Plant Omics Journal POJ

POJ 13(02):78-85 (2020) ISSN:1836-3644
doi: 10.21475/POJ.13.02.20.2271

The variation of volatile compounds emitted from aromatic orchid (*Phalaenopsis bellina*) at different timing and flowering stages

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Abstract

Phalaenopsis bellina, is an orchid widely known for their distinctive fragrance. Of late, fragrant orchids are getting more attention from Orchid breeders for their horticultural market value. Although volatile compounds have been identified in several orchid species, the floral scent emission of *P. bellina* is far from understood. Therefore, this study was carried out to investigate the influence of different timing and floral development stages on the volatile emissions from *P. bellina* flowers using Solid Phase Micro Extraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). The volatile compound was extracted during morning and afternoon sessions from three different floral developmental stages. The volatile compounds emitted from flowers from two different sessions were trapped with SPME fiber for 30 minutes before directly injected into GC-MS for identification. Results showed that approximately 79 volatile compounds were identified, with the terpenoid presented as the major compound class. *P. bellina* had the highest number of volatiles during the morning and full bloom (41), with 29.82% monoterpene and 23.33% sesquiterpenes accounted in total. α-farnesene (19.56%) was abundance during morning emission and remains as the highest volatile in afternoon emission (44.08%), even higher when compared to morning emission. However, a decrease in the volatile compound was observed in afternoon emission, in which only 34 volatile compounds detected. Meanwhile, partial bloom developmental stage revealed linalool as the major terpenoid volatile compounds (25.89%), with only 20 volatile compounds recorded. On the other hand, no volatile compounds were recorded and profiled from flower bud stage. The establishment of a floral scent study provides a brief overview of the regulation of fragrance in *P. bellina*, which can be continued through gene isolation or fragrance-related enzymes study. This information will provide necessary information on orchid floral scent research that useful in boosting horticultural trade of the scented orchids and their function in pollination ecology study.

Keywords: Solid-phase microextraction (SPME); emission; fragrance; *Phalaenopsis bellina*; Orchids.

Introduction

In orchids family, also known as Orchidaceae, several families were known to emit their own signature fragrances such as Cypripedioideae, Orchidoideaea, and Epidendroidea (Phillips et al., 2012). Under Epidendroideaea, the tribe Vandeae were composed of many fragrant such as the *Phalaenopsis* in nature, particularly for *Phalaenopsis bellina. P. bellina*, or also known as "Lundu orchid", or "Norma orchid" is an orchid endemic to the Bornean region (Beaman et al., 2001). The word *Bellina* is a derivative of Latin etymology, which means beauty/lovely (Martin, 2005). Befitting to its name, it is attractive, with brightly coloured flowers and emits a rather fragrant scent (Christenson and Whitten 1995; Mahmood and Chew 2008). Banks (2003) has described its smell as strong, sweet fragrance with a hint of lemony and citrusy note, and appears most prominent during the morning. Due to its active fragrant feature, *P. bellina* is often used as a donor plant to produce novel *Phalaenopsis* varieties with new fragrance (Chuang et al., 2017).

The floral fragrance is one of the essential features of the ornamental orchid and can improve the aesthetic value, quality of flower products and their economic merit. Other than that, floral scents are a volatile chemical that plays a role in pollination to boost reproductive success. However in some cases, the floral fragrance itself is the reward to the pollinator (Bera et al*.,* 2017). The role of floral scent is not only limited to attract pollinators but also acts as a repellant to defend themselves against pathogenic microorganisms, insect attacks and herbivory by other organisms (Cardoso-Gustavson et al*.,* 2017).

Cultivating fragrant orchids is a current trend in orchid breeding, with several studies involving orchid volatile compound analysis and synthesis. *Phalaenopsis, Oncidium, Ophrys* and *Neotinea* were some of the orchids that were studied, with the minimal research into their volatile aromatic compounds. Then, it is challenging to investigate scent production because floral scents are invisible and variable. To our knowledge, Hsiao et al. (2006) were among the first to explore the volatile composition of *P. bellina*

through a dynamic headspace sampling system from days five to days ten post-anthesis flower stage. Monoterpenoids, phenylpropanoids, benzenoids and fatty acids derivatives were detected, in which monoterpenoids were accounted as major compound classes in the emission. After 11 years, Chuang et al. (2017) studied the effect of light types and circadian cycle on volatile emission from *P. bellina*. In this study, white light was reported to increase monoterpene linalool, geraniol and eucalyptol emission. In terms of the circadian cycle, emissions of monoterpene were consistent from 10 am to 4 pm, and decreases afterward for eight days. Similar patterns were observed when the flower was placed under total darkness, albeit with lower emission intensity when compared to flower placed under white light. Further study of volatile composition in *P. bellina* by Chuang et al. (2018) revealed the presence of additional terpenoids, which are limonene, neral and ocimene.

Although the study of *P. bellina* volatiles has been conducted, there are no reports on the timing influence and flowering stages in floral scent emission from *P. bellina* flowers. Therefore, in this study, the flowers of *P. bellina* were trapped using Solid Phase Micro Extraction (SPME) during the morning (9.00 am – 9.30 pm) and afternoon (3.00 pm – 3.30 pm) and in three flowering stages (flower bud, partial bloom and full bloom). The trapped volatiles were then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to profile the volatile diversity between the two emission periods.

Result and discussion

Effect of different observation period on the Phalaenopsis bellina floral scent emission

Volatile emission in *Phalaenopsis bellina* was highly variable during morning and afternoon emission, as profiled in the chromatogram (Figure 1). Approximately, 64 volatile compounds were detected, in which terpenoids contribute significantly towards the aroma of this plant (Table 1). Overall floral scent emission had the largest volatile compounds in the morning (41) and decreased to 34 compounds in the afternoon. α-farnesene (19.56%) were accounted as the highest, followed by 2,6-bis (1,1 dimethylethyl)-4-(1-0)oxopropyl)phenol (15.47%) and 5-isopropyl-2,4-imidazolidinedione (9.89%) that contributed proportion to the scent in the morning. Meanwhile, α-farnesene (44.08%), linalyl anthranilate (9.20%) and linalyl formate (5.60%) accounted for the highest compounds in the afternoon. The emission of α-farnesene compound was high during morning, and increased in relative abundance during afternoon emission. In terms of compound classes, morning emission shows a total terpenoid abundance of 58.9%, which comprises of monoterpene (24.2%), sesquiterpene (29.2%) and triterpene (5.5%) (Figure 2). Other compound groups were detected as well such as phenols, ketones, fatty acid derivatives, esters, ether, halogen-containing groups, and hydrocarbons, which made up the volatile components. On the other hand, afternoon emission showed the highest abundance of terpenoid (88.5%) that encompasses of 82.4% monoterpene and 6.1% sesquiterpene. However, the number of volatile fractions detected was reduced, in which only esters, halogen-containing groups, hydrocarbons, phenols, alcohols and different compounds were detected.

Several orchid species also exhibited terpenoid as their main constituents in their volatile compositions. Manzo *et al*. (2014) reported the presence of terpenoids as the major constituents of *Ophrys sphegodes* subsp. *Sphegodes* and *Orphys bertolonii* subsp. *Benacensis.* Similar research was also reported by Yeh *et al*., (2014) in *Phalaenopsis* Nobby's Pacific Sunset, in which terpenoids also exist as significant components in its volatiles.

On the contrary, *Neotinea tridentate* revealed the presence of aldehydes, hydrocarbons and esters as its major volatiles (Manzo et al. 2014). Variation in volatile mixture across orchid species allows for reduced attractiveness to individuals and species, thereby increasing specificity to only selected few pollinator species, such as in bees for pollination of *Catasetum* species (Milet-Pinheiro and Gerlach 2017). Diurnal bees were known as diurnal pollinators for *Phalaenopsis* species (Chiu *et al*., 2017).

A similar trend was also observed in the volatile emission of *Phalaenopsis* Nobby's Pacific Sunset, in which the volatile emission was most active during the morning (Yeh et al. 2014) for emission of linalool, geraniol, and α-farnesene. Chiu *et al*. (2017) also reported the highest odor emission in the morning from 10 am to 12 pm for *Oncidium* Rosy Sunset orchid. Interestingly, a similar study conducted by Chuang *et al*. (2017) revealed otherwise, where emissions of volatiles monoterpene linalool and eucalyptol from *P. bellina* was detected in the afternoon. Yeh *et al*. (2014) proposed that flower odour was changed at different times to attract different pollinators.

Interestingly, the emission of α-farnesene was detected consistently high in both morning and afternoon sessions of full bloom of *P. belina* flower. Several studies have been recorded on the critical functions of α-farnesene, especially as a pollinator attraction. Contrary finding, however, α-farnesene was reported as a poor pollinator attractant (Hetherington-Rauth and Ramírez 2016). However, the emission of a-farnesene together with other potent scents compounds (linalool and phenylethyl salicylate) increased the numbers of pollinators such as bees and flies which acting like a behavioral modifier to filter out certain species of pollinators.

Solid Phase Micro Extraction (SPME) was a method that was widely used to trap and capture emitted volatiles for analysis (Shi et al. 2019). Compared to standard headspace extraction that was conducted previously by Hsiao *et al*. (2006) and Chuang *et al*., (2017), this method managed to profile a broader range of volatiles, in which several new compounds were managed to identify such as copaene, bergamiol, and linalyl anthranilate. Fan *et al*. (2019) have indeed agreed to this method since this approach is indeed sensitive and effective in finding scent-related volatiles of *Camellia* species. On the contrary, (Reis et al. 2004) reported SPME is not effective method to trap volatiles emitted from *Stanhopea, Polystachya* and *Epidendrum* orchids, which may suggest SPME performance was varied across species.

Effect of flowering development stages on the Phalaenopsis bellina floral scent emission

It is difficult to determine which flowering stages produced the most potent scent and to prove the specific volatile composition of the scents (Dudareva and Pichersky, 2000). The chromatogram profile of volatile emission in different flowering development was shown in Figure 3. Emission of volatile compounds from *Phalaenopsis bellina* has yielded 20 volatiles chemical compounds in partial bloom and increased into 41 volatile chemical compounds in full bloom (Table 2). However, no volatile compounds were detected in flower

Table 1. Identification of volatile compounds emitted from *P. bellina* flowers at morning and afternoon.

No.	Compound name[a]	Chemical formula	Chemical class	Morning emission (9.00am – 9.30am) RA(%)[b]	Afternoon emission (3.00pm - 3.30pm)RA(%)[b]
1	.gamma.isogeraniol	$C_{10}H_{18}O$	Monoterpene alcohol	0.24	-
2	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	Phenol	2.33	-
3	17-Acetoxy-20,20-dimethylandrost-4-en-3-one, 19,2-oxymethano-	$C_{24}H_{34}O_4$	Others	-	0.61
4	1H-Indene, 5,5'-(1,10-decanediyl)bis[octahydro-	$C_{28}H_{50}$	Hydrocarbon	0.03	-
5	1S-à-Pinene	$C_{10}H_{16}$	Monoterpene hydrocarbon	4.89	1.16
6	2(5H)-Furanone, 5,5'-(1,4-phenylenedinitrilo)bis-	$C_{14}H_8N_2O_4$	Others	-	0.05
7	2,4-Quinolinedicarboxylic acid	$C_{11}H_7NO_4$	Carboxylic acid	7.47	-
8	2,5-Cyclohexadiene, 1,4-diethyl-1,4-dimethyl-	$C_{12}H_20$	Hydrocarbon	-	0.17
9	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	$C_{17}H_{26}O_2$	Phenol	15.47	0.24
10	2,6-Difluoro-3-methylbenzoic acid, eicosyl ester	$C_{28}H_{46}F_2O_2$	Others	-	4.10
11	2-Amininonadecane	$C_{19}H_{41}N$	Halogen containing group	0.32	-
12	2-Butene-1,4-diol, diacetate	$C_8H_{12}O_4$	Ketone	0.42	-
13	2-menthene	$C_{10}H_{18}$	Monoterpene ketone	-	1.25
14	3-Buten-1-amine, N,N-dimethyl-	$C_6H_{13}N$	Halogen containing group	-	0.10
15	5-Isopropyl-2,4-imidazolidinedione	$C_6H_{10}N_2O_2$	Ketone	9.89	-
16	à-Farnesene	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	19.56	44.08
17	á-Pinene	$C_{10}H_{16}$	Monoterpene hydrocarbon	-	4.08
18	bergamiol	$C_{12}H_{20}O_2$	Monoterpene alcohol	1.22	0.10
19	Borneol	$C_{10}H_{18}O$	Monoterpene alcohol	0.16	0.12
20	Caproic Acid	$C_6H_{12}O_2$	Carboxylic acid	0.21	-
21	Carane	$C_{10}H_{18}$	Monoterpene hydrocarbon	3.44	-
22	Cholest-5-en-3-ol (3á)-, tetradecanoate	$C_{27}H_{46}O$	Ester	0.08	-
23	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)-	$C_{29}H_{50}O_2$	Ether	0.05	-
24	cis-á-Terpineol	$C_{10}H_{18}O$	Monoterpene alcohol	-	0.56
25	cis-Linaloloxide	$C_{10}H_{18}O_2$	Monoterpene alcohol	0.06	-
26	citral	$C_{10}H_{16}O$	Monoterpene aldehyde	1.38	3.02
27	Copaene	$C_{15}H_{24}$	Sesquiterpene cyclic	-	0.38
28	Diethyl Phthalate	$C_{12}H_{14}O_4$	Ester	-	1.41
29	farnesol	$C_{15}H_{26}O$	Sesquiterpene alcohol	0.66	-
30	fenchol	$C_{10}H_{18}O$	Monoterpene alcohol	1.55	1.78
31	Fenchyl acetate	$C_{12}H_{20}O_2$	Monoterpene ketone	1.81	-
32	Geraniol butyrate	$C_{14}H_{24}O_2$	Monoterpene ketone	0.53	-
33	Isoborneol	$C_{10}H_{18}O$	Monoterpene alcohol	-	2.51
34	Isoborneol, pentamethyldisilanyl ether	$C_{15}H_{32}OSi_2$	Sesquiterpene alcohol	0.24	-
35	Limonen-6-ol, pivalate	$C_{15}H_{24}O_2$	Ester	0.25	-
36	Limonene	$C_{10}H_{16}$	Monoterpene hydrocarbon	0.15	-
37	linalool	$C_{10}H_{18}O$	Monoterpene alcohol	0.42	-
38	Linalool formate	$C_{10}H_{18}O_2$	Monoterpene aldehyde	-	5.61
39	Linalool oxide	$C_{10}H_{18}O_2$	Monoterpene alcohol	-	1.18
40	Linalyl anthranilate	$C_{17}H_{23}NO_2$	Sesquiterpene ketone	5.59	9.20
41	Linalyl isobutyrate	$C_{14}H_{24}O_2$	Monoterpene ketone	-	0.11
42	meso-5,6-Decanediol	$C_{10}H_{22}O_2$	alcohol	-	0.23
43	Methanone,(3-amino-2-benzofuryl)(4-fluorophenyl)-	$C_{15}H_{10}FNO_2$	alcohol	-	0.24
44	N-Bromo-2,2-dimethylaziridine	C_4H_8BrN	Halogen containing group	0.40	-
45	Nerol	$C_{10}H_{18}O$	Monoterpene alcohol	4.87	3.69
46	nerolidol	$C_{15}H_{26}O$	Sesquiterpene alcohol	2.61	-
47	nerolidol isobutyrate	$C_{19}H_{32}O_2$	Sesquiterpene ketone	0.23	-
48	Ocimene	$C_{10}H_{16}$	Monoterpene hydrocarbon	1.40	0.49
49	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	Ketone	0.15	-
50	Phenylethyl salicylate	$C_{15}H_{14}O_3$	Ester	0.08	-
51	p-Isopropenylphenol	$C_9H_{10}O$	Phenol	2.87	4.09
52	R-(+)-β-Citronellol	$C_{10}H_{20}O$	Monoterpene alcohol	-	5.70
53	RS-2,3-hexanediol	$C_6H_{14}O_2$	Alcohol	-	0.11
54	Santalol	$C_{15}H_{24}O$	Sesquiterpene alcohol	0.27	-
55	Squalene	$C_{30}H_{50}$	Triterpene hydrocarbon	5.51	-
56	sulcatone	$C_8H_{14}O$	ketone	1.09	-
57	trans-á-Terpinyl butanoate	$C_{14}H_{24}O_2$	Monoterpene ester	-	0.14
58	Trifluoroacetyl-à-fenchol	$C_{10}H_{18}O$	Monoterpene alcohol	0.16	-
59	α-citral	$C_{10}H_{16}O$	Monoterpene aldehyde	1.55	1.46
60	B-camphor	$C_{10}H_{16}O$	Monoterpene ketone	-	1.75
61	β-Citral	$C_{10}H_{16}O$	Monoterpene aldehyde	0.41	-
62	B-farnesene	$C_{15}H_{24}$	Sesquiterpene hydrocarbon	-	0.08
64	Diethyl malate	$C_8H_{14}O_5$	ester	-	0.20
			Total	100	100

[a]*Volatile compounds were listed in elution time order from capillary VF-WAXms column* [b]*Note*: RA - relative abundance

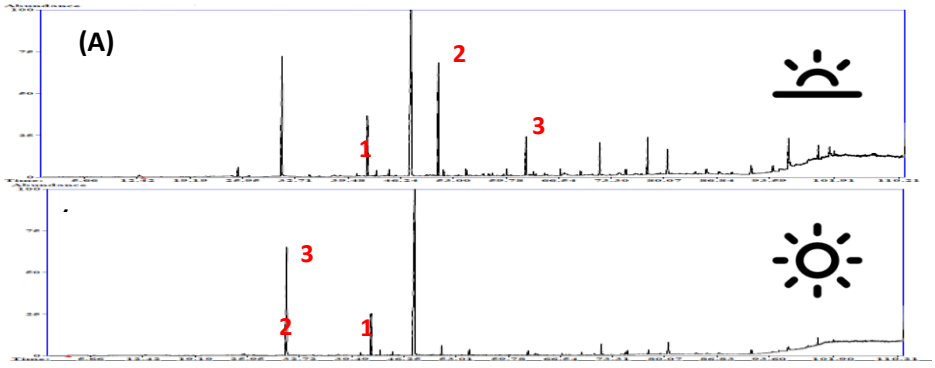

Fig 1. (A) chromatogram of morning emission of *P. bellina*. Top three compounds were labelled in numbers, **(1)** α-farnesene, **(2)** 2,6-bis(1,1-dimethylethyl)-1-(1-oxopropyl)phenol and **(3)** 5-isopropyl-2,4-imidazolidinedione **(B)** Chromatogram of afternoon emission of *P. bellina*. Top three compounds were labelled in numbers, **(1)** α-farnesene, **(2)** linalyl anthranilate, **(3)** linalyl formate.

Table 2. Identification of volatile compounds emitted from *P. bellina* fully open flowers at different developmental stages, which were floral bud, partial bloom and full bloom.

No.	Compound name[a]	Chemical formula	Chemical class	Floral bud RA(%)[b]	Partial bloom RA(%)[b]	Full Bloom RA(%)[b]
1	Ocimene	$C_{10}H_{16}$	Monoterpene hydrocarbon	-	-	1.40
2	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	$C_{10}H_{18}O_2$	Furan	-	0.26	-
3	β-ocimene	$C_{10}H_{16}$	Monoterpene hydrocarbon	-	0.48	-
4	17-Octadecen-14-yn-1-ol	$C_{18}H_{32}O$	alcohol	-	12.75	-
5	bergamotene	$C_{15}H_{24}$	sesquiterpene hydrocarbon	-	0.08	-
6	Tetradecanedioic acid, bis(tert-butyldimethylsilyl) ester	$C_{26}H_{54}O_4Si_2$	Hydrocarbon	-	0.41	-
7	Isophthalic acid, 2,3-dichlorophenyl ethyl ester	$C_{19}H_{18}Cl_2O_4$	aromatic hydrocarbon	-	4.72	-
8	N-Morpholinomethyl-isopropyl-sulfide	$C_8H_{17}NOS$	Halogen containing group	-	4.75	-
9	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	Hydrocarbon	-	2.12	-
10	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{17}H_{50}O_7Si_7$	Hydrocarbon	-	1.15	-
11	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-n	$C_{16}H_{48}O_{10}Si_9$	Hydrocarbon	-	3.75	-
12	ethylbutenol	$C_6H_{12}O$	alcohol	-	0.43	-
13	1,3-Dioxane, 4,4-dimethyl-	$C_6H_{12}O_2$	ether	-	2.95	-
14	2,6-Difluoro-3-methylbenzoic acid, nonadecyl ester	$C_{27}H_{44}F_2O_2$	Ester	-	3.19	-
15	S-Benzoyl(thiohydroxylamine)	C_7H_7NOS	Halogen containing group	-	2.42	-
16	Phthalic acid, butyl 2-pentyl ester	$C_{17}H_24O_4$	ester	-	0.74	-
17	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	Hydrocarbon	-	0.58	-
18	cis-Linaloloxide	$C_{10}H_{18}O_2$	Monoterpene alcohol	-	-	0.06
19	Limonene	$C_{10}H_{16}$	Monoterpene hydrocarbon	-	-	0.15
20	1S-à-Pinene	$C_{10}H_{16}$	Monoterpene hydrocarbon	-	-	4.89
21	bergamiol	$C_{12}H_{20}O_2$	Monoterpene alcohol	-	-	1.22
22	borneol	$C_{10}H_{18}O$	Monoterpene alcohol	-	-	0.16
23	Geraniol butyrate	$C_{14}H_{24}O_2$	Monoterpene ketone	-	-	0.53
24	sulcatone	$C_8H_{14}O$	ketone	-	-	1.09
25	linalyl anthranilate	$C_{17}H_{23}NO_2$	Sesquiterpene ketone	-	24.01	5.59
26	linalool	$C_{10}H_{18}O$	Monoterpene alcohol	-	32.09	0.42
27	farnesol	$C_{15}H_{26}O$	Sesquiterpene alcohol	-	-	0.66
28	nerolidol isobutyrate	$C_{19}H_{32}O2$	Sesquiterpene ketone	-	-	0.23
29	nerolidol	$C_{15}H_{26}O$	Sesquiterpene alcohol	-	-	2.61
30	β-Citral	$C_{10}H_{16}O$	Monoterpene aldehyde	-	-	0.41
31	Citral	$C_{10}H_{16}O$	Monoterpene aldehyde	-	-	1.38
32	α-Citral	$C_{10}H_{16}O$	Monoterpene aldehyde	-	-	1.55
33	à-Farnesene	$C_{15}H_{24}$	Sesquiterpene	-	-	19.56
34	carane	$C_{10}H_{18}$	Monoterpene hydrocarbon	-	-	3.44
35	.gamma.isogeraniol	$C_{10}H_{18}O$	Monoterpene alcohol	-	-	0.24
36	fenchol	$C_{10}H_{18}O$	Monoterpene alcohol	-	-	1.55
37	nerol	$C_{10}H_{18}O$	Monoterpene alcohol	-	-	4.87
38	Fenchyl acetate	$C_{12}H_{20}O_2$	Monoterpene ketone	-	-	1.81
39	Trifluoroacetyl-à-fenchol	$C_{10}H_{18}O$	Monoterpene alcohol	-	-	0.16
40	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	$C_{17}H_{26}O_2$	Phenol	-	-	15.47
41	2-Butene-1,4-diol, diacetate	$C_8H_{12}O_4$	Ketone	-	-	0.42
42	1H-Indene, 5,5'-(1,10-decanediyl)bis[octahydro-	$C_{28}H_50$	Hydrocarbon	-	-	0.03
43	Caproic Acid	$C_6H_{12}O_2$	Carboxylic acid	-	-	0.21
44	5-Isopropyl-2,4-imidazolidinedione	$C_6H_{10}N_2O_2$	Ketone	-	-	9.89
45	Isoborneol, pentamethyldisilanyl ether	$C_{15}H_{32}OSi_2$	Sesquiterpene alcohol	-	-	0.24
46	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	Phenol	-	-	2.33
47	Limonen-6-ol, pivalate	$C_{15}H_{24}O_2$	Ester	-	-	0.25
48	Phenylethyl salicylate	$C_{15}H_{14}O_3$	Ester	-	-	0.08
49	2-Amininonadecane	$C_{19}H_{41}N$	Halogen containing group	-	-	0.32
50	p-Isopropenylphenol	$C_9H_{10}O$	Phenol	-	3.11	2.87
51	2,4-Quinolinedicarboxylic acid	$C_{11}H_7NO_4$	Carboxylic acid	-	-	7.47
52	N-Bromo-2,2-dimethylaziridine	C_4H_8BrN	Halogen containing group	-	-	0.40
53	Cholest-5-en-3-ol (3á)-, tetradecanoate	$C_{27}H_{46}O$	Ester	-	-	0.08
54	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)-	$C_{29}H_{50}O2$	Ether	-	-	0.05
55	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	Ketone	-	-	0.15
56	Squalene	$C_{30}H_50$	Triterpene hydrocarbon	-	-	5.51
57	Santalol	$C_{15}H_{24}O$	Sesquiterpene alcohol	-	-	0.27
			Total	0.00	100.00	100.00

[a] *Volatile compounds were listed in elution time order from capillary VF-WAXms column*

[b] *Note*: RA - relative abundance

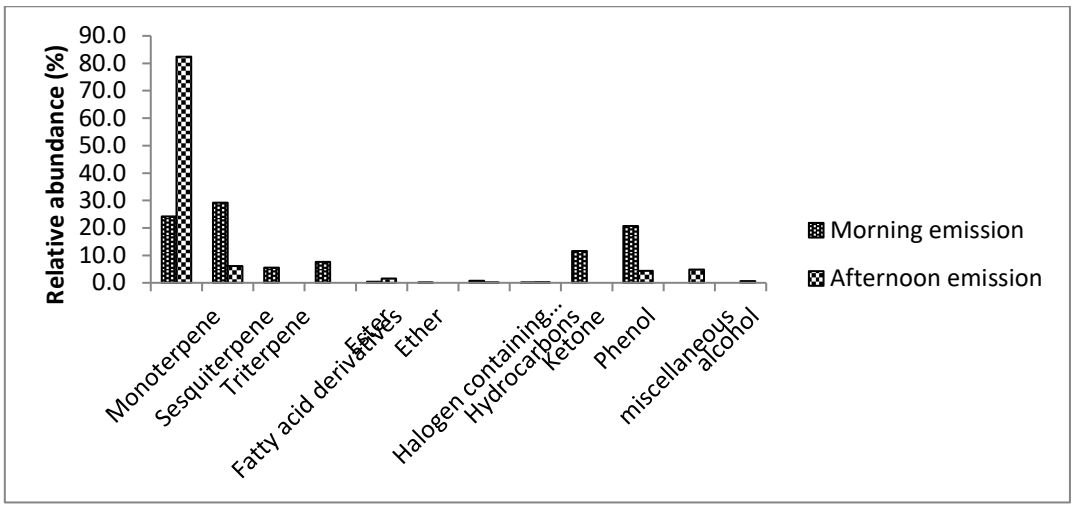

Fig 2. The volatile content comparison in different floral developmental stages. Aromatic compounds mainly include terpenoids (monoterpene, sesquiterpene and triterpene), alcohols, esters, aldehydes, ketones and phenols.

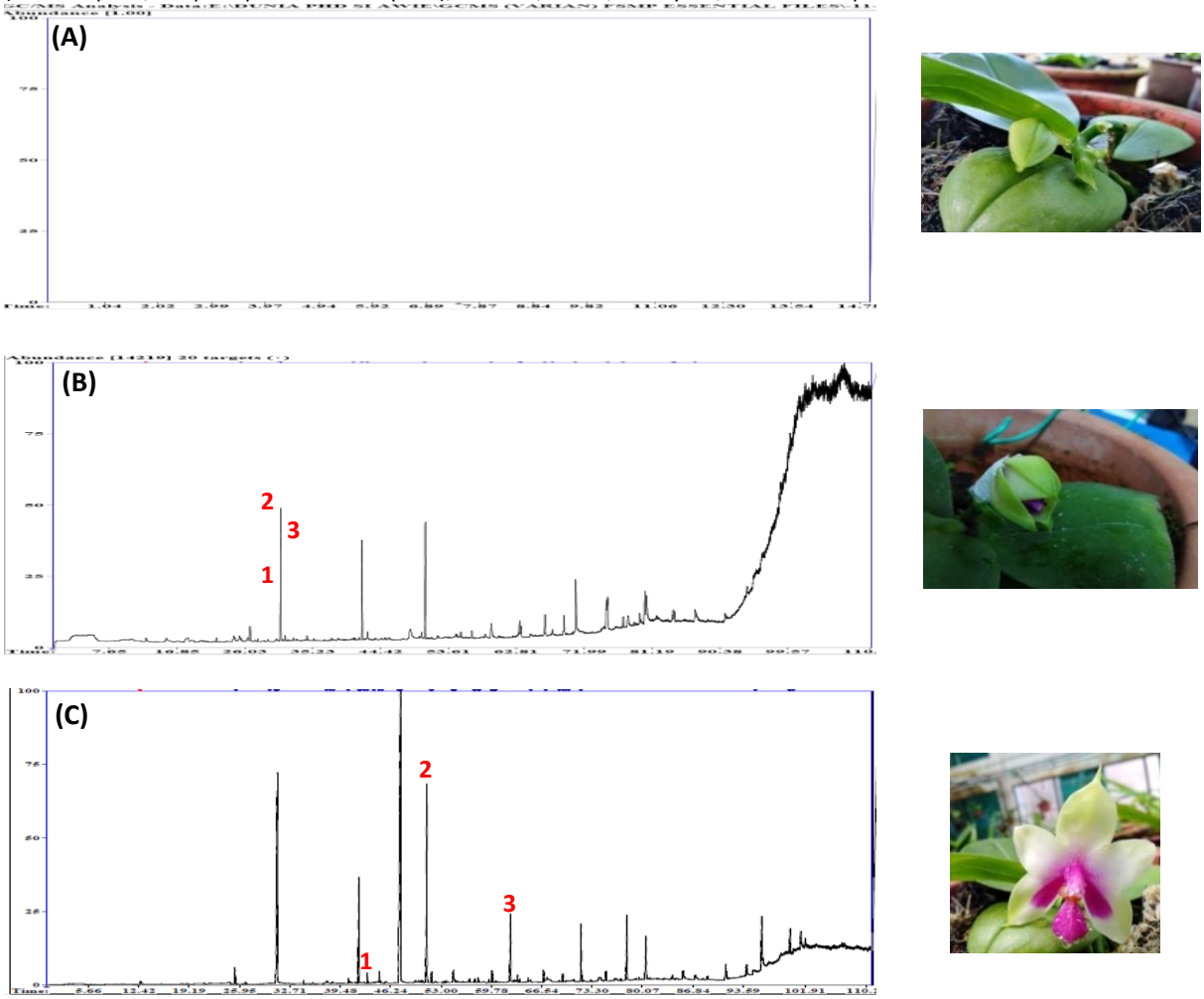

Fig 3. Comparison of chromatogram profile emission by *Phalaenopsis bellina* at three different floral developmental stages. **(a)** flower bud, **(b)** partial bloom, numbers on the graph represent the following compound **(1)** linalool, **(2)** linalyl anthranilate and **(3)** 17-octadecan-14-yn-1-ol and **(c)** full bloom, numbers on the graph represent the following compound **(1)** α-farnesene, **(2)** 2,6-bis(1,1-dimethylethyl)-1-(1-oxopropyl)phenol and **(3)** 5-isopropyl-2,4-imidazolidinedione

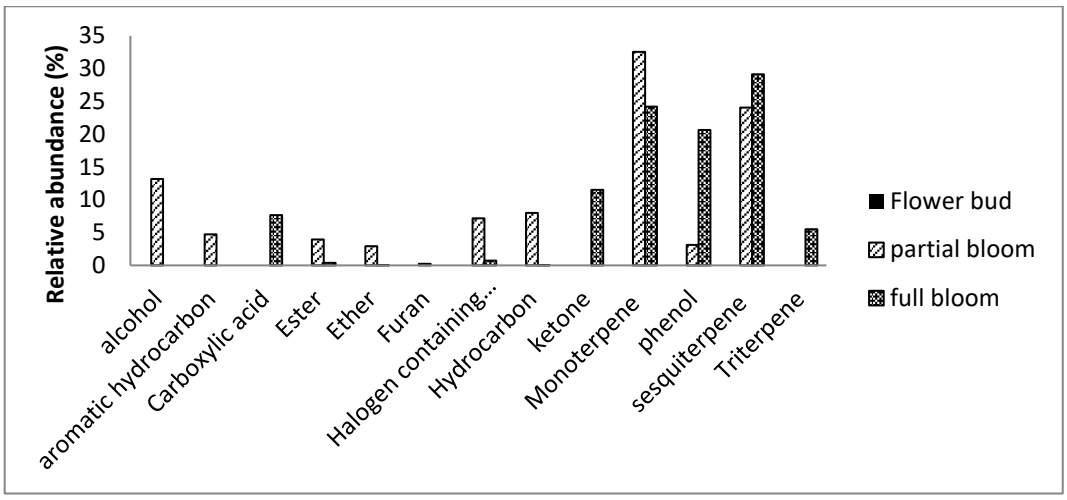

Fig 4. The volatile content comparison in different floral developmental stages. Aromatic compounds mainly include terpenoids (monoterpene, sesquiterpene and triterpene), alcohols, esters, aldehydes, ketones and phenols.

Fig 5. Solid Phase Micro Extraction (SPME) setting to trap the floral volatile emitted by *P. bellina.*

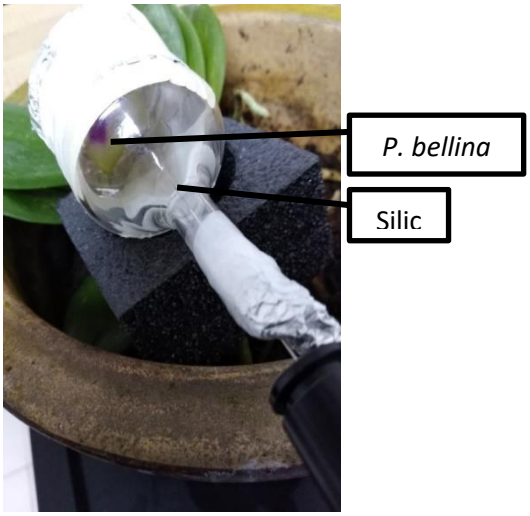

Fig 6. Close up figure of Solid Phase Micro Extraction (SPME) setting to trap the floral volatile emitted by *P. bellina.*

bud of *P. bellina*. Progressing into full bloom, total monoterpene was reduced to 24.23%, while sesquiterpene emission was raised to 29.16%. Triterpenes were also released during full bloom, with an abundance of 5.51%. Partial bloom of *P. bellina* revealed emission of linalool as the highest abundance (32.09%), followed by linalyl anthranilate (24.01%) and 17-octadecan-14-in-1-ol (12.75%). However, linalool emission was decreased drastically into 0.42%, as well as linalyl anthranilate (5.59%) moving into full bloom. In full bloom, α-farnesene becomes the highest abundant compound (19.56%). By looking into the emission of compound classes of *P. bellina,* the flower starts to emit its volatiles during partial bloom stage, in which terpenoid abundance was the highest (56.6%) and increases to 58.9% during full bloom (Figure 4). Other volatile fractions that were detected during partial bloom were alcohols, hydrocarbons, esters, ethers, furans, halogen-containing groups and phenols. Additional volatile fractions, which were carboxylic acids, ketones and terpenoid triterpenes, were identified during full bloom. The results shown were comparable to a similar study conducted by Li *et al*. (2017) for volatile emission from *Luculia yunannensis.* The highest compound diversity was observed during full bloom (37 volatile compounds) compared to partial bloom (31 volatile

compounds), with monoterpenes 3-carane and sesquiterpene α-copaene being the dominant compounds emitted during the blooming period.
According to Steenhuisen *et al*. (2010), scent production was limited to certain flowering times and stages such as anthesis and receptivity also limit the unnecessary use of resources into producing scent after pollination. This might explains the emission profile of partial bloom of *P. bellina* in which a low number of volatile compounds were detected. Meanwhile, plants can also rapidly alter their volatile floral production in response to volatile floral cues from their flowering neighbours (Caruso & Parachnowitsch, 2016; Ninkovic *et al*., 2016). This mechanism can increase the pollination rate and mating by increasing floral volatile emission to attract more pollinators (Li et al. 2017).

Materials and methods

Plant materials

Phalaenopsis bellina is an ornamental orchid with a sweet scent, which was purchased from local vendors in Malaysia. Volatile components were identified during the flowering period, cultivated in the Institute for Tropical and Biology Conservation greenhouse, controlled at 25 ± 3°C. A sampling of volatiles from *P. bellina* was performed during the morning (9.00 am – 9.30 am) and afternoon (3.00 pm – 3.30 pm). Sampling was also done in different floral developmental stages, which were on floral bud, partial bloom, and full bloom stages.

Solid Phase Microextraction (SPME)

Volatiles emitted by a single flower was absorbed by Solid Phase Micro-Extraction (SPME)(Supelco, USA), according to (Mohd-Hairul et al. 2010) for volatile study from *Vanda* Mimi Palmer, with slight modifications on incubation period. The SPME with silica fiber that was coated with 100 µm polydimethylsiloxane (PDMS) was used to absorb the volatiles emitted by *P. bellina* flower. A single flower was put into a modified funnel without detaching the flower from the flower stalk (Figure 5 and 6). The back of the funnel was covered with aluminum foil to ensure no volatiles were escaped during trapping. The SPME holder was pressed to allow the silica fiber in the SPME to emerge from the SPME syringe and captured the volatiles produced by the flower for 30 minutes at ambient room temperature (25 ± 2°C).

Gas Chromatography-Mass Spectrometry Analysis of Volatiles

The SPME fiber that has trapped the volatiles was thermally desorbed for 3 minutes at 250°C in an injector Port of Varian GC-MS (Varian 450GC) equipped with Agilent J & WVF-Waxms Capillary column (30m x 0.25mm x 0.25 µm). The oven temperature was: from 40°C, hold 4 minutes, and then temperature ramp was set to 5°C/min until it reaches 250°C, where it is held for 10 minutes. The injections were performed in splitless mode, while the carrier gas used was helium at a constant flow of 1 mL/min. The transfer line to the mass spectrometer was maintained at 230°C, and the ion source temperature was set at 250°C. The mass spectra were obtained by using a mass selective detector with the electronic impact at 70eV, in which the m/z range was from 30-500. Compounds were identified by comparison with the National Institute of Standards and Technology 2008 (NIST 08). Volatile compound measurements were carried out by peak area normalization (expressed in percentage).

Conclusion

The floral scent is an essential component of the trait repertoire that increases the value of orchids in the horticulture industry. However, scent traits of *Phalaenopsis bellina* have received limited attention. This study revealed differences in the volatile profile in *P. bellina* flower in morning and afternoon emission, indicating timing influence and flowering stages on the volatile emission. Therefore, future work may focus on elucidating the molecular mechanisms of fragrance-related genes related to the terpenoid biosynthetic pathway.

Acknowledgments

This study was conducted with support from the Fundamental Research Grant Scheme (FRG0466-2017) from the Ministry of High Education (MOHE), Malaysia and UMSGREAT Research Grant (GUG0280-2/2018) from Universiti Malaysia Sabah. The authors also express gratitude to all who are directly and indirectly involved in this study.

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