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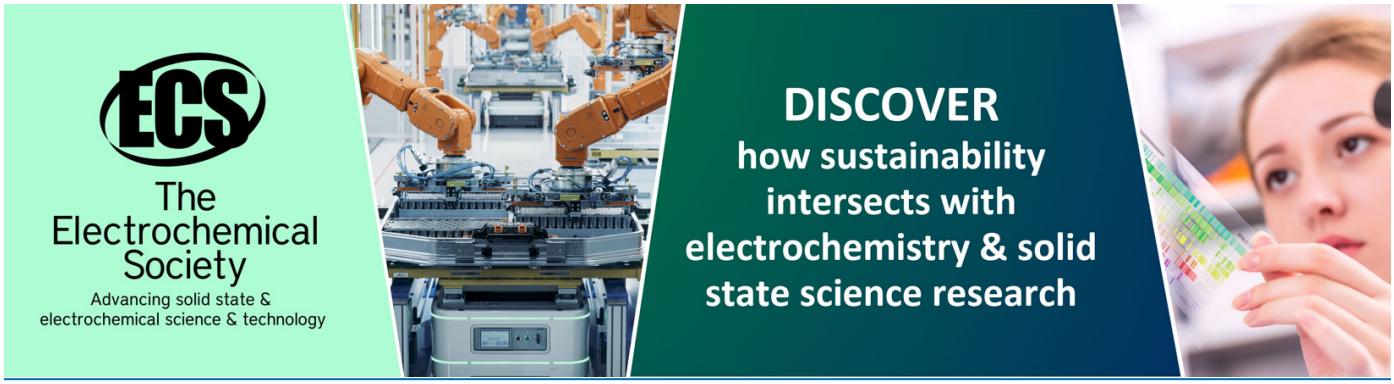

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Assessing the Floral Volatile Constituents of Male and Female *Rafflesia Kerri* Meijer from Lojing Highlands, Peninsular Malaysia

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Abstract. *Rafflesia kerri* Meijer is a gigantic parasitic flowering species, endemic in Peninsular Malaysia and Thailand. The flower reported to emit a foul smell, mimicking rotten meat to attract pollinators from the Calliphoridae to visit the male and female flower. Beside the olfactory factor, the visual display with red brownish tinge colour and the enormous size believed to act as secondary attractant in luring the pollinators. However, the study of pollination biology on this species remain limited and information obtained scanty. Herein, this study was aimed to evaluate the presence of chemical compounds by qualitative phytochemical analysis and screen the floral volatile constituents (FVCs) emitted through Head Space – Solid Phase Microextraction – Gas Chromatography – Mass Spectrometry (HS-SPME-GC-MS). The phytochemical screening of both extracts showed the presence of alkaloids, triterpenoids/ steroids, flavonoids and tannins, whereas the tentative floral volatile constituents (FVCs) identified were from various chemical classes such as long chain hydrocarbon, organosilicon compound, primary alcohol, aromatic acid as well as miscellaneous compound. The finding suggests that the presence of phytochemical compounds and combination of vast floral volatile constituents identified are believed to contribute in scent emission and attract the pollinators to visit the flower.

1. Introduction

Rafflesia is a genus of parasitic flowering plants from the family of Rafflesiaceae. This gigantic flower grown in the tropical rainforest of Southeast Asia, mainly in Malaysia, Thailand, Indonesia and the Philippines [1, 2]. In Malaysia, the flower commonly known as *Pakma*. Seven species of *Rafflesia* reported from Peninsular Malaysia: *Rafflesia kerri, R. azlanii, R. cantleyi, R. su-meiae, R. sharifah-hapsahieae, R. parvimaculata and R. tuanku-halimii* [3, 4], where *R. kerri* recognized as the largest species in Peninsular Malaysia [5, 6]. The early discovery of this species reported in 1929, found in Ranong, Province by A.F.G. Kerr and in 2008, 26 populations of *R. kerri* were mapped in Lojing Highlands, Kelantan, Peninsular Malaysia [7].

Studies on pollination biology of this species reported by Hor et al. [8] and Banzinger et al. [2]. As sessile organism and unisexual flower, *Rafflesia* is dependent on pollinators for the pollination process to occur, transferring the pollen from the male flower to the stigmatic area of the female

flower. A success pollination process resulted in fruit formation, contained thousands of miniscule seeds, ready to be dispersed by the dispersal agents [2, 9] and thus, maintain the continuity of the species. *Rafflesia* is a sapromyophilous flower, emit the foul scent mimicking the rotten flesh, and the colour displayed has deceit the carrion flies for food/brood sites [9]. However, Banzinger et al. [2] believed *R. kerri* is not a deceptive flower due to the availability of slimy mush (anther exudates) under the disc rim in male flower that can be suck by the flies. Besides, the pollen is source of carbohydrates, lipids and protein. Recent study by Hor et al. [8] identified potential pollinators for *R. kerri* in Lojing Highlands were by calliphorid flies from the genera *Chrysomya, Lucilia* and *Hypopygiopsis*, whereas diptera (Rhagonidae, Syrphidae, Anthomyiidae, Tachinidae, Sarchophagidae, Musciade, Conopidae, Ulidiidae, Drosophilidae) and hymenoptera (Formicidae, Apinae and Andrenidae) known as visitors visiting the blooming flower. [10] listed the female carrion flies *Chrysomya villeneuvei* Patton, *C. rufifacies* (Macquart), *C. defixa* Walker, *C. chani* Kurahashi, *C. pinguis* Walker as pollinators for *R.kerri* in Thailand. Previous studied by Patino et al. [11] showed *Raffesia* is an endothermic flower, and the production of CO_2 and other volatile constituents was likely to influence the scent emission emitted by the flower, attracting the pollinators to visit the flower. However, the data obtained is scant to conclude the mechanism of pollination. Moreover, the rarity to encounter the blooming flower has baffled researcher to study the floral scent of this flower. Therefore, this research was carried out to fill the gaps in knowledge by determining the phytochemical compounds and screening the floral volatile constituents (VCs) emitted by the *Rafflesia* which believed responsible in attracting the pollinator to visit the flower.

2. Materials and methods

2.1 Plant materials collection

The samples of *R. kerri* collected from Rafflesia Conservation Area (RCA), Lojing Highlands, Kelantan. The site lies between latitude of 4^0 32' to 4^0 47 N and longitude of 101^0 20' to 101^0 34' E with an altitude ca. of 1, 400 m above sea level (a.s.l.) [7]. The central disc of the flower was choosed as plant materials due to differences in the structure as shown in Figure 1.

2.2 Chemicals and reagents

Anhydrous sodium sulphate, sulphuric acid, potassium iodide, mercury dichloride, acetic acid, acetic anhydride and glacial acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Organic solvents (AR grade) - absolute ethanol, methanol, ammonia, chloroform were from HmbG (Orioner Hightech Sdn. Bhd, Malaysia).

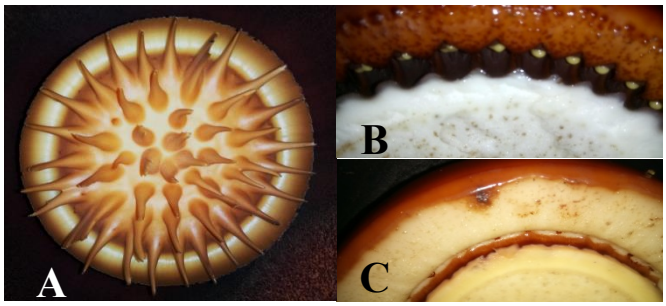

Figure 1. A (Upper view of central disc with 48 processes pointing upward); **B** (male central disc with the presence of pollen); **C** (female central disc with stigmatic area, without the presence of pollen).

2.3 Ethanolic extract preparation

The fresh samples collected were weighed, cut into small pieces and directly place into a sampling bottle contained absolute ethanol, macerated for three days at room temperature. Then, the solution filtered and evaporated using rotary evaporator (Heildolph, Germany). The concentrated ethanolic

extract kept in airtight container and labelled as MCD for male central disc ethanolic extract and FCD for female central disc ethanolic extract.

2.4 Qualitative phytochemical screening

The qualitative phytochemical test was carried out as described by Harborne et al. [12] to determine the presence of bioactive compounds (alkaloids, saponins, triterpenoids/ steroids, flavonoids and tannins) according to the protocols.

2.5 Volatile constituents (VCs) screening

In this study, two different fibres namely; 100 μm PDMS and 50/30 μm Divinylbenzene Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) were used to obtain wide range of the volatile constituents. Prior to operate, all fibres conditioned at 250 °C for 2 min. About 0.5g of each ethanolic extract was placed into 20 mL vial and tightly capped. The vials were introduced in a thermostatic bath 60 s at 50 °C and vibrated for 5 s every 2 s. The extraction time was fixed at 60 s to permit volatile compounds to be trapped. After sampling, the fibre was immediately inserted into the GC injector for 1 min. The non-polar column, HP5-MS was used. The temperature programme was set as follows: the initial temperature was 40 °C for 10 min, and then increased to 180 °C at rate 3 °C/min. Total running programme was 71.67 min. The volatile constituents were qualitatively identified by matching the mass spectrum with database Wiley7Nist05.L and HPCH2205.L library.

4. Results

Based on the current study, 100 g of fresh sample of the male and female *R. kerri* central disc extracted with ethanol solvent has obtained extract yields approximately from 5 to 6 g. Figure 2 shown the physical form of the extract found to be viscous solid resin-like with dark-brown colour and it has undescribed scent arise.

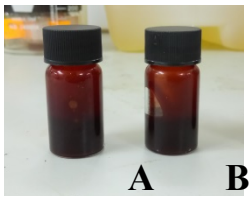

Figure 2. A) Male ethanolic extract (MCD) and B) Female ethanolic extract (FCD) from *R. kerri* central disc.

4.1 Qualitative phytochemical

Through phytochemical qualitative screening, both MCD and FCD ethanolic extracts showed the presence of alkaloids, triterpenoid/ steroid, flavonoids and tannins (Table 1).

Table 1. Results of qualitative phytochemical screening of the MCD and FCD ethanolic extract.

Chemical group tested	MCD	FCD
Alkaloids	+	+
Saponins	-	-
Triterpenoids/ steroids	+	+
Flavonoids	+	+
Tannins	+	+

*Scale '+' and '-' used to indicates the presence and absent of alkaloids, saponins, triterpenoids/ steroids, flavonoids and tannins.

4.2 Floral volatile constituents (FVCs)

The results obtained by using different fibres (PDMS and DVB/CAR/PDMS) on MCD and FCD ethanolic extract shown in Table 2 and Table 3, respectively. The PDMS fibres are for non-polar

compounds whereas DVB/CAR/PDMS are for semi-polar compounds [13]. Through the study conducted, more peaks detected from PDMS fibres compared to DVB/CAR/PDMS fibres. This indicates that the majority of the VCs are from the non-polar compounds.

In MCD ethanolic extract, about 45 of VCs were detected by using PDMS fibres, where 1-acetoxy-6a-methyl-1,1a-dihydrocycloprop[a]inden-6(6aH)-one (2.08%) and 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol (3.63%) were identified and the rest were unknown VCs. Through DVB/CAR/PDMS fibres, majority VCs detected are organosilicon compounds: Hexamethylcyclotrisiloxane (1.44%), Octamethylcyclotetrasiloxane (4.77%), Decamethylcyclopentasiloxane (2.02%), Dodecamethylcyclohexasiloxane (4.08%), and Tetradecamethylcycloheptasiloxane (3.62%). Followed by 1,2-benzenedicarboxylic acid, diethyl ester (11.39%); 4-octadecyl morpholine (19.07%); 2-ethyl-1-hexanol (5.42%) and the rest 8 peaks were unknown VCs.

In FCD ethanolic extract, about 68 peaks were detected consist of long chain hydrocarbon groups: Tetradecane (1.85%), 1-(2-hydroxyethoxy) tridecane (1.27%), 1-tetradecene (0.64%), 1-nonadecene (17.47%), n-Octadecane (6.20%), 1,1'-oxybis dodecane (1.20%), 1,1,3-trimethyl cyclopentane (0.59%), 2-hydroxy-16-cyani-hexadecane (0.47%). Followed by 2-dodecanol (2.79%), 13-tetradecen-1-ol acetate (9.78%), 1-octadecanol (0.20%); Methyl 4-cyanothiochroman-trans-3-carboxylate (0.29%); Bis-(3,5,5-trimethylhexyl) ether (0.33%), and the rest were unknown VCs. Through the DVB/CAR/PDMS fibres, 2,4-diisocyanato-1-methylbenzene (37.58%) was detected.

Table 2. The volatile constituents of MCD ethanolic extract

No.	RT	%	COMPOUNDS	No.	RT	%	COMPOUNDS
1.	7.566	0.79	Unknown	24.	54.939	1.52	Unknown
2.	38.927	2.08	1-acetoxy-6a-methyl-1,1a-dihydrocycloprop[a]inden-6(6aH)-one	25.	55.125	1.52	Unknown
3.	41.576	0.32	Unknown	26.	55.201	1.29	Unknown
4.	42.033	0.39	Unknown	27.	55.419	3.98	Unknown
5.	42.085	0.16	Unknown	28.	55.496	2.43	Unknown
6.	42.499	0.26	Unknown	29.	55.749	8.58	Unknown
7.	42.838	0.32	Unknown	30.	56.071	2.16	Unknown
8.	45.185	0.32	Unknown	31.	56.347	9.68	Unknown
9.	49.113	0.24	Unknown	32.	56.690	2.12	Unknown
10.	50.937	3.63	2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	33.	56.839	0.83	Unknown
11.	51.026	0.22	Unknown	34.	57.081	2.17	Unknown
12.	51.171	0.37	Unknown	35.	57.220	1.53	Unknown
13.	52.320	1.17	Unknown	36.	57.610	3.09	Unknown
14.	52.557	1.07	Unknown	37.	58.105	3.30	Unknown
15.	53.521	1.63	Unknown	38.	58.506	1.74	Unknown
16.	53.581	0.45	Unknown	39.	58.594	0.98	Unknown
17.	53.607	0.32	Unknown	40.	58.997	2.94	Unknown
18.	53.644	0.35	Unknown	41.	59.349	3.75	Unknown
19.	53.664	0.90	Unknown	42.	59.891	3.28	Unknown
20.	53.764	0.46	Unknown	43.	60.261	1.51	Unknown
21.	54.057	1.59	Unknown	44.	60.473	6.62	Unknown
22.	54.219	1.26	Unknown	45.	60.687	0.81	Unknown
23.	54.803	7.94	Unknown				

No.	RT	%	COMPOUNDS	No.	RT	%	COMPOUNDS
1.	6.899	1.44	Hexamethylcyclotrisiloxane	9.	46.970	0.23	Unknown
2.	19.735	4.77	Octamethylcyclotetrasiloxane	10.	49.379	11.39	1,2-benzenedicarboxylic acid, diethyl ester;
3.	21.708	5.42	2-ethyl -1-hexanol	11.	50.928	21.64	Unknown
4.	28.948	2.02	Decamethylcyclopentasiloxane	12.	52.004	2.68	Unknown

5.	37.553	4.08	Dodecamethylcyclohexasiloxane	13.	52.306	1.33	Unknown
6.	41.243	0.80	Unknown	14.	58.00	1.87	Unknown
7.	41.736	0.74	Unknown	15.	60.493	19.07	4-octadecyl morpholine
8.	45.182	3.62	Tetradecamethylcycloheptasiloxane	16.	65.973	0.78	Unknown

Table 3. The volatile constituents of FCD ethanolic extract

No.	RT	%	COMPOUNDS	No.	RT	%	COMPOUNDS
1.	36.668	0.21	Unknown	35.	52.850	0.23	Unknown
2.	39.675	0.42	Unknown	36.	53.376	0.81	Unknown
3.	42.240	0.14	Unknown	37.	53.455	0.31	Unknown
4.	42.381	2.79	2-dodecanol	38.	53.822	1.92	Unknown
5.	42.675	1.85	Tetradecane	39.	54.435	0.22	Unknown
6.	42.910	0.34	Unknown	40.	54.690	0.48	Unknown
7.	44.232	0.29	Methyl 4-cyanothiochroman-trans-3-carboxylate	41.	54.975	1.60	Unknown
8.	44.753	0.21	Unknown	42.	55.379	0.67	Unknown
9.	45.447	0.29	Unknown	43.	55.525	0.18	Unknown
10.	45.598	0.18	Unknown	44.	56.399	0.59	Unknown
11.	46.790	0.14	Unknown	45.	56.555	0.35	Bis –(3,5,5-trimethylhexyl)ether
12.	47.968	0.30	Unknown	46.	56.862	0.75	Unknown
13.	48.289	1.27	1-(2-hydroxyethoxy)tridecane	47.	56.929	0.54	Unknown
14.	48.532	1.70	Unknown	48.	57.022	1.20	1,1'-oxybis dodecane,
15.	48.624	0.64	1-tetradecene	49.	57.452	1.65	Unknown
16.	48.840	0.53	Unknown	50.	57.625	0.72	Unknown
17.	48.879	0.33	Bis-(3,5,5-trimethylhexyl)ether	51.	57.666	0.59	1,1,3-trimethyl cyclopentane
18.	49.200	2.18	Unknown	52.	57.900	0.68	Unknown
19.	49.334	2.15	Unknown	53.	58.240	0.24	Unknown
20.	49.519	0.46	Unknown	54.	58.372	0.43	Unknown
21.	49.683	1.77	Unknown	55.	58.615	2.00	Unknown
22.	49.954	4.70	Unknown	56.	58.755	0.35	Unknown
23.	50.091	17.47	1-nonadecene	57.	58.844	1.01	Unknown
24.	50.334	6.20	n-octadecane	58.	58.888	1.11	Unknown
25.	50.604	3.67	Unknown	59.	59.500	1.52	Unknown
26.	50.860	0.15	Unknown	60.	60.002	3.52	Unknown
27.	50.892	0.11	Unknown	61.	60.245	9.78	13-tetradecen-1-ol acetate
28.	51.009	1.74	Unknown	62.	60.685	2.29	Unknown
29.	51.160	0.15	Unknown	63.	61.252	1.79	Unknown
30.	51.242	0.14	Unknown	64.	62.486	0.47	2-hydroxy-16-cyani-hexadecane
31.	51.296	0.12	Unknown	65.	63.153	0.58	Unknown
32.	51.321	0.19	Unknown	66.	63.532	0.37	Unknown
33.	52.071	0.48	Unknown	67.	63.854	0.22	Unknown
34.	52.783	1.08	Unknown	68.	64.180	0.20	1-octadecanol
No.	**RT**	**%**	**COMPOUNDS**	**No.**	**RT**	**%**	**COMPOUNDS**
1.	38.985	37.58	2,4-diisocyanato-1-methylbenzene	4.	50.244	34.84	Unknown
2.	41.656	7.23	Unknown	5.	50.403	16.35	Unknown
3.	41.702	4.00	Unknown				

5. Discussion

Plant secondary metabolites are organic compounds produced by plant that play roles in defense system, anti-microbial, antioxidant, nitrogenous storage, attractant to pollinators and repellent against plant herbivores [14]. The common organic compounds synthesize by plant are; alkaloids, amines, cyanogenic glucosides, glucosinolates, non-protein amino acids, organic acids, terpenoids, phenolics, quinones, polyacetylenes and peptides.

Alkaloid is one of the bioactive compounds commonly found in plants. Goyal [15] listed the role of alkaloids in plant as: (1) end products of metabolism, (2) source of nitrogen, (3) plant defence, (4) growth regulators and (5) alternative source of minerals in plant. The presence of white precipitate in the test conducted indicates *R. kerri* central disc part contained alkaloids. The similar results also reported by Sofiyanti et al. [16] for *R. hasseltii*, where two alkaloid compounds (nicotine – pyrrolidine group and caffeine – purine group) detected. The presence of this compound possibly influences the behaviour of insect toward the flower.

Triterpenoids/ steroids is another chemical compounds found in plants. This natural product was recognized to have potential as sex hormone, anti-tumor, plant growth hormone regulator and antibacterial properties [17].

Flavonoids are polyphenolic structures that found in all plant parts [18, 19]. The weak transparent yellow from the test conducted indicates the central disc part containing flavonoids. Sofiyanti et al. [16] listed three phenolic compounds (catechin, proanthocyanidin and phenolic acid) identified from *R. hasseltii* where proanthocyanidin (anthocyanidin) believed responsible in *Rafflesia* visual display, attracting pollinators to approach the flower. This statement supported by Wink [14] and Griesbach [20], explained the presence of flavonoids, anthocyanins and carotenoids believed to give colour to the flower whereas terpenoids, amines and phenylpropanoids contribute to scent mixtures luring pollinators to visit the flower and thus assist in pollination process.

Tannins are polyphenolic compounds with the presence of sufficient hydroxyls and other suitable group (e.g carboxyl) to create strong complexes with proteins and other macromolecules. Tannins can be sub-divided into two groups known as hydrolysable tannins and condensed tannins [21]. The test conducted exhibited strong blue precipitate indicates the extracts contained hydrolysable tannins. Hydrolysable tannins are derived from simple phenolic groups such as gallotannins – gallic acid or ellagitannins – ellagic acid. Previous studied by Kanchanapoom et al. [22] identified four tannin compounds together with phenylpropanoid glucoside in *R.kerri*. Tannins play roles in plant defense activities by; 1) act as anti-microbial against plant pathogen Schultz et al. [23] and the astringent taste of tannins help in feeding repellents – herbivores [24].

Plants emit variety of volatile organic compounds (VOCs) as response to the biotic and abiotic stresses. The VOCs are released by various plant organs such as, flower, fruit, leaves, and root [25, 26]. The VOCs have chemical properties; low molecular weight, high vapour pressure and low boiling point. The diverse VOCs can be grouped into isoprene, terpenoids, phenylpropanoids/benzenoids, fatty acid, amino acid derivatives, alcohols, alkanes, alkenes, and esters [27, 28, 29]. All of these VOCs has various roles and were likely to influence the chemical activity of the plant. Floral volatile constituents (FVCs) play a crucial role in pollination biology by emitting a strong scent, luring pollinators to visit the flower [30]. Other than serve as attractant, the FVCs released serve in defense system against the herbivores and other plant pathogens includes bacteria, virus and fungi.

Long chain hydrocarbons is one of the groups detected from GC-MS chromatogram, which includes alkanes (Tetradecane, 1-(2-hydroxyethoxy)tridecane, n-Octadecane, 1,1'-oxybis dodecane, 1,1,3-trimethyl cyclopentane and 2-hydroxy-16-cyani-hexadecane) and alkenes (1-tetradecene and 1-nonadecene). Ayasse et al. [31] reported the long chain hydrocarbons (alkanes and alkenes) are the common compounds found in plant and insect and play role as male-sex pheromones in many species. Doi et al. [32] found alkenes as the chemical signal used by many flies species and Schiestl and Cozzolino [33] reported the flower that produced alkenes responsible in the attraction of the pollinator behaviour through false host or mating signal. Thus, the composition of floral scent with the presence

of long chain hydrocarbon group believed to attract pollinators and other visitors to locate the *Rafflesia* and increase the pollination success rate.

Cyclic siloxanes are belongs to the organosilicon group (colourless, odourless and slightly volatile) and widely used in cosmetics, plastics, hygiene products, etc. [34]. The presence of this compound in the extract believed to give the viscous resin-like characteristic to the extract. Yugandhar and Savithramma [35] identified the listed organosilicon compounds (Table 2) in the medicinal plant *Syzygium alternifolin* stem bark extract except Tetradecamethylcycloheptasiloxane. Wang et al. [36] explained Hexamethylcyclotrisiloxane was used in liquid silicones preparation and as chemical ingredients in lotions, fragrances and skin care products, whereas the Octamethylcyclotetrasiloxane was found to have mild estrogenic activity in mice and also was used in industrial silicone polymers and personal care appliances [37]. Tetradecamethylcycloheptasiloxane (oxygenated monoterpens) is a major compound identified in *Mesembryanthemum edule* (L.) essential oil [38], where this compounds believed to be valuable than the monoterpene hydrocarbons due to contribution in the fragrance of the essential oil [39].

The presence of 2-ethyl-1-hexanol, 2-dodecanol and 1-octadecanol were grouped into primary alcohol. 2-ethy-1-hexanol is mild, oily, sweet, and has slightly rose floral odor [40]. The VCs of 2-ethyl-1-hexanol also was identified in other Rafflesiaceae genus: *Rhizanthes lowii* [41] where these two genera attracting the same calliphorid flies (*Lucilia, Chrysomya* and *Hypopygiopsis* [2, 9, 42, 43]. Besides, 1-octadecanol identified in the extract of the Lamiaceae; *Micromeria kosaninii* [44] and also from the hairpencil glands of males of *Heliothis virescens* (F.) – Lepidoptera [45]. The presence of this constituent not only could be found in the plant extract but also from the insect gland (receptor), which can explain to us of how plant-insect communicate and able to locate the host plant.

Aromatic acid includes; 1,2-benzenedicarboxylic acid, diethyl ester, Bis-(3,5,5-trimethylhexyl)ether, 2,4- diisocyanato-1-methylbenzene, 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol and 13-tetradece-1-ol acetate. 1,2-benzenedicarboxylic acid, diethyl ester is one of the derivatives of benzoic acid which has very slight and aromatic odour. This constituent was identified in apricots and plums [46]. This finding support by Banzinger [2] statement, when he explained the weaker fruity fragrance (dried apricots and peaches) comes from the tube cavity. Besides, 2,4-diisocyanato-1-methylbenzene is a chemical with sweet, fruity and pungent odour and was found in the faeces, urine and tissue (carcass, gastrointestinal tract content) [47], which possibly contribute in attracting the carrion fly to visit *Rafflesia*. 2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl) phenol is one of the predominant constituents emitted by *Muscodor kashayum* give rise to the pungent smell and act as anti-microbial [48]. Besides, Williams et al. [49] discovered 13-tetradece-1-ol acetate constituent as sex pheromones to attract the male click beetle *Melanotus communis* (Coleoptera). The presence of this constituents blend in with other compounds was likely being the reason for other visitors to visit the *Rafflesia*.

1-acetoxy-6a-methyl-1,1a-dihydrocycloprop[a]inden-6(6aH)-one, 4-octadecyl morpholine and Methyl 4-cyanothiochroman-trans-3-carboxylate were group into miscellaneous compound. The information regarding all of these VCs were limited and thus need a further study.

6. Conclusions

This research has found both of the extract to contain alkaloids, tannins, triterpenoids and flavonoids as plant secondary metabolites, which was likely to contribute in pollination biology of *Rafflesia*. The volatile constituents of MCD and FCD extracts had combinations of different chemical constituents from different chemical classes. It can be concluded the mixture of the volatiles constituents emitted by *Rafflesia* influenced the species of pollinators and visitors visiting the flower. More research activities recommended to be conducted in completing this study such as; 1) identification of collected insect specimens into species level, 2) identification of the unknown volatile constituents, and 3) field test to determine which volatile constituents responsible in attracting the pollinators.

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