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Volatile compounds of essential oil from different stages of *Michelia alba* (cempaka putih) flower development

[Sebatian meruap minyak pati daripada pelbagai peringkat perkembangan bunga cempaka putih (*Michelia alba*)]

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Key words: *Michelia alba,* volatile compounds, essential oil, gas chromatography-mass spectrometry, flower development

Abstract

Seven developmental stages of *Michelia alba* (cempaka putih) flowers namely Stage 5 to 11 (S5–S11) were investigated for their volatile compounds. The essential oil was isolated by Simultaneous Distillation Extraction (SDE) technique and the oil obtained was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. In total, 78 compounds representing 93–98% of the overall *M. alba* volatiles were identified. Thirty-three of these compounds belonged to isoprenoids group which comprised 30–50% of the total volatile compounds detected throughout S5–S11, whereas the remaining belonged to fatty acid derivatives, benzenoid, phenylpropanoid and other hydrocarbon compounds. The major compounds which represented more than 10% of the essential oil at each stage were dihydrocarveol (S5–S8), linalool (S9–S11), butanoic acid-2-methyl, methyl ester (S9) and cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl) (S6–S7). In this study, variations in the compounds of essential oil as well as their level in percentage within the flower development stages were observed. Dihydrocarveol was the most abundant compounds detected in S5–S8 (44–65%), while linalool was the most abundant compound detected in S9–S11, which accounted for 59–89% of the total essential oil obtained. Based on the profile of both compounds, it might suggest that dihydrocarveol was one of the compounds that contributed significantly during bud development through S5–S8 in which the bud became yellowish and started to swell until the petal just began to open, whereas linalool might contribute significantly to the characteristic fragrance through S9–S11 in which during these phases, the aroma of *M. alba* fragrance was very much intense.

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Introduction

Michelia alba, commonly known in Malaysia as 'cempaka putih' belongs to the family of Magnoliaceae. This higher plant, which is a native to tropical and subtropical Southeast Asia, is often cultivated by Chinese and Malays for its fragrant and ivory-coloured flowers. The flowers are formed in clusters, star-like shape and have a pleasant and sweet floral scent. *Michelia* (cempaka) species is commonly known to produce high value essential oil. The popularity of the fragrance has resulted with many species of *Michelia* being grown all over the world. It was reported that the essential oil of *Michelia* has been used as a key topnote in *Joy* and *J'adore*, which are among the world's most expensive perfumes (Anon. 2000).

As a starting material for perfume production, this essential oil of *M. alba* is expensive which is due to its low yield through preparation from the whole plants (Lai and Lee 1994). Hence, the use of plant cell culture technology might be an attractive solution to overcome this predicament. Thus, it is desirable that the essential oil of *Michelia* species can be producible from cell cultures using its flower parts through a bioreactor system. However, before such production system could be initiated, established information on the chemical profiles of the fragrant through various stages of flower need to be understood.

Previous researchers (Euyama et al. 1992; Shang et al. 2002) have extensively investigated the volatile compositions of *M. alba* flower and leaf. However, studies on the volatile compounds present at different stages during the flower development has never been carried out elsewhere. This paper reports the results of our investigation on the volatile compounds of essential oils from seven developmental stages of *M. alba* flowers.

Materials and methods

Plant materials

Fresh samples of seven developmental stages i.e. S5–S11 (*Plate 1*) of *M. alba* flowers (Sanimah et al. 2002) were collected from Bangi, Selangor. The flower samples were then cut separately into small pieces and weighed prior to extraction.

Isolation of essential oil by Simultaneous Distillation Extraction (SDE) apparatus

About 300 g of fresh *M. alba* flower sample and 300 ml of distilled water were placed in a 500 ml round flask, and 40 ml of 99% (v/v) pentane (BDH, Germany) was placed in 250 ml round flask. All these were connected to the modified Likens-Nickerson micro SDE apparatus (Suri 2001). The extraction was carried out at 100 °C for 4 h. The essential oil obtained was dried over 10 g of anhydrous sodium sulphate overnight, filtered and concentrated by blowing with pure nitrogen gas.

Identification of volatile composition in essential oils

Analyses of the volatile compounds were performed by using gas chromatography-mass spectrometry (GC-MS) with scan mode. The GC-MS analysis was carried out by using a Shimadzu GC-17A with capillary injector and interface to QP 5050A Shimadzu mass spectrometer (Shimadzu, Japan). The capillary column used was DB–5 MS with 0.25 mm x 30 mm film thickness (Supelco, USA). The GC operated at 200 °C in injector temperature 60–240 °C (3 °C/min) (programmed), with total run time of 80 min, and the carrier gas was helium at linear velocity of 36 cm/s. Sample was injected in split mode at a ratio of 1:20.

Results and discussion

Flowers of *M. alba* were selected for volatile compounds at seven stages: S5 = Bud became yellowish (colour break) and swelled; S6 = Bud changed to greenish-cream, swelled and elongated; S7 = Buds changed to full cream colour and the bracts opened;

Plate 1. Eleven development stages of ***Michelia alba*** *flower*

S8 = Quarter bloom, outer whorl of petals opened; S9 = Half bloom, outer and middle whorl of petal opened; S10 = Full bloom, outer, middle and inner whorl of petal opened and S11 = Stamen turned brown, some petals fell; as defined by Sanimah et al. (2002). The yield of essential oil was in the range of 0.18–0.25 % (w/v). *Figure 1* shows the total ion chromatogram from S11 of *M. alba* flowers.

Table 1 shows the volatile compounds in the essential oil of *M. alba* with their relative peak area. A total of 78 compounds were detected at seven different stages of *M. alba* flowers, in which 18–34 compounds were detected in all the stages. Based on the reference list of Knudsen et al. (1993), 33 of these compounds belonged to isoprenoids group which made up 30–50% of the total volatile compounds detected at various stages whereas the remaining compounds belonged to fatty acid derivatives, benzenoid, phenylpropanoid and other hydrocarbon compounds. Twenty-five major volatile compounds (appeared in bold face) which represented more than 1% of the total essential oil in at least one stage, were identified throughout S5–S11. The compounds which consisted in more than 10% of essential oil were dihydrocarveol (S5–S8), linalool (S9–S11), butanoic acid-2-methyl, methyl ester (S9) and cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl) (S6–S7).

Apparently, the essential oils obtained in seven different stages of flower development showed distinct differences in fragrance composition among themselves as well as their yield in percentage of peak area. Most of the compounds including major constituents were only present in several stages, but they were not detected in the other stages. Some of the compounds were exclusively present in only one particular stage *(Table 1)*. In this study, cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl) and eugenol methyl ether, which belonged to the fatty acid derivatives

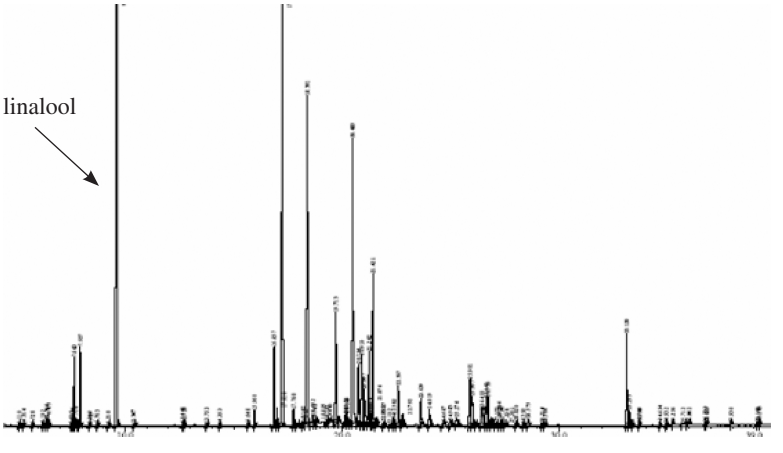

Figure 1. Total ion chromatography of volatile compounds extracted by SDE in ***Michelia alba*** *flower (stage 11)*

Table 1. Compounds of the essential oil in seven different stages of *Michelia alba* flower development

No.	Identified components	Peak area of gas chromatogram (%)						
		S5	S6	S7	S8	S9	S10	S11
	Isoprenoids							
1.	**(+) - α-terpineol (p-menth-1-en-8-ol)**	**0.79 ± 0.42**	**1.28 ± 0.01**	**0.69 ± 0.05**	**0.51 ± 0.08**	–	–	–
2.	(z)-β-farnesene	–	–	0.3 ± 0.05	–	–	–	–
3.	α-cadinol	–	–	–	–	0.10 ± 0.01	–	–
4.	**α-caryophyllene**	**1.08 ± 0.01**	**0.69 ± 0.01**	**0.38 ± 0.05**	**0.91 ± 0.05**	**0.3 ± 0.10**	–	–
5.	α-santalene	–	0.32 ± 0.01	0.35 ± 0.03	–	–	–	–
6.	α-cubebene	0.2 ± 0.01	0.37 ± 0.01	–	–	–	–	–
7.	α-pinene	–	–	–	–	–	–	0.27 ± 0.09
8.	IR-α-pinene	–	–	–	–	–	0.40 ± 0.00	–
9.	β-farnesene	0.33 ± 0.01	–	–	–	–	–	–
10.	**Cadina-1-(10),4-diene**	–	**1.24 ± 0.03**	–	**1.23 ± 0.80**	–	–	–
11.	Cadina-1,3,5-triene	–	–	0.26 ± 0.04	–	–	–	–
12.	Camphene	–	–	–	–	0.23 ± 0.02	0.24 ± 0.78	0.18 ± 0.03
13.	α-caryophyllene	–	–	–	–	–	0.23 ± 0.60	–
14.	**Caryophyllene**	–	–	**2.34 ± 0.50**	**1.68 ± 0.14**	**0.49 ± 0.02**	–	–
15.	**Caryophyllene oxide**	–	**0.73 ± 0.12**	**0.98 ± 0.2**	–	**1.11 ± 0.01**	**0.35 ± 0.60**	**1.23 ± 0.03**
16.	Isocaryophyllene	–	–	–	–	–	0.11 ± 1.2	–
17.	**Chamigrene**	**0.76 ± 0.01**	**0.68 ± 0.01**	**1.23 ± 0.19**	**0.94 ± 0.05**	–	–	–
18.	**Cineol**	–	**0.89 ± 0.02**	–	–	**1.14 ± 0.08**	**1.51 ± 0.01**	**1.33 ± 0.12**
19.	Cis-Geraniol	–	0.28 ± 0.01	0.21 ± 0.01	–	–	–	–
20.	Cis-Z-Bisabolene epixode	–	–	0.31 ± 0.02	–	–	–	–
21.	Cis-β-Ocimene	–	–	–	–	–	0.75 ± 0.36	–
22.	D-Limonene	–	–	–	–	0.26 ± 0.32	–	–
23.	**Germacrene D**	**2.39 ± 0.3**	**1.52 ± 0.07**	**1.25 ± 0.08**	**2.48 ± 0.6**	**0.26 ± 0.06**	–	–
24.	Isocineol	0.86 ± 0.06	–	–	–	–	–	–
25.	**Linalool**	–	–	–	–	**59.12 ± 0.05**	**74.12 ± 0.07**	**79.42 ± 0.12**
26.	Mycrene	–	–	0.83 ± 0.02	–	–	–	–
27.	Nerolidol	–	–	–	0.25 ± 0.04	–	–	–

(*cont.*)

Table 1. (*cont.*)

No.	Identified components	Peak area of gas chromatogram (%)						
		S5	S6	S7	S8	S9	S10	S11
28.	**Nopinene**	**0.47 ± 0.04**	**0.56 ± 0.19**	–	–	**1.5 ± 0.06**	**1.56 ± 0.12**	**0.85 ± 0.07**
29.	Sabinene	–	–	–	–	0.49 ± 0.09	0.51 ± 0.35	0.3 ± 0.14
30.	tau-muurolol	–	–	0.73 ± 0.1	0.73 ± 0.19	0.31 ± 0.03	–	–
31.	Ylangene	–	–	0.21 ± 0.6	–	–	–	–
32.	**Dihydrocarveol**	**60.33 ± 0.06**	**64.52 ± 0.4**	**58.82 ± 0.08**	**43.81 ± 0.19**	–	–	–
33.	**Ocimene**	**5.03 ± 0.05**	**1.43 ± 0.06**	**0.84 ± 0.09**	**2.95 ± 0.15**	–	**0.16 ± 0.45**	–
Fatty acid derivatives/Benzenoid/Phenylpropanoid/Miscellaneous								
34.	**3-butenamide**	–	–	–	–	–	**1.11 ± 0.85**	–
35.	1-decanol, 5,9-dimethl	–	–		–	–	0.29 ± 0.00	–
36.	1,6,10-dodecatriene, 7,11-dimethyl-3-methylene	–	0.36 ± 0.13	–	–	–	–	–
37.	2,6-dimethyl-1,6-heptadiene -4-ol-acetate	–	–	–	–	–	–	0.11 ± 0.15
38.	2,3-Dimethylcyclohexanol	–	–	–	–	–	0.11 ± 0.32	–
39.	3-ethenylheptan-2,6-dione	–	–	–	–	–	0.11 ± 0.25	–
40.	3,7-octadien-2-ol, 2,6-dimethyl	–	–	0.13 ± 0.06	–	0.24 ± 0.17	–	0.64 ± 0.08
41.	12-oxabicyclo [9.1.0] dodeca-3-7-diene, 1,5,5	–	–	–	–	0.18 ± 0.12	–	0.22 ± 0.15
42.	7-methylenebicyclo[3.2.0] hept-3-en-2-one	–	–	–	–	–	0.06 ± 0.22	–
43.	**Bicyclo[3.1.1]hept-2-ene,2, 6,6, trimethyl**	**3.61 ± 0.1**	**0.63 ± 0.32**	–	**1.93 ± 0.33**	**0.54 ± 0.25**	–	–
44.	**Bicyclo[7.2.0]undec-4-ene, 4,11,11- trimethyl-8-methylene**	**4.8 ± 0.04**	**3.52 ± 0.13**	**1.52 ± 0.26**	**3.24 ± 0.32**	**0.63 ± 0.72**	**0.51 ± 1.2**	–
45.	**Butanoic acid-2-methyl, methyl ester**	–	–	**6.44 ± 2.7**	**17.8 ± 0.5**	**20.46 ± 0.08**	**8.24 ± 0.56**	**2.08 ± 0.13**

(*cont.*)

Table 1. (*cont.*)

No.	Identified components	Peak area of gas chromatogram (%)						
		S5	S6	S7	S8	S9	S10	S11
46.	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene	–	0.36 ± 0.02	–	–	–	–	–
47.	2,6-Dimethyl-1,6-heptadiene -4-ol-acetate	–	–	–	–	–	–	0.11 ± 0.15
48.	3,7-Octadien-2-ol, 2,6-dimethyl	–	–	0.13 ± 0.06	–	0.24 ± 0.17	–	0.64 ± 0.08
49.	Cyclobutane, 1,2-bis (1-methylethenyl) trans	–	–	0.47 ± 0.17	–	–	–	–
50.	**Cyclohexane,1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)**	**9.74 ± 0.05**	**13.46 ± 0.5**	**10.75 ± 1.5**	**9.63 ± 0.7**	**2.03 ± 0.4**	**0.99 ± 0.3**	**0.93 ± 0.5**
51.	Cyclopentanol, 1,2-dimethyl -3-(1-methylethenyl)	–	–	0.35 ± 0.3	–	–	–	–
52.	Cyclopropane, 1-(2-methylene-3-butenyl)- 1-(1-methylenepropyl)	–	–	0.57 ± 0.17	–	–	–	–
53.	Hexanoic acid, 2-methyl, methyl ester	–	–	–	–	0.21 ± 0.09	0.22 ± 0.45	0.25 ± 0.02
54.	**Isobutanol**	–	–	–	–	**3.81 ± 0.09**	–	–
55.	Isooctane, (ethenyloxy)	0.53 ± 0.04	0.47 ± 0.01	0.53	0.61 ± 0.04	–	–	–
56.	**Octadiene**	–	–	**1.2 ± 0.08**	–	–	–	–
57.	Octanoic acid	–	–	0.64 ± 0.23	–	–	–	–
58.	**Octane, 1-(ethenylthio)**	–	–	–	**2.46 ± 0.6**	–	–	–
59.	**Pentane**	–	–	–	–	–	**4.64 ± 0.45**	**1.25 ± 0.23**
60.	Propanoic acid, 2, 2-dimethyl-,2-phenylethyl ester	–	–	–	0.54	0.11 ± 0.15	–	0.28 ± 0.08
61.	Tridecane	–	–	0.22 ± 0.32	–	–	–	–

(*cont.*)

Table 1. (*cont.*)

No.	Identified components	Peak area of gas chromatogram (%)						
		S5	S6	S7	S8	S9	S10	S11
62.	Benzene, 1-methyl-2 (1-methyl)-	–	–	–	–	–	–	0.1 ± 0.07
63.	Benzene, 1-methoxy-4-(2-methyl)-	–	–	–	–	–	–	0.37 ± 0.12
64.	Benzene, 1-methoxy-4-(2-propenyl)	–	–	–	–	0.25 ± 0.14	0.23 ± 0.35	–
65.	Ethanon, 1-(3-ethylcyclobutyl)	–	–	–	–	0.3 ± 0.03	–	0.63 ± 0.50
66.	**Phenylethyl alcohol**	–	–	**0.22 ± 0.01**	**2.66 ± 0.07**	**1.29 ± 0.03**	**1.28 ± 0.56**	–
67.	**Eugenol methyl ether**	**3.13 ± 0.01**	**2.63 ± 0.01**	**3.07 ± 0.66**	**3.69 ± 0.47**	**1.76 ± 0.45**	**1.36 ± 0.75**	**1.98 ± 0.42**
68.	**Isoeugenol methyl ether**	**0.62 ± 0.05**	**0.51 ± 0.05**	**1.14 ± 0.06**	–	–	0.06 ± 0.70	–
69.	1H-cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methyl	0.51 ± 0.01	0.37 ± 0.03	0.9 ± 0.13	0.46 ± 0.06	–	–	0.37 ± 0.06
70.	2H-Pyran-3-ol, 6-ethanyltetrahydro-2	–	–	–	–	–	–	0.41 ± 0.01
71.	1- Napthalenol, decahydro-1, 4a-dimethyl-7-(1-methylethylidene)	0.45 ± 0.01	0.4 ± 0.15	–	0.53 ± 0.13	0.25 ± 0.32	–	–
72.	1,3-bis- (2-cyclopropyl, 2-methylcyclopropyl) – but-2-ene-1-one	–	0.38 ± 0.04	0.28 ± 0.05	0.3 ± 0.17	–	–	–
73.	**2-furanmethanol, 5-ethenyltetrahydro-alpha-5-trimethyl- trans**	–	**0.35 ± 0.1**	**0.97 ± 0.01**	**0.43 ± 0.01**	**1.03 ± 0.06**	**0.54 ± 0.45**	**3.34 ± 0.01**
74.	5H-1-Pyrindine	–	–	–	–	0.11 ± 0.14	–	–
75.	Isopropyltoluene	–	–	–	–	0.13 ± 0.25	–	–
76.	Ledene oxide	–	–	–	0.13 ± 0.6	–	–	–

(*cont.*)

Table 1. (*cont.*)

No.	Identified components	Peak area of gas chromatogram (%)						
		S5	S6	S7	S8	S9	S10	S11
77.	Naphthalene, 1,2,3,4,4a,5,6, 8a, - octahydro-4a	–	–	–	–	0.18 ± 0.45	–	–
78.	Tetracyclo[6.1.0.0(2,4).0(5, 7] nonane, 3,3,3,9, 9-hexamethyl-Z,Z,E	–	–	–	0.19 ± 0.9	–	–	–

Compounds appeared in bold face were the major compounds which represented more that 1% of the total essential obtained in at least one stage
Means ± s.d (n = 3)

and phenylpropanoid respectively, were the only compounds produced by all stages of S5 to S11.

The variation of the volatile compositions in different stages of *M. alba* flower development found in this study was in agreement with Vendramini and Trugo (2000), which reported that the chemical composition including the distribution of volatile compounds of plant species and their organs are much dependent on their stage of maturity. Verdoonk et al. (2003) also reported the same phenomena when they studied the volatile emission in few stages of Petunia flower development. They found that the peaks of methylbenzoate and the two sesquiterpenes (germacrene D and cadina-3,9-diene) became more obvious during the expansion of the corolla limbs and opening of the flower, however when the flower developed further, the emission of both sesquiterpenes decreased while that of benzenoid increased.

The result also indicated that dihydrocarveol was the most abundant compound detected in S5 to S8 (44–65 %) while in S9 to S11, linalool was the most abundant compound, accounting for 59–89% of the total essential oil. Previous research works by Euyama et al. (1992) also reported that linalool was the major compound detected in essential oil of *M. alba* flower. This finding was supported by Shang et al. (2002) who found that linalool was one of the major compounds isolated when the essential oil of *M. alba* fresh flowers was analysed by headspace SPME–GC-MS. However, among the main compounds (α-mycrene, (*S*)-limonene, eucalyptole, (R)-fenchone, linalool, α-muurolene, camphor, caryophyllene, germacrene D) in which (*S*)-limonene was the most abundant compounds as reported by Shang et al. (2002), only four compounds: mycrene, caryophyllene, linalool and germacrene D were detected in our samples.

One of the main factors that might attribute to these differences was the different methods used for the extraction. In

our case, we used Simultaneous Distillation Extraction (SDE) technique whereas Shang et al. (2002) used a headspace solid phase microextraction technique where the fibres were exposed in the headspace of the fresh flowers together with the constant stirring that might enhance the efficiency of the extraction. However, the SDE technique used for the extraction of our samples was proven effective in our lab in identifying very minute amount of active compounds present in the samples (Suri M., MARDI, Serdang, pers. comm. 2000).

Figure 2 shows the level of five major compounds produced throughout S5–S11 namely 1) linalool; 2) dihydrocarveol; 3) butanoic acid-2-methyl, methyl ester (S9); 4) cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl); and 5) eugenol methyl ether which was produced constitutively in S5–S11. The pattern of dihydrocarveol and linalool level throughout S5–S11 was of particular interest in this study. It showed that the level of dihydrocarveol was slightly increased beginning at S5–S6 but gradually declined when the flower development reached S7 and S8. The level of dihydrocarveol was totally undetected when the flower development reached S9, instead, linalool accumulation was observed which accounted 59% of the total essential oil. Linalool level was markedly increased at S10 and slightly decreased at S11. This result might suggest that dihydrocarveol was one of the constituents that contributed significantly during bud development through S5 to S8 while linalool might contribute significantly to the characteristic fragrance since it was first detected when the outer and middle whorl of flower petal opened (S9), at which during this time, the aroma of *M. alba* fragrant was very much intense.

Other volatiles, specifically, cyclohexane and eugenol methyl ether were detected at S5 to S11, but their levels decreased as the flower reached S7 and S9. Butanoic acid was first detected at S7 and slightly increased at S8 and S9, but the level was markedly decreased at S11, at which the petals began to inroll and fell. Schade et al. (2001) reported a similar pattern in carnation flowers. They found that steady state level of 10 volatiles including benzaldehide, benzyl benzoate and caryophyllene change independently as the flowers develop and senesce, suggesting that the synthesis of these volatile compounds was physio-biochemically regulated.

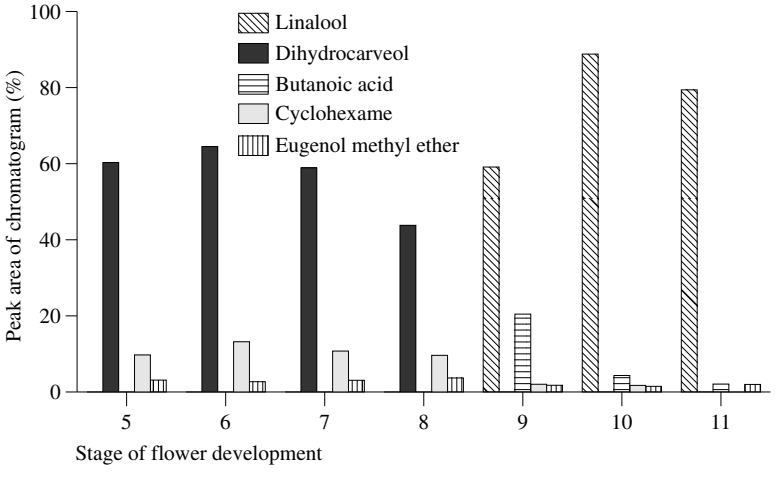

Figure 2. The level of (1) linalool, (2) dihydrocarveol, (3) butanoic acid-2-methyl, methyl ester, (4) cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl) and (5) eugenol methyl ether throughout S5 to S11

As comparison, only 22 compounds namely (+): α-terpineol (p-menth-1-en-8-ol) (S8), α-cadinol (S9), α-santalene(S6–S7), α-cubebene (S5–S6), α-pinene (S11), β-farnesene (S5), caryophyllene (S7–S9), camphene (S9, S11), caryophyllene oxide (S6–S7, S9, S11), cineol (S6, S9–S11), D-limonene (S9), germacrene D-(S5–S9), *cis*-geraniol (S6–S7), ocimene (S5–S8), linalool (S9–S11), nerolidol (S8), sabinene (S9), τ-muroolol (S7–S9), bicyclo[3.1.1] hept (S5–S6, S8–S9), isobutanol (S9), phenylethyl alcohol (S7–S9), eugenol methyl ether (S5–S11), which were present in at least one stage throughout S5–S11, were similar to the volatile compounds in *M. alba* as previously reported by Euyama et al. (1992) and Shang et al. (2002), whereas the remaining were totally new compounds.

These conflicting results suggested that variability of the oil composition in different population of the same plant species might be due to genetic diversity (Skoula et al. 1999). In the present work, we have used the same species but because the plants were grown in Malaysia, there could be a change in the chemical composition or may be in the biosynthesis pathway as described by Pala-Paul et al. (2004). Previous report of several higher plant species also showed different chemical compositions among the same species growing in different places under different conditions (Skoula et al. 1996; Yu et al. 2003).

The high variation in the compositions of essential oil between the stages made the task of identifying the constituents responsible for characteristic fragrance of *M. alba* difficult. However, since each stage has its own characteristic compositional pattern, these data are potentially useful for our current research activity toward producing fragrance essential oil in *M. alba* cell cultures.

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Abstrak

Tujuh peringkat perkembangan bunga cempaka iaitu peringkat 5 hingga 11 (S5–S11) telah dikaji kandungan sebatian meruapnya. Minyak pati bunga cempaka ini telah diasingkan menggunakan kaedah Pengekstrakan Penyulingan Serentak dan dianalisis menggunakan alat Kromatografi Gas Spektrometri Jisim (GC-MS). Keseluruhannya 77 sebatian yang mewakili 93–98% minyak pati meruap telah dapat dikenal pasti. Sejumlah 33 sebatian ini tergolong dalam kumpulan isoprenoid yang mewakili 30–50% jumlah keseluruhan sebatian meruap yang dikesan di peringkat S5 hingga S11, sementara bakinya tergolong dalam kumpulan terbitan asid lemak, benzenoid, fenil propanoid dan hidrokarbon lain. Sebatian utama yang mewakili lebih 10% daripada keseluruhan minyak pati di setiap peringkat perkembangan bunga ialah dihidrokarveol (S5–S8), linalool (S9–S11), asid butanoik-2-metil, metil ester (S9) dan sikloheksan, 1-etenil-1-metil-2,4-bis (1-metiletenil) (S6–S7). Hasil kajian ini menunjukkan variasi yang tinggi dari segi komposisi dan peratusan sebatian meruap antara satu peringkat perkembangan bunga dengan peringkat perkembangan bunga yang lain. Dihidrokarveol ialah sebatian meruap yang utama di peringkat S5 hingga S8 iaitu 44–65%, sementara linalool ialah sebatian meruap utama di pringkat S9 hingga S11 iaitu 59–89% daripada jumlah minyak pati yang diperoleh. Berdasarkan profil penghasilan dua sebatian ini, besar kemungkinan dihidrokarveol adalah salah satu sebatian utama yang menyumbang secara signifikan dalam peringkat S5–S8 iaitu semasa putik bunga cempaka bertukar kekuningan dan membengkak sehinggalah kelopak bunga mula terbuka, sementara linalool berkemungkinan besar penyumbang secara signifikan terhadap ciri kewangian dalam peringkat S9–S11. Pada peringkat ini, kewangian bunga cempaka ialah yang paling tinggi.

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