

Secretory Cells in *Etlingera elatior* (Jack) R. M. Smith (Zingiberaceae): Morphology, Histochemistry, and Essential Oil Composition

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ABSTRACT

Etlingera elatior is a perennial, aromatic herb with attractive flavour and fragrance afforded by organic compounds that are stored and released from specialised structures; however, the secretory structures remain undefined. Thus, this study was carried out to determine the secretory cells in the leaves, inflorescences, and peduncles of *E. elatior* by using scanning electron and light microscopies. Histochemical tests were performed to localise and ascertain the nature of secretion materials, while gas chromatography-mass spectrometry was used to characterise the composition of essential oils (EO). Findings indicate the presence of a heterogeneous mixture comprising EO, mucilaginous and/or lipophilic substances in the secretory cells. A total of 50 compounds were identified in the EO, with the predominance of alcohol. The presence of several terpenic compounds (α -pinene, (*E*, *E*)- α -farnesene, (*E*)-caryophyllene, *E*- β -farnesene, (*E*)-nerolidol) suggests a potential involvement of the secretory structures in plant signalling. The widespread distribution of secretory cells throughout the plant tissues indicates adaptive features of the plant's secretory system. These cells emerged as the main secretory system of *E. elatior* that renders the EO.

Keywords: Gas chromatography-mass spectrometry, light microscopy, scanning electron microscopy, secretory cells, secondary metabolites

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INTRODUCTION

The aromatic properties of a plant are afforded by the presence of secretory structures as the site capable of synthesising, accumulating, and/or storing volatile organic compounds. The plant volatiles comprised of secondary metabolites that constitute the “essence” of the plant (Elshafie et al., 2023),

imparting not only a strategic adaptation for the plant to compensate its immobility for mediating with external cues such as in plant-herbivore and plant-pollinator interactions (Lucas-Barbosa et al., 2016) but also provide human with valuable natural plant products for multitudes of purposes, from medicine to flavours and fragrances (Lee & Ding, 2016).

Ecologically, specialised structures such as glandular trichomes, secretory cavities, and oil cells produce specific secretion products that are often tailored to fulfil the functional aspects of defence against herbivory attacks or even attracting pollinators. Their localisation in the plant body also varies with their function, consistent with their diverse structures (Watts & Kariyat, 2021). For instance, an adaptive feature exists in the structural and localisation of floral nectaries in the legume flower with their specific pollinators (Sinjushin et al., 2022). The glandular trichomes of *Etilingera elatior* true flowers release mucilage, terpenes, and phenolic compounds that are able to secure its anthesis. In contrast, the non-glandular trichomes were involved in the floral development by providing physical and mechanical protection to the flowers (Lee & Ding, 2024).

Alternatively, the mixtures of secondary metabolites offer humans as sources of medicinally important substances or compounds with high biotechnological interests. The specialised cells possess the remarkable capability to manufacture metabolites in large quantities relative to their microscopic size, thereby presenting it as a potential utility as “green factories” for molecular farming and functional targets for plant metabolic engineering (Huchelmann et al., 2017; Muthulakshmi et al., 2023). Therefore, a detailed description of the localisation, morphology and histochemistry of the secretory structures would provide not only useful information for ecological, taxonomic, and chemosystematic purposes (Cassola et al., 2019) but also a new opportunity, particularly for plant breeders to exploit the plant by modifying gland metabolism to enhance yield and improve the composition of essential oil (EO) for commercial production (D’Amelia et al., 2021).

Etilingera elatior, commonly known as torch ginger, is an aromatic herb native to Southeast Asia. The plant comprises a leafy and flowering shoot system that grows in a clump of pseudostems emerging from the rhizome. The leafy shoot can grow up to 3-4 m in height, whereas the flowering shoot comprises an inflorescence borne on a peduncle that can reach up to 1.5 m (Choon & Ding, 2017) (Figures 1A-B). In Malaysia, the inflorescence bud is a staple ingredient for food flavourings in popular local cuisines such as ‘asam laksa’, ‘nasi kerabu’ and ‘nasi ulam’ (Choon & Ding, 2016). Despite the extensive characterisation of the chemical profile of its EO (Bezerra-Silva et al., 2016; Juwita et al., 2018; Sungthong & Srichaikul, 2018), identification of the type and morphological characteristics of the secretory structures that afforded their aromatic properties remains poorly understood. Besides, the biological activities of EO derived from torch ginger have indicated their potential for various pharmacological purposes (Juwita et al., 2018). Bezerra-Silva et al. (2016) demonstrated the promising potential of the EO to deter ovipository activities against

Aedes aegypti, the vector of the dengue virus. Most recently, the EO of torch ginger was incorporated into a starch-based edible film as an active packaging to improve the quality of chicken meat during the chilled storage period (Marzlan et al., 2022).

This study aims to identify the micromorphological characteristics of the secretory structures in leaves, inflorescences, and peduncles of *E. elatior*. We also investigated the secretory structures' histochemical contents and the chemical composition of the EO.

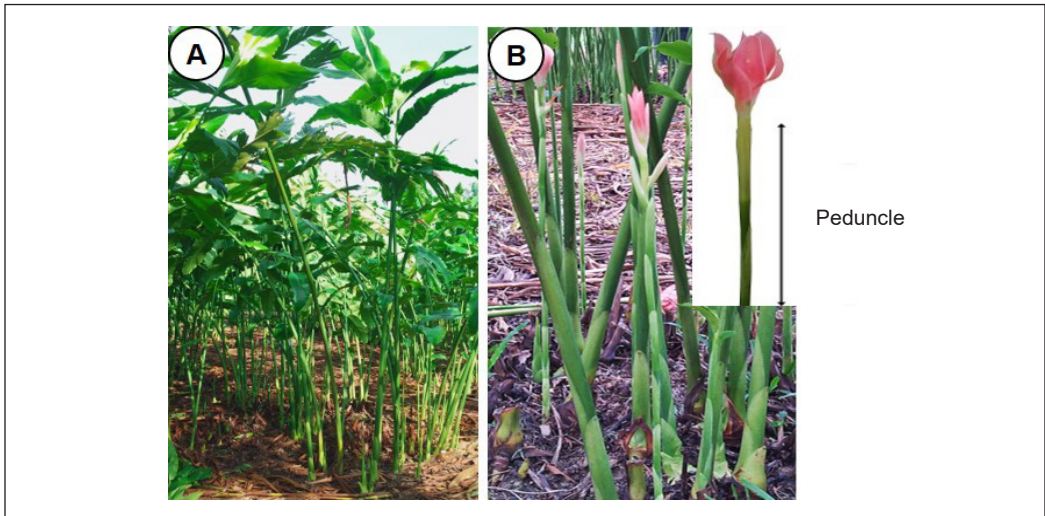


Figure 1. General view of *Etlingera elatior* plant. (A) Leafy shoot and (B) inflorescence shoot with a long peduncle

MATERIALS AND METHODS

Plant Materials

The *E. elatior* young, fully expanded leaves (Figure 2A) and inflorescence shoot at full bloom stage (Figure 2B), along with its peduncle, were collected from five-year-old plants grown in Field 2, Faculty of Agriculture, Universiti Putra Malaysia (3°00'28' N, 101°42'10' E). The sample collection was carried out between eight and nine in the morning during sampling days. The true flowers of the fully bloomed *E. elatior* inflorescence were removed to obtain its inflorescence axis (Figure 2C).

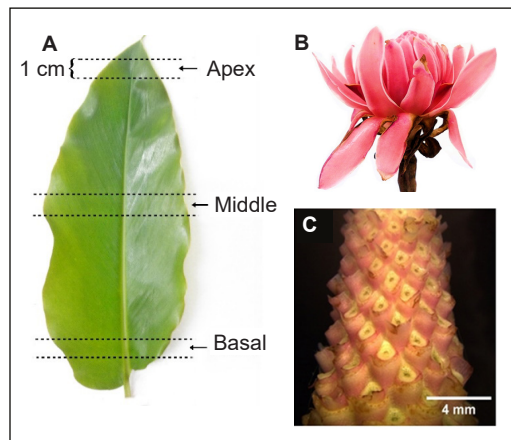


Figure 2. A young and fully expanded *Etlingera elatior* leaf lamina; (B) A full bloom of *E. elatior* inflorescence indicated by the opening of the true flowers in dark red colour (arrow); and (C) The inflorescence axis of *E. elatior* after the removal of true flowers

Scanning Electron Microscopy (SEM)

The middle part of the leaf, inflorescence axis, and peduncle were fixed in FAA (10% formaldehyde, 5% acetic acid, 50% ethanol, which were purchased from Sigma Aldrich®, Germany) for 24 hr and placed in a vacuum to remove air from the tissue as described by Lee and Ding (2024). The samples were rinsed thoroughly with distilled water and then post-fixed in 1% osmium tetroxide (Sigma Aldrich®, Germany) for 24 hr. After a series of dehydration in graded ethanol (50%, 60%, 70%, 80%, 90% and 100%), the samples were subjected to critical point drying (Leica EM CPD030, Vienna) and then sputter-coated (Bal-Tec SCD 050, Netherlands) with gold. Observations were carried out using JEOL JSM-5610V SEM (JEOL Ltd., Japan) at an accelerating voltage of 15 kV.

Histochemical Analysis

Fresh plant materials were hand-sectioned using ethanol-cleaned razor blades and then subjected to the following histochemical tests: Nadi reagent for EO and terpenes (Caissard et al., 2004), ruthenium red for mucilage and pectin (Johansen, 1940) and Sudan IV for lipids (Jensen, 1962). Distilled water and lipid removal solution of methanol, chloroform, water and chloride acid mixture (66:33:4:1) (Machado et al., 2006) were performed as positive and negative control procedures, respectively. All sections were mounted on a glass slide with a cover slip and then examined with a light microscope (Meiji Techno, Japan) equipped with a digital single-lens reflex (DSLR) camera (Olympus E-420, Japan).

EO Extraction and Chemical Analysis

The EO from fresh leaves, inflorescence, and peduncles were extracted in triplicate by hydro distillation using Dean-Stark apparatus for 4–5 hr. The yields were determined as % w/w on a fresh weight basis. Collected EO were first diluted in 1:100 HPLC grade methanol (Sigma Aldrich®, Germany) prior to analysis by gas chromatography coupled with mass spectrometry (GC-MS). The EO of 1 μ l was injected into a GCMS-QP2010 Ultra (Shimadzu Co., Japan). The chemical composition was separated on a BPX5 silica column (30 m length \times 0.25 mm internal diameter \times 0.25 μ m film thickness). The temperature conditions were programmed as follows: initial temperature of the column oven at 50°C, heated to 300°C at the rate of 3°C per minute, and then held constant at 300°C for a further 10 min. Helium was used as a carrier gas. Mass spectra were recorded with an ion source temperature of 200°C and interface temperature of 250°C. The mass scan parameters included a start time of 2.50 min and an end time of 93.0 min, and the data were collected at a to-charge ratio (m/z) between 40 and 700. Identification of the individual compounds was based on a comparison of their retention times and mass spectra with those from the National Institute of Standards and Technology (NIST) 08, Flavour and Fragrance Natural Synthetics and Compounds (FFNSC) version 1.3 and Wiley 229 registry of the mass spectral library. The

relative abundance of each compound in EO was quantified by dividing the area response of a particular peak by the absolute responses from peak areas of the total ion chromatogram.

Data Analysis

The diameter of secretory structures was measured on the digitally recorded micrographs using Image J software (National Institute of Health, Bethesda, MD, USA). The means were calculated from $n = 10$ measurements \pm SD.

RESULTS

Morphology and Distribution of the Secretory Structures

The secretory cells of *E. elatior* were scattered on the spongy mesophyll of leaves and the ground tissues of the inflorescence axis and peduncle (Figures 3A-C), each measuring an average diameter size of 27.0 ± 3.2 , 35.1 ± 1.9 and 29.4 ± 2.7 μm , respectively (Table 1). The translucence of the oil accumulating in a sac-like structure further augmented the appearance of isodiametric-shaped secretory cells that can be readily distinguished from the neighbouring cells (Figures 3B-C). Superficial observation of the secretory cells revealed that a membrane binds an extra-plasmatic space containing the secretory materials similar to a large central vacuole. Upon detailed examination, the cells consisted of tripartite cell walls: (1) outer wall, an intermediary suberised wall that enclosed the oil, and (2) inner wall with tiny protuberance identified as cupule (Figure 3D).

Table 1

Diameter of Etlingera elatior secretory cells at different plant parts

	Plant parts		
	Leaves	Inflorescence axis	Peduncle
Secretory cell diameter (μm) \pm SD	27.0 ± 3.2	35.1 ± 1.9	29.4 ± 2.7

Note. SD = Standard deviation; $n = 10$

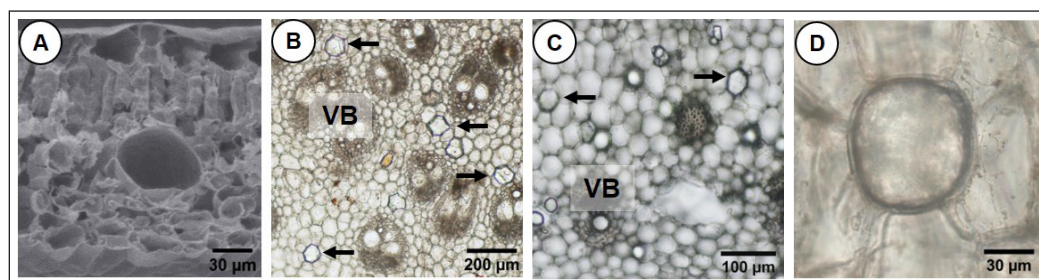


Figure 3. Secretory cells of *E. elatior*. (A) Scanning electron micrograph of leaf transverse section showing the secretory cell in spongy mesophyll. (B–C) Distribution of secretory cells (arrows) occurring alongside the vascular bundles (VB) in the (B) inflorescence axis and (C) peduncle. (D) Isodiametric shape and translucence of secretory cell accumulating secretory content in a sac-like structure

Histochemical Analysis

The secretory materials contained in the secretory cells reacted strongly with the Nadi reagent and Sudan IV tests (Figures 4A–B). The reaction produced intense colourations that indicated the presence of EO and lipophilic substances. The secretory contents, however, yielded negative reactions with Ruthenium red for mucilage. During the Sudan IV histochemical test, observation revealed that the oil droplets were liberated to the adjacent cell via an aperture identified as a cupule (Figure 4C). The paradermal section of the peduncle revealed six to eight elongated cells radially surrounding the secretory, forming a rosette (Figure 4D). Surface analysis of the peduncle showed that the secretory cells were lodged as slight protrusions on the epidermal surface (Figure 4E).

EO Yield and Chemical Composition

The EO obtained from fresh leaves yielded 0.11%, whereas the inflorescence and peduncle yielded 0.09% and 0.05%, respectively (Table 2). A total of 50 constituents were identified in the EO, accounting for 96.6%–99.3% of the total compositions. Overall, the EO from aerial parts of *E. elatior* were rich in alcohols and aldehydes. The major compounds detected in the EO were 1-dodecanol (32.1%–36.9%), n-dodecanal (10.2%–33.9%), dodecyl acetate (2.9%–9.2%) and n-tetradecanol (3.4%–5.5%).

The EO from different plant parts can be distinguished by the higher occurrences of dodecyl acetate (9.2%), (*E*)-caryophyllene (6.3%), n-tetradecanol (5.5%), 1-decanol (4.9%) and (*E*)- β -farnesene (3.6%) in the leaves compared to inflorescences and peduncle (Table 2). In the flowering shoot, the occurrences of n-dodecanol (27.4%–33.9%) and dodecanoic acid (4.8%–4.9%) were higher in the inflorescences and peduncles compared to the leaves (2.1%).

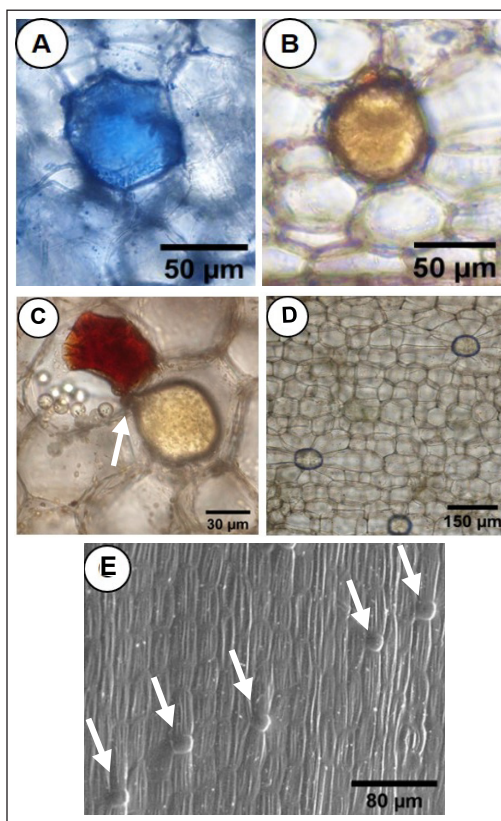


Figure 4. Histochemistry of the secretory structures. (A–B) Secretory cells tested positive for (A) essential oils and (B) lipophilic substances after strong reactions from the Nadi reagent and Sudan IV, respectively. (C) Oil droplets stained with Sudan IV exit the cell into the adjacent cell via a tiny aperture identified as a cupule (arrow). (D) The paradermal section of the peduncle shows the secretory cells surrounded by 6–8 cells arranged radially, forming a rosette. (E) Scanning electron micrograph of peduncle surface showing the secretory cells slightly lodged as protrusions (arrows)

Table 2

GC-MS profiles of essential oils from leaves, inflorescences, and peduncles of *Etilingera elatior*

	Name	RT	RI	Relative abundance (%)		
				Leaves	Inflorescence	Peduncle
1	α -Pinene	8.0	936	-	0.4	4.9
2	D-Limonene	9.9	1034	-	-	0.5
3	<i>cis</i> -Pinocampphone	19.3	1191	0.2	-	-
4	α -Terpineol	20.2	1210	0.5	-	-
5	<i>n</i> -Decanal	20.5	1217	1.0	2.6	1.6
6	1-Decanol	23.6	1286	4.9	4.2	1.9
7	2-Undecanone	24.6	1304	0.7	1.2	1.4
8	Methyl myrtenate	24.9	1309	0.3	-	-
9	2-Undecanol	25.0	1312	-	0.2	-
10	Undecanal	25.4	1316	-	0.1	0.9
11	Geranic acid methyl ester	26.0	1332	0.3	-	-
12	β -Elemene	28.9	1396	0.2	-	-
13	<i>cis</i> -Dodec-5-enal	29.2	1398	-	0.2	0.4
14	(<i>Z</i>)-9-Tetradecenal	29.5	1410	0.3	-	-
15	<i>n</i> -Dodecanal	30.0	1427	10.2	27.4	33.9
16	(<i>E</i>)-Caryophyllene	30.3	1433	6.3	1.3	0.4
17	(<i>E</i>)- β -Farnesene	31.6	1459	3.6	-	-
18	(<i>E, E</i>)- α -farnesene	31.7	1496	-	0.4	0.4
19	α -Humulene	32.0	1468	0.7	0.2	-
20	(<i>Z</i>)-8-Dodecen-1-ol	32.3	1476	2.2	1.4	0.4
21	1-Dodecanol	32.9	1496	32.2	36.9	32.1
22	2-Tridecanone	33.7	1512	1.4	1.3	1.3
23	<i>E</i> -Nerolidol	36.4	1573	0.4	-	-
24	Dodecanal dimethyl acetal	36.8	1581	0.4	-	-
25	Dodecanoic acid	37.0	1585	2.1	4.9	4.8
26	Dodecyl acetate	38.2	1620	9.2	5.7	2.9
27	Tetradecanal	38.7	1625	-	0.4	1.1
28	Tetradec-(9 <i>Z</i>)-en-1-ol	40.4	1682	1.9	0.5	0.3
29	Dec-(5 <i>Z</i>)-en-1-yl acetate	40.5	1792	2.5	-	-
30	<i>cis</i> -9-Tetradecen-1-ol	40.6	1677	2.8	1.7	1.0
31	<i>n</i> -Tetradecanol	41.2	1689	5.5	4.4	3.4
32	<i>trans</i> -Farnesol	42.6	1729	0.1	-	-
33	Tetradecanoic acid	44.4	1779	-	0.3	1.1
34	(<i>Z</i>)-5-Tetradecen-1-yl acetate	45.0	1794	0.3	-	-
35	(<i>E</i>)-9-Tetradecen-1-ol acetate	45.4	1804	-	0.2	-
36	Tetradec-(9 <i>E</i>)-en-1-yl acetate	45.4	1805	0.6	-	-
37	Eicosyl acetate	45.9	1818	1.2	-	-
38	Trifluoroacetoxy hexadecene	46.0	1813	-	0.7	-

Table 2 (continue)

	Name	RT	RI	Relative abundance (%)		
				Leaves	Inflorescence	Peduncle
39	Phytol	55.9	2117	0.5	-	-
40	Tricosyl heptafluorobutyrate	65.3	2422	-	0.4	0.3
41	Tricosyl trifluoroacetate	67.8	2512	-	0.3	
42	Dodecanoic acid, dodecyl ester	69.2	2576	1.9	1.0	0.7
43	Tetracosyl trifluoroacetate	70.0	2619	-	-	0.5
44	1,54-Dibromo-tetrapentacontane	70.2	2425	0.4	-	-
45	1-Hentetracontanol	73.6	2622	0.3	-	-
46	Dodecanoic acid, tetradecyl ester	74.4	2771	-	0.6	-
47	13-Bromotetradecanoic acid	75.7	2855	1.1	-	-
48	Myristyl myristate	74.3	2776	0.5	-	-
49	Hexadecanoic acid, dodecyl ester	79.4	2973	-	0.2	-
50	Triacontyl heptafluorobutyrate	82.8	3558	-	-	0.5
Total identified (%)				96.9	99.3	96.6
Oil yield (%)				0.11	0.09	0.05
Acids				2.1	5.3	5.9
Alcohols				49.5	49.1	39.2
Aldehydes				11.5	30.6	37.5
Diterpene				0.5	0.0	0.0
Esters				15.6	6.9	3.6
Ketones				2.2	2.4	2.7
Monoterpenes				0.8	0.4	5.4
Sesquiterpenes				11.3	1.9	0.7
Others				3.4	2.7	1.7

Note. RT=Retention time; RI=Retention indices relative to BPX5 column; Dash indicates not detected

Further, the EO profiles from the flowering shoot can be distinguished by the higher monoterpene constituents of α -pinene in the peduncles (4.9%) compared to the inflorescences (0.4%).

DISCUSSION

Morphological Characteristics of Secretory Structures

The secretory cells found in *E. elatior* corresponded to the morphological characteristics described in the leaves of *Laurus nobilis* (Maron & Fahn, 1979) and *Piper umbellatum* (Marinho et al., 2011) and flower of *Magnolia sirindhorniae* (Ghosh et al., 2021) in which these plants are notable species with aromatic properties. The secretory cells are characterised by extra-plasmatic space enclosed by a membrane similar to a large central vacuole where the secretion is accumulated. The tri-lamellar structure is a typical

feature that differentiates oil-secreting from mucilage-secreting cells, with the latter only characterised by an outer cellulosic wall (Marinho et al., 2011). The presence of a suberised wall presumably acts as a seal to the oil cell to prevent leakage of potentially toxic substances to the surrounding cells (Evert, 2006).

Interestingly, the occurrence of cupule has been a subject of debate, with some researchers indicating their presence as a fixation artefact where convincing evidence must be supplemented with ultrastructural studies as demonstrated by Maron and Fahn (1979) and Marinho et al. (2011) on oil cells of *L. nobilis* and *P. umbellatum*, respectively. Our finding, on the contrary, accords with Geng et al. (2012) on observing cupule under the light microscope as opposed to requiring ultrastructural studies. Our investigation using fresh, free-hand sections of the plant materials may have resulted in minimal changes in the structure and dimension of the tissue and, hence, affording a close *in situ* observation of the morpho-characteristic of the tissues compared to the conventional FAA or other fixative solutions that may lead to misinterpretation of tissue shrinkage as artefacts. Furthermore, documentation of tissue morphology depends on the methods of tissue processing with solvent fixation using methanol, which has been reported to preserve tissue better than conventional FAA and/or glutaraldehyde-based fixation that usually causes tissue shrinkage (Talbot & White, 2013).

The discovery of the release method of secretion materials from secretory cells via an aperture is unexpected. One possible reason to attribute the observation is the affinity of Sudan IV to react with the lipophilic nature of the secretory contents (Jensen, 1962). While it may be beneficial to perform an ultrastructural examination of the secretory structures to elucidate the secretion mechanism and transport of the oil in and out of the cells, it goes beyond the scope of this study. Notwithstanding, further comparison between secretory cells with their content liberated versus intact revealed that oil droplets exited out to the adjacent cells via the cupule. Therefore, we hypothesise the newly discovered function of the cupule as an aperture that allows the release of secretion content. Previously, the cupule has been functionally described as a peg that attaches the oil sac to the wall (Geng et al., 2012; Postek & Tucker, 1983).

Functional Significance, Taxonomic Relevance, and Potential Exploits

The present study is the first report to comprehensively characterise the secretory structures, especially in Zingiberaceae. The occurrence of secretory cells in all of the plant parts examined in *E. elatior* indicates their prevalence and, therefore, emerges as the main secretory structures of the plant. Their presence as an internal secretory system suggests reservoirs of chemical defence that can be dispensed immediately upon herbivory attack or injury to the organ (Costa et al., 2021; Kromer et al., 2016).

Furthermore, chemical analysis of the EO, particularly from the inflorescence, revealed the presence of several compounds that are commonly indicative of plant signalling,

such as α -pinene, (*E,E*)- α -farnesene and (*E*)-caryophyllene. These compounds have been shown to either attract pollinating bees (Giuliani et al., 2020; Perkins et al., 2023), enhance the repellence of aphids (Ahmed et al., 2019; Dieudonné et al., 2022), or attract natural enemies (Ninkuu et al., 2021).

The secretory cells in the leaves, inflorescence axis, and peduncle of *E. elatior* in this study further underline its importance as a structure taxonomically acquainted with Zingiberaceae, thereby corroborating the previously reported in ginger (*Zingiber officinale*) and shell ginger (*Alpinia zerumbet*) (Remashree et al., 1999; Jezler et al., 2013; Victorio et al., 2011)

CONCLUSION

The present study is the first report to comprehensively characterise secretory structures in Zingiberaceae. Secretory cells have emerged as the main secretory system of *E. elatior* that produces EO, and their presence is widely distributed throughout the leaves, inflorescences, and peduncles. Although an elaborate field experiment is necessary to demonstrate the mechanism of plant signalling in mediation with their environment, the present study has established the foundation for further research avenues, particularly those that utilise secretory structures for agricultural advancement.

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