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Comparative analysis reveals distinct floral scent patterns in three *Durio* species

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Abstract. *Mae CY, Shaipulah NFM, Zalipah MN. 2025. Comparative analysis reveals distinct floral scent patterns in three* Durio *species. Biodiversitas 26: 3136-3143.* The floral scents of three durian species were collected using the dynamic headspace method and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). A total of 52 volatile compounds from nine different chemical classes were detected from *Durio zibethinus*, *D. lowianus*, and *D. graveolens* flowers. The floral profiles of *D. zibethinus* were dominated by alkanes and sesquiterpenes, whereas those of *D. lowianus* and *D. graveolens* were dominated by triterpenes and sesquiterpenes, respectively. The hexane eluates extracted more sesquiterpenes than hexane:ethyl acetate eluates. In *D. zibethinus* flowers, squalene was identified as the predominant volatile compound in hexane extracts, while β-caryophyllene dominated in hexane:ethyl acetate extracts. Squalene was the primary volatile component in hexane extracts of *D. lowianus* flowers, whereas (E,E)-α-farnesene predominated in the hexane:ethyl acetate fraction. (E,E)-α-farnesene was the dominant volatile compound emitted from *D. graveolens* flowers, prevailing in both hexane and hexane:ethyl acetate extracts. Sulfur-containing compounds were absent from all three species. The diverse terpene composition, bright red petals, and extended flowering period of *D. lowianus* suggest a generalist strategy targeting multiple pollinator groups across both day and night. This study revealed different pollination strategies among three durian species through their distinct floral volatile profiles, highlighting the intricate and multifaceted nature of plant-pollinator relationships in tropical ecosystems.

Keywords: Durian, GC-MS, plant-pollinator relationship, tropical, volatile organic compounds

Abbreviations: GC-MS: Gas Chromatography-Mass Spectrometry; VOCs: Volatile Organic Compounds

INTRODUCTION

The genus *Durio* is native to Southeast Asia, including Malaysia. Durian trees thrive in tropical climates with annual rainfall exceeding 2000 mm (Ketsa et al. 2020). There are approximately 29 species in the genus *Durio*, with at least 23 species native to Malaysia (Salma 2011). Due to the distinct shape and aroma of the durian fruit, it has become popular globally as an edible fruit. Nine durian species are found to produce edible fruits, namely *Durio dulcis* Becc., *D. grandiflorus* (Mast.) Kosterm. & Soegeng, *D. graveolens* Becc., *D. kinabaluensis* Kosterm. & Soegeng, *D. kutejensis* (Hassk.) Becc., *D. lowianus* Scort. ex King, *D. oxleyanus* Griff., *D. testudinarum* Becc., and *D. zibethinus* Murray (Ketsa et al. 2020). However, only *D. zibethinus* fruits were domesticated and cultivated commercially, with over a hundred cultivars recognized in Malaysia (Department of Agriculture 2021). *Durio zibethinus* flowering is influenced by specific environmental conditions, requiring annual rainfall followed by a brief dry period (Ketsa et al. 2020). The flowering season typically lasts two to three months, during which individual flowers take one to two months to develop fully. Inflorescences occur in clusters of three to 30 flowers, exhibiting nocturnal blooming patterns. Flower opening begins in the late afternoon and completes around 07:00 pm, with anthesis lasting less than 24 hours (Ng et al. 2020). Following anthesis, flowers typically abscise the next morning, leaving only the stigma if pollination was successful. Despite the abundance of flowers, only one to two per inflorescence typically develop into fruits after pollination. This extended flowering phase, coupled with low fruit set, presents an opportunity to investigate floral traits and their potential influence on pollination success.

Durio zibethinus flowers are dichogamous, in which stamens and pistils of the hermaphrodite flower mature at different times, preventing self-pollination (Ng et al. 2020). Various studies indicated that self-pollinated flowers were not able to produce successful mature fruit sets (Wayo 2018). Wind pollination was considered not ideal due to sticky and clumped pollen. Thus, the flowers require biotic pollination, where flowers employ various strategies to attract floral visitors, including petal color, floral scent, and nectar content. The large, creamy white petals, strong scent, and evening anthesis period of *D. zibethinus* flowers strongly suggest that nocturnal floral visitors are likely the primary pollinators of these flowers (Ng et al. 2020). Nectarivorous bats such as the cave nectar bat (*Eonycteris spelaea* (Dobson, 1871)) and frugivorous bats such as the island flying fox (*Pteropus hypomelanus* (Temminck, 1853)) and lesser short-nosed fruit bat (*Cynopterus brachyotis* (Müller, 1838)) have been observed visiting durian flowers and subsequently pollinating the flowers (Aziz et al. 2017; Low et al. 2021). The distinct and pungent aroma of durian flowers suggests that floral scent plays a crucial role in attracting the pollinators. Bat-pollinated flowers are typically associated with unpleasant smells, due to the presence of sulfur-containing compounds (Farré-Armengol et al. 2020).

Volatile Organic Compounds (VOCs) are naturally occurring chemicals that are present in the atmosphere as they are released as gases during metabolic activities in living organisms. In plants, VOCs are derived from primary metabolic processes and are often classified as secondary metabolites due to their role in ecological interactions rather than direct growth or reproduction (Picazo-Aragonés et al. 2020). VOCs are emitted from leaves, roots, fruits, and flowers, serving as a key medium for communication within and between species. Among these, floral volatile organic compounds hold significant ecological importance, particularly in mediating plant-pollinator interactions. By attracting specific pollinators, these compounds play a critical role in facilitating the sexual reproduction of plants, ensuring genetic diversity and species survival (Murray et al. 2024).

Although there is much literature on VOC emissions of the durian fruit (Hasmadi et al. 2021; Sujang et al. 2023), the specific compounds emitted by durian flowers and their role in pollinator interactions remain poorly understood. Understanding the precise chemical cues involved in this interaction is essential to fully elucidate the pollination dynamics of durian species. This study, therefore, aimed to analyze and compare the floral VOCs emitted by three edible durian species, *Durio zibethinus*, *D. lowianus*, and *D. graveolens*. By identifying these compounds, this research seeks to uncover the specific chemical mechanisms that facilitate the attraction and interaction of bats with durian flowers, contributing valuable insights into the reproductive ecology of these economically and culturally significant plants.

MATERIALS AND METHODS

Plant species and sampling site

Flower samples were collected from the Malaysian Research and Development Institute (MARDI), Jerangau Station (4°57'22"N, 103°10'43"E), located in Terengganu, Peninsular Malaysia. MARDI Jerangau cultivates eight different species of durian trees distributed across the entire orchard, approximately 400 hectares of land. Among the eight species, three species of durian flowers were selected for this study, namely *D. zibethinus*, *D. lowianus,* and *D. graveolens* (Figure 1). Durian flowers are complete flowers, with all four whorls present within an individual. The inflorescence of *D. zibethinus* is often found in small clusters containing one to 20 flowers. Each flower features five white petals and contains densely filled white filaments and anthers. The inflorescence of *D. zibethinus* is nocturnal, in which the flower begins to bloom at approximately 03:00 pm and reaches peak anthesis at 07:00 pm. The inflorescences of *D. lowianus* are found tightly packed in large clusters containing 20 to 50 flowers. The flowers comprise five bright red petals. Similar to *D. zibethinus* flowers, filaments are dense within an individual flower. The flowers of *D. graveolens* are found on short pedicles, which are closely attached to the trunk or lateral branches. Clusters are small, containing one to ten flowers. *D. graveolens* flowers exhibit a pentamerous white corolla. The floral morphology of *D. graveolens* differs from *D. zibethinus* by its elongated, sparse filaments compared to the dense filaments of the latter. Female receptivity begins as early as 02:00 pm, followed by male receptivity several hours later (Ng et al. 2020). Both female and male receptivity end the following morning. Anthesis in *D. lowianus* flowers initiates at approximately 02:00 pm and concludes by 10:00 am the following day.

Procedures

Volatile organic compounds collection

Flower samples were collected prior to the beginning of anthesis for all three species. Flower buds with splitting at the epicalyx but not yet beginning to bloom were selected for this study. A total of six flowers per species were collected; two flowers from three different trees. All flower samples were collected from 02:00 pm to 04:00 pm. The unopened flowers selected were cut at the pedicle approximately 1 cm from the epicalyx of the flower buds and immediately placed into a 10-mL glass vial containing 0.1% sucrose solution, and then brought to the lab for the collection of volatile organic compounds. Two flowers at the beginning of anthesis per species were placed in a desiccator. The collection of the volatiles was conducted using the push-pull headspace system method as described in Shaipulah et al. (2016). The opening of the glass vial was enclosed with a layer of aluminum foil to prevent volatile compounds from the sucrose solution from escaping into the headspace. For control, volatiles were collected from completely enclosed glass vials containing 0.1% sucrose solution. The volatiles in the headspace of the flowers were collected by trapping the air on 150 mg Tenax® TA from 07:00 pm until 07:00 am the following morning. Volatile collection was maintained at 24-25°C in darkness throughout 12 hours. The floral volatiles were extracted by eluting Tenax® TA with 1 mL hexane or hexane: ethyl acetate (80:20) containing 50 pg/µL of benzyl acetate as the internal standard. Three independent replicates were performed for each species with each solvent condition, hexane and hexane: ethyl acetate.

Figure 1. Flowers of: A. *Durio zibethinus*, B. *Durio lowianus*, C. *Durio graveolens*

Gas Chromatography-Mass Spectrometer (GC-MS) and data analysis

The volatiles of the flowers were analyzed using a SHIMADZU QP2010 Ultra Gas Chromatograph-Mass Spectrometer. Splitless injection of a 1 μL sample was carried out at 50°C, with the injector pot directly heated to 300°C at a rate of 240°C min^{-1}. The gas chromatographic parameters were as follows: the initial temperature was fixed at 50°C for 1 min, then increased at a rate of 5°C min^{-1} to 300°C, then further increased to 320°C at 5°C min^{-1}. Compounds were separated on a SHIMADZU SH-5 capillary (30 m × 0.25 mm I.D. × 0.25 μm film thickness) with Helium as carrier gas at a flow rate of 1 mL/min. The MS scan range was set at 50-600 m/z; a 70 eV n-alkane standard, C_7-C_{30} (Sigma-Aldrich), was used and run using the same parameters described above prior to GC-MS analysis.

Data analysis

The compounds were identified using the National Institute of Standards and Technology (NIST) Library database and retention indices. The retention indices were calculated using the method as described in Van Den Dool and Kratz (1963). Experimental Retention Indices (RI) were compared with reported RI in NIST (https://webbook.nist.gov/chemistry/). The peak area of the samples was normalized using the peak area of benzyl acetate. The volatile composition was expressed as the percentage of peak area relative to the total peak area of each compound.

RESULTS AND DISCUSSION

Species-specific volatile profiles

A total of 50 major volatile compounds were identified from all three durian species using the dynamic headspace method and analyzed with Gas Chromatography-Mass Spectrometry (GC-MS), as shown in Table 1. *Durio zibethinus* flowers were detected to emit 11 volatile compounds each in the hexane and hexane:ethyl acetate extracts, respectively. Of these, only four volatile compounds, 1-dodecene, 1-tetradecene, caryophyllene, and humulene, were identified in both hexane and hexane:ethyl acetate extracts. Squalene was found to be the highest emitted volatile in hexane extracts, accounting for 23.84% of the total emissions. In contrast, caryophyllene was identified as the predominant volatile compound in the hexane:ethyl acetate extract, comprising 47.34% of the total emissions. The selective extraction of squalene in hexane, but not in the hexane:ethyl acetate mixture, can be attributed to the increased polarity of the latter solvent system, which reduces its efficiency in extracting non-polar compounds (Ramli et al. 2018; Ardhyni et al. 2022). Flowers of *D. lowianus* emitted 25 volatile compounds; 11 and 19 compounds were isolated in the hexane and hexane:ethyl acetate extracts, respectively. Five volatile compounds, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene, (E,E)-α-farnesene, cetene, and 1-octadecene, were identified from both extracts. Similar to *D. zibethinus* flowers, squalene represented the highest emission in the hexane extract, accounting for 41.06%. (E,E)-α-farnesene exhibited the highest emission, representing 28.97% of the peak area in the hexane:ethyl acetate solvent. The flowers of *D. graveolens* emitted 34 volatile compounds, the highest number of volatile components detected compared to *D. zibethinus* and *D. lowianus*. Out of the 34 volatile compounds, 22 and 21 compounds were detected in the hexane and the hexane:ethyl acetate extracts, respectively. Twelve compounds, 2,2,6-trimethyloctane, 1-decene, 1-dodecene, 1-tetradecene, β-ylangene, γ-muurolene, (Z,E)-α-farnesene, (E,E)-α-farnesene, (E)-γ-bisabolene, (E)-β-farnesene epoxide, 1-octadecene, and squalene, were identified from both extracts. For *D. graveolens* flowers, the sesquiterpene (E,E)-α-farnesene was the predominant compound with the highest emission identified in both hexane and hexane:ethyl acetate solvents, with a peak area of 83.34% and 91.20%, respectively. Other volatiles emitted by *D. graveolens* flowers were represented as having less than a 3.00% peak area.

Chemical analysis revealed species-specific Volatile Organic Compound (VOC) profiles in *Durio* flowers. Seven, nine, and 13 compounds were detected being emitted by only *D. zibethinus, D. lowianus,* and *D. graveolens* flowers, respectively. *Durio zibethinus* flowers exhibited unique emissions of octadecane, (9E)-9-hexadecen-1-ol, β-myrcene, 3,7-dimethylnonane, eucalyptol, and E-14-hexadecenal. The floral scent profile of *Durio lowianus* was distinguished by the presence of nonadecane, acetic acid n-octadecyl ester, α-ethylcaproaldehyde, benzaldehyde, linalool, 7-epi-sesquithujene, tetradecane, pentadecane, and 1-eicosane. The flower of *Durio graveolens* was uniquely composed of anisole, germacrene D, trans-farnesal, eicosane, heneicosane, tricosane, benzyl salicylate, β-ylangene, γ-muurolene, (Z,E)-α-farnesene, (E)-γ-bisabolene, and (E)-β-farnesene epoxide.

Chemical classification and compositional analysis

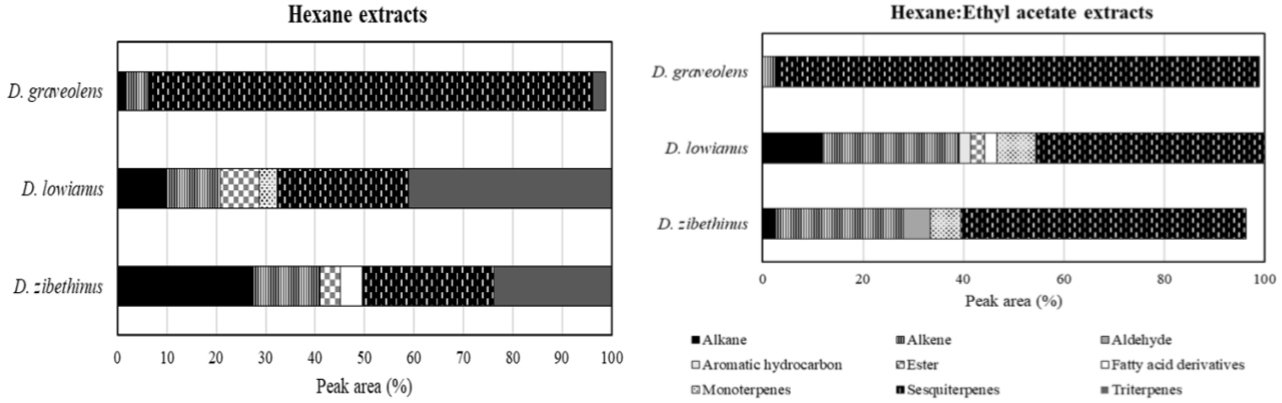
Volatile compounds profiles from *Durio* spp. flowers comprise nine distinct chemical classes, with aliphatic hydrocarbons and terpenes representing the predominant classes (Figure 2). Terpene volatiles, comprising monoterpenoids, sesquiterpenes, and triterpenes, constituted the majority of the peak area across all three *Durio* species examined. In hexane eluates, sesquiterpenes dominated the floral volatile profiles, accounting for 26.6%, 26.6%, and 90.2% of the total peak area in *D. zibethinus, D. lowianus*, and *D. graveolens*, respectively (Figure 2.A). Elution with a hexane:ethyl acetate mixture significantly altered the sesquiterpene proportions, particularly in *D. zibethinus* and *D. lowianus*. The sesquiterpene fraction increased 56.8% in *D. zibethinus* and 45.7% in *D. lowianus*, representing around a 2-fold increase for both species, while *D. graveolens* exhibited a slight rise to 95.8% (Figure 2.B). This enhanced extraction can be attributed to the moderate polarity of ethyl acetate and its hydrogen bonding capabilities, which better facilitate the extraction of slightly polar terpenoids containing oxygen-functional groups compared to hexane alone (Jiang et al. 2016). The floral emissions of *D. graveolens* exhibited a highly specialized volatile profile, with a single compound, (E,E)-α-farnesene, contributing to more than 80% of the total sesquiterpene peak area. Furthermore, triterpenes were solely detected in hexane eluates (Figure 2.A), which suggests a solvent-dependent elution pattern for this class of compounds.

Table 1. Major volatile compounds identified from flowers of *Durio zibethinus*, *D. lowianus,* and *D. graveolens* (n = 3)

Compounds	RT	RI	RI_{ref}	Hexane (peak area in %)			Hexane:ethyl acetate (peak area in %)			Class of compound
				DZ	DL	DG	DZ	DL	DG	
Anisole	6.47	920	920			0.31				Aromatic hydrocarbon
α-ethylcaproaldehyde	7.61	963	959					2.43		Fatty acid
2,2,6-trimethyloctane	7.718	966	964			trace	1.38	3.02	trace	Alkane
Benzaldehyde	7.92	973	975					2.16		Aromatic hydrocarbon
2,2,4,6,6-pentamethylheptane	8.55	993	995					1.62	trace	Alkane
β-myrcene	8.56	994	993				3.15			Monoterpene
1-decene	8.61	995	992			0.35	1.91	4.24	trace	Alkene
Ethyl hexanoate	8.82	1002	1002						trace	Fatty acid
3,7-dimethylnonane	9.793	1037	1042				1.00			Alkane
Eucalyptol	9.858	1040	1035				1.14			Monoterpene
Linalool	11.81	1101	1101					2.08		Monoterpene
Nonanal	11.98	1107	1105						trace	Aldehyde
(E)-4,8-dimethyl-1,3,7-nonatriene	12.20	1115	1105		3.65		1.41	5.59		Monoterpene
1-dodecene	14.56	1192	1192	5.45		1.25	9.68		0.79	Alkene
7-epi-sesquithujene	20.26	1389	1406					3.14		Sesquiterpene
1-tetradecene	20.35	1392	1392	4.77		1.61	12.31		0.97	Alkene
Tetradecane	20.57	1399	1400					3.02		Alkane
β-ylangene	21.18	1423	1423			0.50			0.24	Sesquiterpene
Caryophyllene	21.26	1425	1417	21.66	12.21		47.34	3.79		Sesquiterpene
γ-muurolene	21.51	1436	1435			0.45			0.20	Sesquiterpene
Cis- β -farnesene	21.99	1454	1452						trace	Sesquiterpene
Humulene	22.23	1463	1460	4.95	3.24		9.42			Sesquiterpene
Germacrene D	22.87	1486	1486			0.38				Sesquiterpene
(Z,E)-α-farnesene	22.99	1491	1491			0.28			0.39	Sesquiterpene
Pentadecane	23.24	1499	1500					4.35		Alkane
(E,E)-α-farnesene	23.36	1504	1506		11.19	83.34		28.97	91.20	Sesquiterpene
β-himachalene	23.64	1515	1508						trace	Sesquiterpene
δ-cadinene	23.79	1522	1522			2.42		2.03		Sesquiterpene
Nerolidol	24.82	1563	1565					2.27	0.29	Sesquiterpene
Cetene	25.58	1592	1593		6.77			13.82	0.65	Alkene
(E)-γ-bisabolene	25.67	1595	1577			0.32			0.58	Sesquiterpene
(E)-β-farnesene epoxide	26.18	1617	1624			1.54			2.91	Sesquiterpene
trans-farnesol	28.54	1716	1720			0.62		5.50		Sesquiterpene
trans-farnesal	29.06	1739	1738			0.30				Sesquiterpene
E-14-hexadecenal	30.27	1792	1808				5.00			Aldehyde
1-octadecene	30.30	1793	1794	3.53	4.00	0.58		6.72	0.32	Alkene
Octadecane	30.44	1799	1800	3.35						Alkane
Diisobutyl phthalate	31.78	1861	1865	4.09	2.90	0.30				Ester
(9E)-9-hexadecen-1-ol	31.85	1865	1862	4.41						Fatty acid
Benzyl salicylate	32.14	1878	1877						0.25	Aromatic hydrocarbon

Nonadecane	32.60	1899	1900		3.39					Alkane
1-eicosene	34.53	1992	1993				2.41			Alkene
Eicosane	34.66	1998	2000			0.37				Alkane
Heneicosane	36.63	2098	2100			0.37				Alkane
Docosane	38.54	2199	2200	5.98	6.58	0.44				Alkane
Acetic acid n-octadecyl ester	38.68	2207	2211		5.02					Ester
Tricosane	40.33	2298	2300			0.54				Alkane
Bis(2-ethylhexyl) adipate	41.87	2386	2390				2.84		trace	Ester
Pentacosane	43.77	2499	2500	17.97						Alkane
Squalene	48.63	2793	2809	23.84	41.06	2.54			trace	Triterpene

Note: RT: Retention Time, RI: Retention Index, RIref: Retention Index in reference, DT: *Durio zibethinus*, DL: *Durio lowianus*, DG: *Durio graveolens*



A B

Figure 2. Chemical classes of volatile compounds in the: A. Hexane extracts, and B. hexane:ethyl acetate extracts of *Durio zibethinus*, *Durio lowianus*, and *Durio graveolens* (n = 3)

Aliphatic hydrocarbon volatiles, comprising alkanes and alkenes, were present in abundance after terpenes, specifically in hexane eluates of *D. zibethinus* and *D. lowianus* flowers. In hexane eluates, alkanes accounted for 27.3%, 9.97%, and 1.72% of the peak area in *D. zibethinus*, *D. lowianus*, and *D. graveolens*, respectively (Figure 2.A). Elution with a hexane:ethyl acetate mixture yielded contrasting results for alkane composition, with *D. zibethinus* exhibiting a substantial reduction to 2.38% of the total peak area, whereas *D. lowianus* showed an increase to 12.01% (Figure 2.B). Alkanes were detected in trace amounts, comprising less than 1% of the total peak area in hexane eluates of *D. graveolens* floral volatiles, but were completely absent in the corresponding hexane:ethyl acetate solvent mixture. Besides that, alkenes accounted for 13.75% of the peak area in *D. zibethinus*, 10.77% and 3.79% in *D. lowianus*, and *D. graveolens*, respectively, in hexane eluates (Figure 2.A). In the hexane:ethyl acetate mixture, both *D. zibethinus* and *D. lowianus* showed an increase in peak areas, to 23.9% and 27.19%, while *D. graveolens* exhibited a slight decrease to 2.73% (Figure 2.B).

In the hexane eluates, fatty acid derivative volatiles constituted a small percentage of the peak area only in *D. zibethinus*. The fatty acid compound was identified to be the source of a single fatty alcohol, (9E)-9-hexadecen-1-ol, which accounted for 4.41% of the total peak area (Figure 2.A). Similarly, a single fatty aldehyde volatile was detected in only *D. lowianus*, accounting for only 2.43% of the total peak area in the hexane:ethyl acetate eluates (Figure 2.B). Aromatic hydrocarbons were present in the flowers of *D. lowianus* and *D. graveolens*. In the hexane eluates, anisole contributed only 0.31% of the peak area in *D. graveolens* flower emission (Figure 2.A). Analysis of the hexane:ethyl acetate eluates revealed distinct aromatic compounds, with benzaldehyde constituting 2.16% of the total peak area in *D. lowianus*, while benzyl salicylate comprised 0.25% in *D. graveolens* (Figure 2.B).

Discussion

Species-specific pollination strategies and volatile profiles

The terpene volatile bouquet in hexane:ethyl acetate extracts reveal distinct pollinator attraction strategies among the *Durio* species. *D. lowianus* exhibits the most diverse terpene profile, characterized by a blend of (E,E)-α-farnesene, linalool, and other terpenes. Although two pteropodid bats*, Cynopterus brachyotis* and *Eonycteris spelaea,* were identified as pollinators of *D. lowianus* in the study area (Low et al. 2021), the mixed bouquet, particularly the presence of linalool, suggests adaptation to multiple pollinator groups. Various bee species, including *Andrena Lasioglossum*, *Nomada*, and *Apis cerana* (Fabricius, 1793)*,* show sensitivity to linalool (Braunschmid et al. 2017; Lukas et al. 2019). Interestingly, in *Penstemon digitalis* Nutt., *Bombus impatiens* (Cresson, 1863) prefer floral nectar with weakly scented linalool, suggesting complex selective pressures on floral scent emission in natural environments (Burdon et al. 2020). The diverse terpene composition of *D. lowianus* aligns with its morphological and temporal adaptations - bright red petals attractive to diurnal visitors and extended anthesis period beyond dawn with dehisced anthers remaining after 07:00 am hours (Unpublished data), unlike the strictly nocturnal flowering of other *Durio* species. The bright red colors of its petals suggested the attraction of diurnal birds and insects that rely on visual cues to locate flowers (Krauss et al. 2017; Chen et al. 2020; Akter et al. 2024), whereas bat pollination is indicated by nocturnal anthesis, exposed flowers through cauliflory, and strong floral scent (Muchhala et al. 2024). This combination suggests a generalist pollination strategy that maximizes reproductive success through multiple pollinator groups across both nocturnal and diurnal periods. In contrast, *D. zibethinus* exhibits a specialized volatile bouquet with β-caryophyllene as the primary compound, which potentially attracts honey bees (*A. cerana*) and specific moth species such as the European grape berry moth (Zhang 2018). *Apis cerana* was observed visiting the flowers of *D. zibethinus* between 05:00 pm and 06:00 pm (Wayo et al. 2018). Therefore, it is plausible that caryophyllene serves to attract honey bees, such as *A. cerana*, as potential pollinators. The emission of caryophyllene has also been examined in moth-pollinated flowers in the Neotropics, with a specific focus on Sphingophilous plants (Moré et al. 2021). Sphingid moths were noted feeding on *D. zibethinus* flowers during nighttime, coinciding with the flowers' blooming period (Unpublished data). *D. graveolens* exhibits a specialized volatile profile rich in (E,E)-α-farnesene, which may serve to attract specific beetle and bee pollinators. This specialization is evidenced by observations of pollen beetles and honey bees visiting *D. graveolens* flowers during the anthesis period (Ng et al. 2020). According to Li et al. (2024), there is a correlation between floral petal color and volatile emissions. White-petaled species (*D. zibethinus* and *D. graveolens*) emit high levels of single compounds attractive to nocturnal visitors, while bright red flowers like those of *D. lowianus* emit volatiles that attract diurnal visitors. This pattern of high abundance of individual compounds is often associated with nocturnal pollination syndromes (Fenske and Imaizumi 2016).

Durio spp. is commonly pollinated by Old World bats in Malaysia, including *P. hypomelanus*, *E. spelaea*, and *C. brachyotis* (Aziz et al. 2017; Low et al. 2021). The apparent absence of sulfur-containing compounds in these *Durio* species, compared to New World bat-pollinated flowers, could be due to two factors: evolutionary differences in bat pollination systems between Old and New World, where Old World bats rely less on olfactory cues (Pettersson et al. 2004), and methodological limitations, as sulfur-containing compounds are typically better extracted using diethyl ether rather than the hexane and hexane:ethyl acetate solvents used in this study. The lack of sulfur-containing volatiles might be explained by these Old-World bats feeding in less dense vegetation, thus relying less on olfactory cues (Pettersson et al. 2004). Alternatively, this could represent an intermediate evolutionary stage in the development of bat pollination systems, as suggested by previous studies (von Helversen et al. 2000; Fleming et al. 2009). Similarly, two fig species, *Ficus hispida* and *F. scortechinii*, pollinated by Old World bats in Malaysia, also did not detect sulfur-containing compounds from its flowers (Hodgkison et al. 2007). Additionally, *E. spelaea*, a crucial pollinator for economically significant crops in the Southeast Asian region (Bumrungsri et al. 2013; Acharya et al. 2015), did not display an attraction to dimethyl disulfide, a common floral bat attractant (Carter and Stewart 2015).

Extraction efficiency

The observed differences in compound distribution across extraction methods likely reflect both methodological constraints and biological variations. For instance, δ-cadinene was detected in the hexane extract of *D. graveolens* but only in the hexane:ethyl acetate extract of *D. lowianus*, decene was present in all hexane:ethyl acetate extracts while 1-but only in the hexane extract of *D. graveolens*. When ethyl acetate is incorporated into the volatile extraction process of *D. graveolens*, it may facilitate the isolation of a greater number of compounds that effectively reduce both δ-cadinene and 1-decene below detection thresholds due to competition for retention capacity within the extract matrix or analytical column. These solvent-dependent variations may also indicate differential allocation of the precursor farnesyl diphosphate (FPP) resources to various terpene synthases within these Durio species. The biosynthetic complexity varies significantly among these compounds; farnesene synthesis involves a relatively straightforward reaction from FPP, whereas δ-cadinene production requires a more complex reaction sequence including initial isomerization to nerolidyl diphosphate, followed by cyclization, 1,3-hydride shift, secondary ring closure, and deprotonation (Wang et al. 2021). As demonstrated by Tholl (2015), key enzymes upstream in the terpene biosynthetic pathway, such as 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGR), can become rate-limiting when multiple downstream synthases are active, potentially explaining the differential presence of these compounds under varying extraction conditions.

In conclusion, this study determined the volatile compounds emitted by the flowers of three durian species, with each species releasing a unique set of compounds that facilitate pollination. Even though prior research revealed durian flowers rely on bats for pollination, they do not emit sulfur-containing compounds that typically attract bat visitors. *D. lowianus* displays multiple pollination syndromes to maximize successful pollination, unlike *D. zibethinus* and *D. graveolens*, which emit a high abundance of a single compound at night. The significance of this study lies in the potential it holds for future research. There is a clear need for further investigations that should focus on in-situ volatile collection at regular intervals throughout floral anthesis to understand the rhythmic patterns of emission and their relationship with pollinator activity. Additionally, extracting volatiles with different solvents, particularly diethyl ether, which is better suited for capturing sulfur-containing compounds, could provide a more comprehensive profile of floral scents, especially those associated with bat pollination syndromes. Understanding the co-pollination systems between insects and bats would provide valuable insights into the complementary roles of different pollinators, particularly investigating the interplay between olfactory and visual signals to attract diverse pollinator groups. Comprehensive pollinator surveys would help identify whether our documented volatile compounds serve multiple pollinator groups beyond the primary bat pollinators or represent specialized adaptations to specific taxa. Such studies could reveal how selective pressures from different pollinator groups have shaped the diversification of floral colors and volatile profiles among *Durio* species, ultimately contributing to our understanding of the evolution and maintenance of these complex plant-pollinator relationships in tropical ecosystems.

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