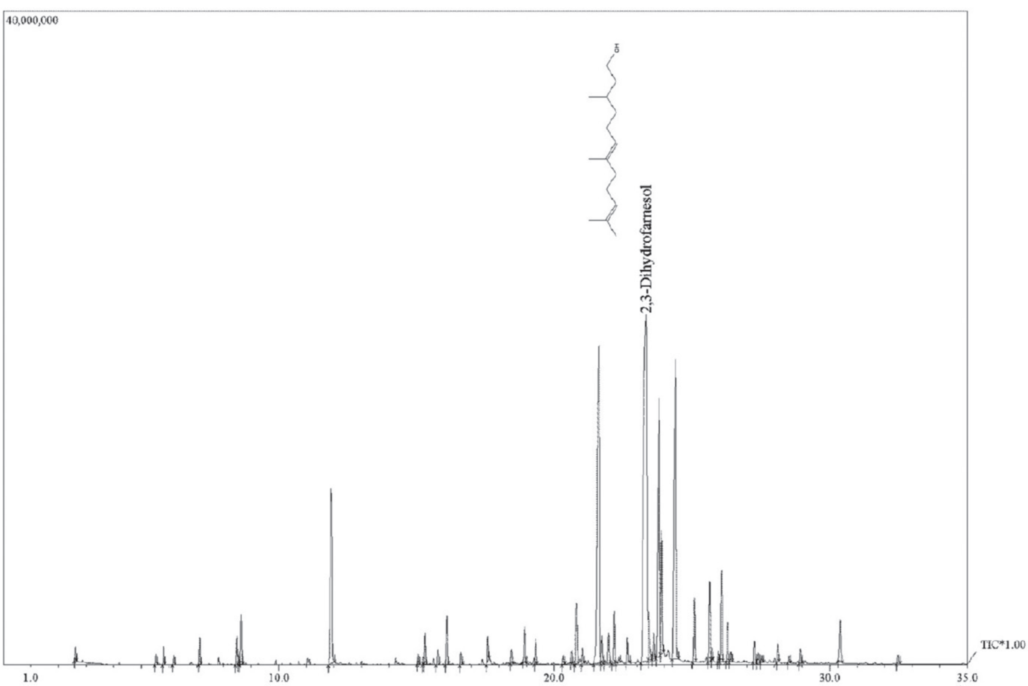
20.0
30.0
35.0
min

b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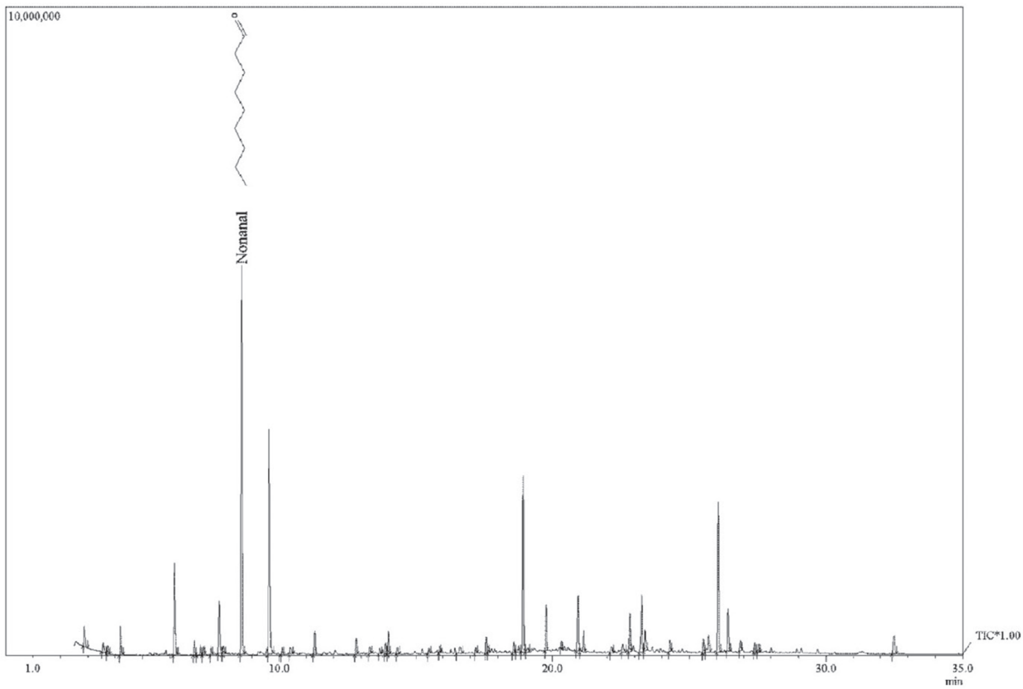
35.0
min

c



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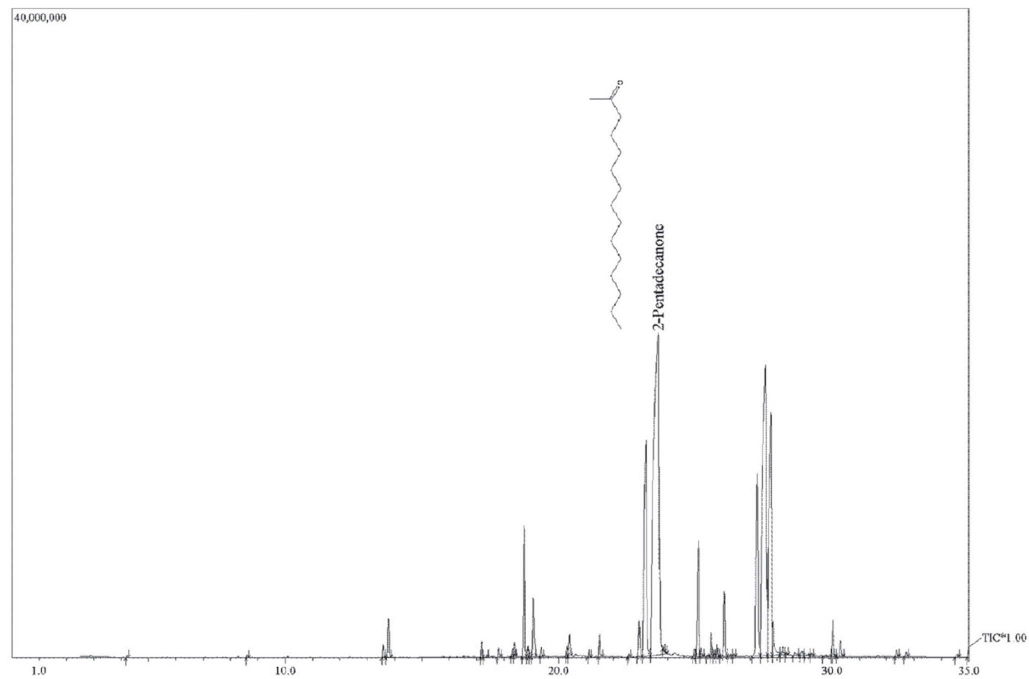

Figure 1. HS-SPME-GC chromatogram of volatile compounds of four Thai fragrant orchid flowers; (a) *R. gigantea*, (b) *R. gigantea* var. *harrisonianum*, (c) *V. coerulea* and (d) *D. parishii.*

Table 1. Volatile composition of *R. gigantea.*

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
1	5.529	978.11	Sabinene	$C_{10}H_{16}$	136	0.79		
2	5.811	991.19	beta-Myrcene	$C_{10}H_{16}$	136	1.83		
3	6.181	1008.44	Octanal	$C_8H_{16}O$	128		2.39	
4	6.768	1035.56	Limonene	$C_{10}H_{16}$	136	0.31		
5	6.778	1036.00	2-Ethylhexanol	$C_8H_{18}O$	130			2.36
6	6.821	1037.89	beta-Phellandrene	$C_{10}H_{16}$	136	0.34		
7	7.129	1051.10	(E)-beta-Ocimene	$C_{10}H_{16}$	136	7.05		
8	8.519	1105.36	Linallol	$C_{10}H_{18}O$	154	0.66		
9	8.618	1109.62	Nonanal	$C_9H_{18}O$	142	0.97	10.16	3.03
10	10.714	1189.83	Terpinen-4-ol	$C_{10}H_{18}O$	154	0.20		
11	11.111	1204.06	alpha-Terpineol	$C_{10}H_{18}O$	154	0.32		
12	11.302	1211.96	Decanal	$C_{10}H_{20}O$	156		6.89	5.17
13	11.963	1238.28	Nerol	$C_{10}H_{18}O$	154	25.42	7.56	13.49
14	12.236	1248.74	Neral	$C_{10}H_{16}O$	152	0.57		1.50
15	12.545	1260.29	Geraniol	$C_{10}H_{18}O$	154	1.73		
16	12.829	1270.66	(2E)-Decenal	$C_{10}H_{18}O$	154		0.94	
17	13.000	1276.79	Geranial	$C_{10}H_{16}O$	152	0.70		2.26
18	13.100	1280.34	Decanol	$C_{10}H_{22}O$	158			1.08
19	13.995	1313.64	Undecanal	$C_{11}H_{22}O$	170		0.73	
20	15.233	1362.54	Eugenol	$C_{10}H_{12}O_2$	164	1.24		
21	15.304	1365.22	Neryl acetate	$C_{12}H_{20}O_2$	196	0.47		
22	15.764	1382.31	alpha-Copaene	$C_{15}H_{24}$	204	0.32	2.60	1.88
23	16.091	1394.15	Tetradecene	$C_{14}H_{28}$	196		0.67	
24	16.290	1401.51	Tetradecane	$C_{14}H_{30}$	198			2.62
25	16.436	1407.80	Hexahydropseudoionone	$C_{13}H_{26}O$	198		1.15	
26	16.607	1415.09	Dodecanal	$C_{12}H_{24}O$	184	0.94		1.28
27	16.918	1428.15	(E)-Caryophyllene	$C_{15}H_{24}$	204		1.24	2.11
28	17.388	1447.45	Isoamyl benzoate	$C_{12}H_{16}O_2$	192	0.15		
29	17.582	1455.27	(E)-Geranylacetone	$C_{13}H_{22}O$	194	0.48	3.17	2.81
30	18.254	1481.68	Dodecanol	$C_{12}H_{26}O$	186	0.14		1.82
31	18.433	1488.56	Germacrene-D	$C_{15}H_{24}$	204	0.28		
32	18.638	1496.34	beta-Selinene	$C_{15}H_{24}$	204		4.72	
33	18.770	1501.57	Pentadecane	$C_{15}H_{32}$	212		2.12	6.22
34	18.918	1508.18	alpha-Farnesene	$C_{15}H_{24}$	204	1.28	3.33	
35	19.309	1525.41	delta-Cadinene	$C_{15}H_{24}$	204	0.42	1.54	
36	20.618	1580.63	(3Z)-Hexenyl benzoate	$C_{13}H_{16}O_2$	204	0.37		
37	20.659	1582.30	1-Hexadecene	$C_{16}H_{32}$	224			1.40
38	20.797	1587.90	Hexyl benzoate	$C_{13}H_{18}O_2$	206	3.94		
39	21.133	1601.64	Hexadecane	$C_{16}H_{32}$	226		2.27	6.70
40	21.515	1619.35	Tetradecanal	$C_{14}H_{28}O$	212	1.71		1.15

Table 1. Continued.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
41	22.613	1668.55	(6Z)-Pentadecen-1-ol	$C_{15}H_{30}O$	226	0.18		
42	22.885	1680.37	Tetradecanol	$C_{14}H_{30}O$	214		1.90	1.88
43	23.382	1701.86	n-Heptadecane	$C_{17}H_{36}$	240		1.39	14.93
44	23.573	1711.14	(2E,6Z)-Farnesol	$C_{15}H_{26}O$	222	0.23		
45	23.751	1719.73	(2Z,6E)-Farnesal	$C_{15}H_{24}O$	220	6.96		
46	23.794	1721.80	Pentadecanal	$C_{15}H_{30}O$	226		1.03	1.66
47	23.874	1725.63	(2E,6E)-Farnesol	$C_{15}H_{26}O$	222	5.10		
48	23.970	1730.21	Hexahydrofarnesol	$C_{15}H_{32}O$	228		5.38	
49	23.976	1730.50	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.85		
50	24.355	1748.40	(2E,6E)-Farnesal	$C_{15}H_{24}O$	220	10.05		
51	25.011	1778.75	Pentadecanol	$C_{15}H_{32}O$	228			2.42
52	25.065	1781.21	Benzyl benzoate	$C_{14}H_{12}O_2$	212	1.44		
53	25.521	1802.04	Octadecane	$C_{18}H_{38}$	254		1.06	5.36
54	25.954	1823.94	Hexadecanal	$C_{16}H_{32}O$	240		1.60	2.17
55	26.283	1840.33	(2Z,6E)-Farnesyl acetate	$C_{17}H_{28}O_2$	264	0.39		
56	26.431	1847.64	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268		26.03	
57	27.270	1888.31	Hexadecanol	$C_{16}H_{34}O$	242		5.71	3.76
58	27.555	1901.90	Nonadacane	$C_{19}H_{40}$	268	0.60	0.88	8.17
59	28.180	1932.07	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	21.11	1.66	2.77
60	28.526	1948.48	linalyl benzoate	$C_{17}H_{22}O_2$	258	0.26		
61	29.103	1975.41	Sandaracopimaradiene	$C_{20}H_{32}$	272	0.17	1.90	

[a]RT retention time (min)
[b]RC relative content (%)
[c]KI Kovats retention indices calculated against n-alkane standards (C_8-C_{20})

Table 2. Volatile composition of *R. gigantea* var. *harrisonianum*.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
1	2.871	802.76	Hexanal	$C_6H_{12}O$	100			1.57
2	5.447	974.18	Benzaldehyde	C_7H_6O	106		0.95	
3	5.536	978.44	Sabinene	$C_{10}H_{16}$	136	0.30		
4	5.818	991.51	beta-Myrcene	$C_{10}H_{16}$	136	0.54		
5	6.175	1008.15	Octanal	$C_8H_{16}O$	128	0.26	1.18	
6	7.128	1051.05	(E)-beta-Ocimene	$C_{10}H_{16}$	136	0.73		
7	8.469	1103.19	Methyl benzoate	$C_8H_8O_2$	136	0.87		
8	8.533	1105.96	Linallol	$C_{10}H_{18}O$	154	0.25		
9	8.630	1110.13	Nonanal	$C_9H_{18}O$	142	1.44	3.75	1.29
10	9.789	1156.56	4-keto-Isophorone	$C_9H_{12}O_2$	152		1.51	
11	9.887	1160.23	Nerol oxide	$C_{10}H_{16}O$	152		2.86	
12	11.045	1201.30	Methyl salicylate	$C_8H_8O_3$	152		6.19	
13	11.302	1211.96	n-Decanal	$C_{10}H_{20}O$	156		1.20	0.78

Table 2. Continued.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
14	11.898	1235.76	Nerol	$C_{10}H_{18}O$	154	8.67	1.89	
15	12.827	1270.58	(2E)-Decenal	$C_{10}H_{18}O$	154		1.49	
16	13.186	1283.37	Methyl norolate	$C_{11}H_{18}O_2$	182			1.29
17	15.063	1356.06	Citronellyl acetate	$C_{12}H_{22}O_2$	198	0.25		
18	15.237	1362.69	Eugenol	$C_{10}H_{12}O_2$	164	0.23		2.72
19	15.312	1365.52	Neryl acetate	$C_{12}H_{20}O_2$	196	0.98		
20	15.774	1382.67	alpha-Copaene	$C_{15}H_{24}$	204	0.49	2.26	
21	16.104	1394.62	(E)-Methyl cinnamate	$C_{10}H_{10}O_2$	162	1.61	2.80	6.82
22	16.287	1401.39	Tetradecane	$C_{14}H_{30}$	198		1.31	
23	16.431	1407.58	Hexahydropseudoionone	$C_{13}H_{26}O$	198		1.41	
24	16.456	1408.66	Vanillin	$C_8H_8O_3$	152			5.09
25	17.589	1455.55	(E)-Geranylacetone	$C_{13}H_{22}O$	194	0.70	3.38	3.52
26	17.686	1459.42	1-methoxy-Naphthalene	$C_{11}H_{10}O$	158		7.58	26.32
27	18.419	1488.02	beta-Ionone	$C_{13}H_{20}O$	192			1.43
28	18.444	1488.98	Germacrene D	$C_{15}H_{24}$	204	0.44		
29	18.637	1496.31	beta-Selinene	$C_{15}H_{24}$	204		4.14	3.52
30	18.768	1501.48	Pentadecane	$C_{15}H_{32}$	212		1.66	3.04
31	18.927	1508.58	alpha-Farnesene	$C_{15}H_{24}$	204	1.22		
32	18.933	1508.85	delta-Guaiene	$C_{15}H_{24}$	204		2.90	1.03
33	19.320	1525.88	delta-Cadinene	$C_{15}H_{24}$	204	0.77	3.11	
34	20.330	1568.78	(E)-Nerolidol	$C_{15}H_{26}O$	222	0.26		
35	20.627	1580.99	(3Z)-Hexenyl benzoate	$C_{13}H_{16}O_2$	204	0.36		
36	20.808	1588.35	Hexyl benzoate	$C_{13}H_{18}O_2$	206	2.87		
37	21.133	1601.64	Hexadecane	$C_{16}H_{34}$	226		1.93	2.09
38	21.517	1619.44	Tetradecanal	$C_{14}H_{28}O$	212			1.31
39	21.767	1630.86	Isopropyl dodecanoate	$C_{15}H_{30}O_2$	242			1.45
40	22.350	1656.99	alpha-Cadinol	$C_{15}H_{26}O$	222	0.21		
41	22.883	1680.28	Tetradecanol	$C_{14}H_{30}O$	214			5.59
42	23.339	1699.79	2,3-Dihydrofarnesol	$C_{15}H_{28}O$	224	34.30	8.31	
43	23.379	1701.71	Heptadecane	$C_{17}H_{36}$	240		2.09	7.79
44	23.432	1704.30	(2Z, 6Z)Farnesol	$C_{15}H_{26}O$	222	1.96		
45	23.617	1713.27	(2E, 6Z)Farnesol	$C_{15}H_{26}O$	222	0.93		
46	23.731	1718.77	(2Z, 6E)Farnesol	$C_{15}H_{26}O$	222		2.28	
47	23.788	1721.51	Pentadecanal	$C_{16}H_{32}O$	240		2.76	1.71
48	23.805	1722.33	(2Z,6E)-Farnesal	$C_{15}H_{24}$	220	12.26		
49	23.897	1726.73	(2E, 6E)-Farnesol	$C_{15}H_{26}O$	222	4.57		
50	24.405	1750.74	(2E,6E)-Farnesal	$C_{15}H_{24}$	220	17.32	5.59	
51	25.091	1782.39	Benzyl benzoate	$C_{14}H_{12}O_2$	212	2.13		
52	25.521	1802.04	Octadecane	$C_{18}H_{38}$	254		1.16	4.95
53	25.957	1824.09	Hexadecanal	$C_{16}H_{32}O$	240	0.32	0.83	1.51
54	26.294	1840.88	(2Z,6E)-Farnesyl acetate	$C_{17}H_{28}O_2$	264	1.19		

Table 2. Continued.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
55	26.428	1847.49	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268		19.53	4.23
56	27.270	1888.31	1-Hexadecanol	$C_{16}H_{34}O$	242	0.78		0.78
57	27.561	1902.20	n-Nonadecane	$C_{19}H_{4}0$	268	0.23		6.56
58	28.119	1929.15	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	0.56	3.93	3.60

[a]RT retention time (min)
[b]RC relative content (%)
[c]KI Kovats retention indices calculated against n-alkane standards (C_8-C_{20})

Table 3. Volatile composition of *V. coerulea.*

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
1	2.867	802.38	Hexanal	$C_6H_{12}O$	100	2.13		
2	3.559	861.06	(3Z)-Hexenol	$C_6H_{12}O$	100	1.06		
3	3.722	873.22	Hexanol	$C_6H_{14}O$	102	0.93		
4	4.195	905.51	Heptenal	$C_7H_{14}O$	114	2.79		
5	6.169	1007.86	Octanal	$C_8H_{16}O$	128	8.61	4.37	
6	6.779	1036.05	2-Ethylhexanol	$C_8H_{18}O$	130		1.71	
7	6.897	1041.20	3-Ethyl-2-methyl-1,3-hexadiene	C_9H_{16}	124	1.40		
8	7.124	1050.89	(E)-beta-Ocimene	$C_{10}H_{16}$	136	0.76		
9	7.233	1055.43	Benzene acetaldehyde	C_8H_8O	120	0.83		
10	7.508	1066.58	(2E)-Octenal	$C_8H_{14}O$	126	0.79		
11	7.801	1078.03	Octanol	$C_8H_{18}O$	130	5.03	3.33	
12	8.532	1105.92	Linalool	$C_{10}H_{18}O$	154			12.18
13	8.627	1110.00	Nonanal	$C_9H_{18}O$	142	34.69	11.29	2.97
14	10.039	1165.85	(3E,6Z)-Nonadienol	$C_9H_{16}O$	140		8.66	
15	10.108	1168.37	(2E)-Nonenal	$C_9H_{16}O$	140	0.64		
16	10.117	1168.70	9-methyl-1-undecene	$C_{12}H_{24}$	168		1.87	
17	10.415	1179.40	Nonanol	$C_9H_{20}O$	144	0.95	1.33	
18	11.119	1204.39	alpha-Terpineol	$C_{10}H_{18}O$	154			1.40
19	11.295	1211.67	Decanal	$C_{10}H_{20}O$	156	2.04	5.58	1.70
20	12.543	1260.21	Geraniol	$C_{10}H_{18}O$	154			0.87
21	12.680	1265.25	EdulanII	$C_{13}H_{20}O$	192		1.17	
22	12.821	1270.37	(2E)-Decenal	$C_{10}H_{18}O$	154	1.45	4.41	1.53
23	13.326	1288.26	Nonanoic acid	$C_9H_{18}O_2$	158	0.81	1.96	
24	13.500	1294.27	alpha-Tridecene	$C_{13}H_{26}$	182		1.42	
25	13.688	1300.84	Dihydroedulan	$C_{13}H_{22}O$	194	0.52	15.45	2.36
26	13.902	1309.79	2-Phenyl nitroethane	$C_8H_9NO_2$	151	1.01		
27	13.990	1313.43	n-Undecanal	$C_{11}H_{22}O$	170	2.09		
28	15.240	1362.80	Eugenol	$C_{10}H_{12}O_2$	164			0.83
29	15.497	1372.45	(2E)-Undecenal	$C_{11}H_{20}O$	168			1.09

Table 3. Continued.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
30	16.099	1394.44	Tetradecene	$C_{14}H_{28}$	196		3.46	
31	16.292	1401.60	Tetradecane	$C_{14}H_{30}$	198		1.75	
32	16.437	1407.84	Hexahydropseudoionone	$C_{13}H_{26}O$	198		1.33	2.01
33	17.197	1439.67	(Z)-alpha-Bergamotene	$C_{15}H_{24}$	204	0.54		1.35
34	17.586	1455.43	(E)-Geranylacetone	$C_{13}H_{22}O$	194	1.55	5.41	0.79
35	17.656	1458.22	(E)-beta-Farnesene	$C_{15}H_{24}$	204	0.59		4.63
36	18.073	1474.66	Acoradiene	$C_{15}H_{24}$	204			1.48
37	18.399	1487.25	alpha-Curcumene	$C_{15}H_{22}$	202			5.76
38	18.587	1494.41	(E)-alpha-Bergamotene	$C_{15}H_{24}$	204	1.01		
39	18.594	1494.68	Pentadecene	$C_{15}H_{30}$	210		2.25	
40	18.771	1501.62	Pentadecane	$C_{15}H_{32}$	212	0.76	3.75	6.99
41	18.929	1508.67	alpha-Farnesene	$C_{15}H_{24}$	204	16.15	1.83	
42	19.043	1513.73	beta-Bisabolene	$C_{15}H_{24}$	204			1.62
43	19.133	1517.70	Tridecanal	$C_{13}H_{26}O$	198	0.62	1.12	
44	19.433	1530.79	beta-Sesquiphellandrene	$C_{15}H_{24}$	204			0.74
45	20.323	1568.49	(E)-Nerolidol	$C_{15}H_{26}O$	222	0.59		
46	21.134	1601.69	Hexadecane	$C_{16}H_{34}$	226	2.01	3.87	4.02
47	21.520	1619.58	Tetradecanal	$C_{14}H_{28}O$	212			0.83
48	22.565	1666.45	alpha-Bisabolol oxide B	$C_{15}H_{26}O_2$	238			2.18
49	22.895	1680.80	Tetradecanol	$C_{14}H_{30}O$	214			8.04
50	23.384	1701.95	Heptadecane	$C_{17}H_{36}$	240	1.96	3.96	25.33
51	25.525	1802.25	Octadecane	$C_{18}H_{38}$	254	1.16	1.62	1.75
52	25.955	1823.99	Hexadecanal	$C_{16}H_{32}O$	240		1.58	
53	26.428	1847.49	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268	3.85	3.87	4.28
54	27.561	1902.20	Nonadecane	$C_{19}H_{40}$	268	0.66	1.62	3.28

[a]RT retention time (min)
[b]RC relative content (%)
[c]KI Kovats retention indices calculated against n-alkane standards (C_8-C_{20})

Table 4. Volatile composition of *D. parishii.*

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
1	2.881	803.70	Hexanal	$C_6H_{12}O$	100		1.55	
2	4.203	906.01	Heptanal	$C_7H_{14}O$	114	0.07	1.17	
3	6.179	1008.34	Octanal	$C_8H_{16}O$	128		3.26	1.96
4	6.898	1041.25	3-Ethyl-2-methyl-1,3-Hexadiene	C_9H_{16}	124		7.22	
5	7.510	1066.66	(2E)-Octenal	$C_8H_{14}O$	126		3.99	1.85
6	7.734	1075.45	Cyclooctanol	$C_8H_{16}O$	128		1.61	
7	7.812	1078.45	Octanol	$C_8H_{18}O$	130		3.07	1.89
8	8.403	1100.31	2,5-Dimethylcyclohexanol	$C_8H_{16}O$	128		1.42	
9	8.617	1109.57	Nonanal	$C_9H_{18}O$	142	0.07	18.02	9.42

Table 4. Continued.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
10	10.112	1168.52	(2E)-Nonenal	$C_9H_{16}O$	140		0.65	
11	10.417	1179.47	Nonanol	$C_9H_{20}O$	144		0.82	
12	11.046	1201.34	Methyl salicylate	$C_8H_8O_3$	152		1.17	
13	11.298	1211.79	Decanal	$C_{10}H_{20}O$	156		5.11	3.81
14	11.752	1230.04	beta-Cyclocitral	$C_{10}H_{16}O$	152		1.46	
15	12.000	1239.71	2-Bornene	$C_{10}H_{16}$	136		1.44	
16	12.826	1270.55	(2E)-Decenal	$C_{10}H_{18}O$	154			2.80
17	13.539	1295.61	Dihydroedulan	$C_{13}H_{22}O$	194		4.16	
18	13.586	1297.21	2-Undecanone	$C_{11}H_{22}O$	170	0.31		
19	13.778	1304.62	Indole	C8H7N	117	1.18		
20	13.991	1313.47	Undecanal	C11H22O	170		2.42	
21	15.008	1353.95	alpha-Cubebene	$C_{15}H_{24}$	204		1.20	
22	15.772	1382.60	alpha-Copaene	$C_{15}H_{24}$	204		3.97	
23	16.291	1401.56	Tetradecane	$C_{14}H_{30}$	198			1.52
24	16.435	1407.76	Hexahydropseudoionone	$C_{13}H_{26}O$	198			4.35
25	16.616	1415.47	Dodecanal	$C_{12}H_{24}O$	184			1.10
26	17.186	1439.22	(E)-alpha-Bergamotene	$C_{15}H_{24}$	204	0.40		
27	17.374	1446.89	beta-Funebrene	$C_{15}H_{24}$	204	0.05		
28	17.587	1455.47	(E)-Geranylacetone	$C_{13}H_{22}O$	194		1.76	
29	17.806	1464.18	alpha-Humulene	$C_{15}H_{24}$	204	0.25		
30	17.927	1468.95	Alloaromadendrene	$C_{15}H_{24}$	204		1.52	
31	18.323	1484.34	Amyl benzoate	$C_{12}H_{16}O_2$	192	0.25		
32	18.384	1486.68	alpha-Curcumene	$C_{15}H_{22}$	202	0.33		
33	18.418	1487.98	beta-Ionone	$C_{13}H_{22}O$	192		2.79	1.66
34	18.652	1496.87	beta-Selinene	$C_{15}H_{24}$	204			1.54
35	18.743	1500.36	2-Tridecanone	$C_{13}H_{26}O$	198	4.16		
36	18.766	1501.39	Pentadecane	$C_{15}H_{32}$	212		0.57	8.71
37	18.883	1506.62	Benzyl tiglate	$C_{12}H_{22}O_2$	198	0.28		
38	19.079	1515.32	beta-Bisabolene	$C_{15}H_{24}$	204	1.89		
39	19.318	1525.80	delta-Cadinene	$C_{15}H_{24}$	204		12.21	
40	19.395	1529.15	alpha-Panasinsen	$C_{15}H_{24}$	204			4.91
41	19.421	1530.27	Calamenene	$C_{15}H_{22}$	202		2.83	
42	19.671	1541.04	Cadine-1,4-diene	$C_{15}H_{24}$	204		4.53	
43	20.315	1568.16	(E)-Nerolidol	$C_{15}H_{26}O$	222	0.23		
44	20.392	1571.35	4-(4-hydroxyphenyl)- 2-Butanone	$C_{10}H_{12}O_2$	164	0.64		
45	21.133	1601.64	Hexadecane	$C_{16}H_{34}$	226	0.19	1.71	4.67
46	22.889	1680.54	Tetradecanol	$C_{14}H_{30}O$	214			16.14
47	23.410	1703.22	2-Pentadecanone	$C_{15}H_{30}O$	226	43.47	4.04	20.51
48	23.793	1721.75	Pentadecanal	$C_{15}H_{30}O$	226	0.08		2.13
49	24.982	1777.42	Pentadecanol	$C_{15}H_{32}O$	228	0.19		

Table 4. Continued.

	RT	KI	Name	Formular	MW	RC		
						flowers	leaves	roots
50	25.114	1783.44	Benzyl benzoate	$C_{14}H_{12}O_2$	212	4.29		
51	25.520	1801.99	Octadecane	$C_{18}H_{38}$	254		1.20	1.96
52	25.581	1805.10	2-Hexadecanone	$C_{16}H_{32}O$	240	0.64		
53	26.433	1847.74	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268	0.08		7.62
54	27.558	1902.05	Nonadecane	$C_{19}H_{40}$	268	26.55	0.95	1.45
55	27.646	1906.34	2-Heptadecanone	$C_{17}H_{34}O$	254	14.40	2.15	

[a]RT retention time (min)
[b]RC relative content (%)
[c]KI Kovats retention indices calculated against n-alkane standards (C_8-C_{20})

DISCUSSION

In the genus *Rhynchostylis,* our study identified nerol and 2,3-dihydrofarnesol as the primary volatile compounds in the flower parts of *R. gigantea* and *R. gigantea* var. *harrisonianum*. This differed from those previously found by Pisutthanan (2008), which identified (Z,Z)-9,12-octadecadienoic acid methyl ester, hexadecanoic acid methyl ester and (Z,Z,Z)-9,12,15-octadecatrienoic acid methyl ester as the primary volatile compounds in the flowers of the same species. This is likely due to the accuracy of our HS-SPME methodology (see discussion below). The primary volatile compounds we found in the flowers of *R. gigantea* and *R. gigantea* var. *harrisonianum* also vary from those found in different species of the same genus. Kaiser (1993) identified (E)-ocimene (47.0%) as the primary volatile compound in flowers of *R. coelestis*. In contrast, the nerol we identified here compares to what others found in some flowers from other genus, including *Michelia alba* (Shang et al., 2002), *Vallaris glabra* (Wongpornchai et al., 2003) and *Rosa damascene* (Ozel et al., 2006).

In the genus *Vanda,* we identified three primary volatile compounds in *V. coerulea*: nonanal, dihydroedulan and heptadecane. The few prior studies of *V. coerulea* only identified some phenolic compounds, such as anthocyanins from the flowers (Tatsuzawa et al., 2004) and stilbenoids from the stems (Simmler et al., 2010), rather than the volatile compounds we found. Kaiser (1993) found different primary volatile constituents in other orchid species of the same genus *Vanda*: *V. coerulescens* (methyl decatrienoate - 27.0%); *V. denisoniana* (linalool - 66.0%) and *V. tessellate* (methyl benzoate - 61.5%). In contrast, the alkyl aldehyde (nonanal) we identified in *V. coerulea* has been found in some flowers from other genus, including *Vallaris glabra* (Wongpornchai et al., 2003), *Anacardium occidentale* (Maia et al., 2000) and *Azadirachta indica* (Aromdee and Sriubolmas, 2006).

In the genus *Dendrobium*, we identified the compound 2-pentadecanone as the primary volatile compound in *D. parishii*. This has also been reported in the plant parts of other flowers in the same genus, such as *D. anosmum* (Kaiser, 1993) and in other flowers of a different genus, the rose hybrid plant (Kim et al., 2000).

The main volatile compounds obtained from the plants studied here likely differed from previous findings due to the choice of methodology. The HS-SPME method used here is able to extract a plant's volatile compounds with high accuracy, without destroying or losing some compounds in the process, providing a close match to what exists in the natural plant. Other conventional methods may lose or destroy compounds during extraction and analysis. None of the research cited above used the HS-SPME method.

To our knowledge, this is the first report on volatile compound identification of the four Thai fragrant orchid species *Rhynchostylis gigantea* Ridl., *Rhynchostylis gigantea* var. *harrisonianum* Holtt., *Vanda coerulea* and *Dendrobium parishii* Rchb. f. Among the isolated volatile compounds, alcohols, aldehydes, alkanes, esters, ethers, ketones, monoterpenes and sesquiterpenes were identified not only from the orchid flowers but also from the leaves and roots as well. These isolates might prove useful for cosmetics because of their aromatic properties. Suggested further research includes identifying the actual quantities of each isolated compound as well as their specific biological activities in order to identify best potential uses medicinally and/or cosmetically.

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