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Comparative Analysis of Leaf Anatomy, Morphology, and Ethnobotanical Uses of *Etlingera* spp. (*Zingiberaceae*) from the Bali Botanical Garden Collection.

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ABSTRACT

Purpose: *Conservative plant identification depends on the morphological and anatomical character. Previously, there was no publication about the leaf anatomical character of Etlingera rubroloba, E. mamasarum, E. hemispaerica and E. sp 1. This research's aim was to describe the anatomical and morphological characters of five Etlingera spp. from the Bali Botanical Garden collection. This research was expected to add taxonomical information to Etlingera. Some ethnobotany information is also described in this paper.*

Research Method: *The research was conducted in Bali Botanical Garden laboratory. Five species of Etlingera spp were used as samples in this research. The morphological characteristics included leaf morphology, ligule morphology, petiole morphology, and inflorescence observations. Meanwhile for anatomical characteristics, transverse sections were made manually with a razor blade as thin as possible from lamina, midrib, leaf margin and petiole. Epidermal peels were prepared by mechanical treatment. Images were captured using Olympus CX31. Ethnobotanical information collected from a literature review.*

Findings: *The results show that the outline pattern of the adaxial surface of the petiole, the hypodermis abaxial layer, the outline of the leaf margin and stomata density show interspecific variation in Etlingera from the Bali botanical garden collection. There are also variations in vegetative and generative morphology. A combination of anatomy, vegetative and generative character enables the differentiation of the species.*

Limitations: *This research was limited to five species of Etlingera from Bali Botanic Garden collection.*

Originality/value: *The research has provided new information on the anatomical characteristic of Etlingera.*

Keywords: *Comparative analysis, leaf anatomy, morphology, ethnobotanical, Etlingera spp .*

INTRODUCTION

Etlingera is one of the largest genera in the Zingiberaceae family. It consists of 150–200 species and is distributed from northeast India, Asia, and Southeast Asia, extending to the Pacific Islands (Poulsen, 2012; Yeats, 2013; Puspitaningrum *et al.*, 2016). The Malesiana region, including Indonesia, is a center of biodiversity in Etlingera.

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Experts have compiled a list of the species of Etlingera found in the Malesian region, including those from Peninsular Malaysia (Khaw, 2001), Java (Poulsen, 2007), Borneo (Smith, 1986; Poulsen, 2006), and Sulawesi (Poulsen, 2012)

Etlingera is a genus belonging to the tribe Alpiniae. The plants can grow up to 6 m tall. The inflorescences can be divided into two groups: the *Phaemeria* group if the inflorescences rise above the ground, and the *Achasma* group if they appear at soil level. Etlingera is a perennial, evergreen, terrestrial herb that keeps its leaves throughout the year. The Etlingera genus is distinguished from the genus in the Zingiberaceae family by the presence of a large sterile bract. This species has tubular and elongated bracteoles, a flower with a unique tube formed by the bases of the labellum and the filament above the attachment of the corolla lobes, and the labellum is curling (Khaw, 2001).

Based on literatures study, Zingiberaceae is a significant family that produces numerous useful items and commercial assets, including food, spices, pharmaceuticals, perfume, colors, essential oils, and cosmetics. The genus Etlingera is known for its medicinal and aromatic properties, as it yields significant quantities of essential oil from all of its organs. Traditional systems of medicine in south-east Asia have suggested the use of these plants for a range of health conditions (Ud-Daula and Basher, 2019). In Indonesia, essential oil research in the Zingiberaceae family has primarily focused on the genus *Curcuma* and *Zingiber*. In contrast, the genus *Etlingera* has not received much attention. The GC-MS method was employed to investigate the essential oils of *Etlingera* elatior, often known as Torch Ginger, Kecombrang, Bakkala, or Patikala, as documented by Wong *et al.* in 2010. The aforementioned species has demonstrated promising medicinal capabilities such as antioxidants (Juwita *et al.*, 2018), antibacterial effects (Chan *et al.*, 2011), and anti-cancer activity (Krajarng et al., 2017). Economically, many species in Etlingera have various potentials such as antioxidant, antibacterial, antifungal, tyrosinase inhibition, cytotoxic, and hepatoprotective activities (Chan *et al.*, 2007; Chan *et al.*, 2011). Many species are also used as ornamental plants because of their varying flowers (Maimulyanti and Prihadi, 2015), and several species are traditionally used for culinary and medicinal purposes (Wijekoon *et al.*, 2011). Therefore, the identification of etlingera species is very important, especially to get the medicinal properties contained therein.

To date, the determination of the genus *Etlingera* has been taxonomically carried out through approaches to morphological variation, especially the morphology of generative organs or flowers. Due to unflowering plants, the identification will be more difficult. The anatomical character investigation in the taxonomy approach has been done by taxonomists since the 19th century. Many anatomical characters have been useful for systematics, particularly the leaf anatomy (Araujo *et al.*, 2010). The leaf anatomical character appears to provide reliable characters for the identification of species. The characteristic of epidermal and stomata can be used as evidence for plant identification (Windarsih *et al.*, 2022). In the *Zingiberaceae* family, Hussin *et al.* (2000) reported that the leaf anatomical evidence can provide some support for taxonomic determination in *Alpinia* spp. Further, the leaf anatomy of *Etlingera mamasarum*, *E. rubroloba*, and *E. hemispaerica* has not been studied. Therefore, the study of the anatomy of *Etlingera* for taxonomic significance is an interesting and challenging theme. Thus, the objective of this study was to describe the leaf anatomy and morphology of *Etlingera* spp. from the Bali Botanical Garden collection.

MATERIALS AND METHODS

Plant materials

Five species were collected from the Bali Botanical Garden collection (Table 1).

Anatomy Procedure for Stomata and Epidermal Characteristics

Fresh mature leaves of *Etlingera rubroloba*, *E. mamasarum*, *E. elatior*, *E. hemispaerica*, and *Etlingera* sp. 1 were obtained from the Zingiberaceae collection block at the Eka Karya Bali Botanical Garden Conservation Center. Each species was repeated three times. Observation of the stomata and epidermis was carried out by tracing the top and bottom surfaces of the leaf using clear nail polish. This method was adapted from Yan *et al.* (2012) and Priyadi and Hendriyani (2016) for the observation of leaf stomatal characters.The stomata and epidermal tracing were made between 9:00 and 10:00 in the morning on sunny days. Both surfaces of the leaf (top and bottom) were smeared with clear nail polish and left to dry. Then, the clear layers of nail polish were separated from the leaf surfaces by using clear tape, affixed to a slide, and observed under a microscope (Olympus CX31, Tokyo, Japan) with magnification between 4000 and 10,000 times.

Table 1: The Five *Etlingera* Species from Bali Botanical Garden Collection

Species	Scientific Name	Collection Number	Origin
Torch ginger	*Etlingera elatior* (Jack) R.M.Sm	VII.C.I.9	Bali Island
Tulip ginger	*Etlingera mamasarum*	VII.C.131	Maros Protected Forest, Kampung Baru Village, Maros Regency, South Sulawesi Province
Honje hutan	*E. hemispaerica* (Blume) R.M.Sm	VII.C.I.95	Lembah Harau Nature Reserve, Harau Regency, Lima Puluh Kota Subdistrict, Sumatera Barat Province
-	*Etlingera* sp. 1	VII.C.I.26	Lore Lindu Forest National Park, Kamamora Village, Palolo Regency, Donggala Subdistrict, Sulawesi Tengah Province
Mandupi	*Etlingera rubroloba*	X.C.115	Pancasila Village, Dompu Regency, Sumbawa Island, West Nusa Tenggara Province

Observation of the Stomata and Epidermal Characteristics

During the observation, several characters were studied, including the type of stomata, density of stomata, stomatal index, epidermal cell shape, and the measurement of epidermal cell size on both the adaxial and abaxial surfaces of the leaf. The stomatal and epidermal cell characteristics were observed across ten unit leaf areas per leaf, with each unit area measured at 314.86 µm × 238.74 µm. The identification of stomatal types was conducted according to Dilcher (1974).

Procedure for Making Transverse Sections of Midrib, Lamina, Leaf Margin, and Petiole

The cross sections of the leaf midrib, lamina, leaf margin, and petiole were made by cutting the middle part of each section as thin as possible using a razor blade. Only fresh, mature leaves (perfectly open) were used. The incisions were placed on glass slides and observed under a microscope (Olympus CX31, Tokyo, Japan) with magnifications of 4000, 10,000, and 40,000 times.

Observation of Leaf Midrib Cross Section

In the transverse section of the leaf midrib, the arrangement type of the vascular bundle was observed. According to Tomlinson (1956), the vascular bundle arrangement in the plant midrib is composed of several types of arcs: Type I arcs are the main vascular bundle; Arc II represents vascular tissue leading to the abaxial surface; Arc III represents vascular tissue leading to the adaxial surface; and Arc IV is vascular tissue positioned very close to the adaxial epidermis.

Observation of lamina cross section

In the transverse section of the leaf lamina, some characters were observed – the shape of epidermis, length of epidermis, width of epidermis, hypodermis both on adaxial and abaxial while the number of palisade layers, only on abaxial.

Observation of leaf margin cross section

In the transverse section of the leaf margin, the shape of the leaf margin was investigated. The reference of leaf margin section based on Hussin *et al.* , (2000) and Talip *et al.* (2005). There are three types of leaf margins: curved up, straight, and curved down.

Observation of petiole cross section

In the transverse section of petiole some characters such as the shape of adaxial and abaxial surface, the type of vascular bundles were observed.

Morphology

The data of morphological characters were collected from observation and documentation. The observed morphological characters are presented in Table 2. The plant height, length and width of leaf were measured with roll meter, while other characters, collected from literature.

Table 2: Traditional uses of Genus *Etlingera*.

Species name	Common name	Plant parts	Traditional use	Reference
Etlingera elatior	• Torch ginger • Kecombrang • Honje hijau (Sunda) • Kincung (Medan) • Bungongkala (Aceh) • Kecicang (Bali) • Combrang (Jawa) • Asam cekala/cikala (Karo) • Bunga rias (Tapanuli) • Wulae (Sulawesi Tenggara)	Leaf	Food/condiment **Traditional medicine:** • Clean wounds • Treat earaches • Remove body odor (postpartum) • Cough medicine	(Naufalin, 2005); (Ibrahim and Setyowati, 1999; Fitmawati, 2020)
		Stem	Food/condiment/spices **Traditional medicine:** • Diarrhea • Treat coughs	(Naufalin, 2005; Fitmawati, 2020)
		Flower	Food/condiment: sour and peppery flavor **Traditional medicine** Soap, shampoo, perfume	(Chan *et al.*, 2007; Lachumy *et al.*, 2010)
		Fruit	**Traditional medicine:** • Relieve fatigue • Treat bruises/swelling • Treat earache • Appetite, canker sores • Coughing, heartburn	(Ibrahim and Setyowati, 1999; Fitmawati, 2020; Vimala *et al.*, 2003; etc.)
Etlingera mamasarum	• Katimbang (Mamasa) • Black Tulip • Helani Tulip Ginger • Tulip Ginger	Fruit	Food	(Ardiyani and Poulsen, 2019)
Etlingera hemispaerica	• Honje hutan • Honje laka (Sunda) • Hinje merah • Honje beureum	Flower	Traditional drink	(Riyanti *et al.*, 2023)
		Stem	Food	(Ibrahim and Setyowati, 2016)
		Fruit	Traditional drink	(Riyanti *et al.*, 2023)
Etlingera rubroloba	• Mandupi (Muna) • Kasimpo (Makassar) • Pane (Bugis)	Stem	**Traditional medicine:** • Joint pain reliever • Treat gout • Increase endurance after typhoid fever	(Jabbar, 2021; Jabbar *et al.*, 2022)

Table 3: Phytochemistry and Biological Activities of Genus *Etlingera*.

Species name	Plant parts	Phytochemicals	Biological activities	Reference
Etlingera elatior	Leaf	Flavonoids, tannins, saponins, and alkaloids (Jabbar *et al.*, 2019)	Antioxidant activity	(Chan *et al.*, 2008)
			Larvacide	(Virgianti and Masfufah, 2015)
			Repellent	(Koraag *et al.*, 2016)
Etlingera elatior	Stem/rhizome	Flavonoids, tannins, saponins, alkaloids, and triterpenoids (Suryani *et al.*, 2019)	Larvacide	(Adityo *et al.*, 2012)
			Antitumor	(Habsah *et al.*, 2005)
Etlingera elatior	Flower	Flavonoids, terpenoids, and tannins (Suwarni *et al.*, 2016)	Antidiabetic	(Nor *et al.*, 2020)
			Antioxidant	(Jackie *et al.*, 2011; Maimulyanti and Prihadi, 2015)
			Anticancer	(Ghasemzadeh *et al.*, 2015)
			Antibacterial	(Valianty, 2002)
			Repellent	(Koraag *et al.*, 2016)
Etlingera elatior	Fruit	Flavonoids, tannins, saponins, steroids, and terpenoids (Isyanti *et al.*, 2019)	Antibacterial	(Naufalin, 2013)
Etlingera mamasarum	-	-	-	-
Etlingera hemispaerica	Leaf	Flavonoids, alkaloids, saponins, and tannins (Ruyani *et al.*, 2019)	Anti-hyperuricemia	(Karyadi *et al.*, 2023)
			Antidiabetic activity	(Ruyani *et al.*, 2014)
			Hepatoprotective	(Ruyani *et al.*, 2017)
			Anti-inflammatory	(Ningtiyas and Ramadhian, 2016)
Etlingera hemispaerica	Flower	Flavonoids, polyphenols, tannins, quinones, monoterpenoids, sesquiterpenoids (Riyanti *et al.*, 2023)	Alpha-Glucosidase Inhibitor	(Riyanti *et al.*, 2023)
Etlingera hemispaerica	Fruit	Flavonoid, alkaloid, saponin, tannin, phenol, sucrose (Karyadi *et al.*, 2023)	Anti-hyperuricemia	(Karyadi *et al.*, 2023)
Etlingera rubroloba	Fruit	Alkaloid, flavonoid, saponin, tannin, and terpenoid (Malik *et al.*, 2022)	Immunomodulator activity	(Malik *et al.*, 2022)
Etlingera rubroloba	Leaf	Phenol, flavonoid (Andila and Nugroho, 2022)	Antibacterial	(Andila and Nugroho, 2022)
Etlingera rubroloba	Rhizome	Phenol, flavonoid (Andila and Nugroho, 2022)	Antibacterial	(Andila and Nugroho, 2022)
Etlingera rubroloba	Stem	-	Xanthine oxidase inhibitory activity, DPPH radical scavenging, antioxidant, anti-inflammatory activity	(Jabbar *et al.*, 2021; Jabbar, 2022)

Table 4: Comparison of Morphological Characteristics of *Etlingera* spp. in Bali Botanical Garden.

Characteristics	**E. rubroloba**	**E. elatior**	**E. hemispaer-ica**	**E. mamasarum**	**Etlingera sp. 1**
Plant height	3–4 m	5 m	2 m	3–4 m	3–4 m
Leaf Morphology					
Leafy shoot height	3–4 m	5–6 m	2–3 m	3–4 m	4–5 m
Number of leaves in a leafy shoot	19–24	22–28	17–25	21–27	21–29
Size of leaf blade	56.5–68 cm length, 12–18 cm width	68–98 cm length, 18.5–20 cm width	43.5–55.7 cm length, 12–14.8 cm width	45–49 cm length, 16.2–16.3 cm width	58.3–59.6 cm length, 12–14 cm width
Leaf color	Adaxial: Greyish olive green; Abaxial: Strong yellow green	Adaxial: Moderate olive green; Abaxial: Moderate yellowish green	Adaxial: Greyish olive green; Abaxial: Dark red (young), Green to dark red (mature)	Adaxial: Strong yellow green; Abaxial: Strong yellow green	Adaxial: Greyish olive green; Abaxial: Dark red (young), Green to dark red (mature)
Leaf surface	Pubescent	Pubescent	Pubescent	Hirsute	Pubescent
Leaf base shape	Rounded	Oblique	Rounded	Rounded	Oblique
Ligule Morphology					
Size of ligule	1.5–1.6 cm height, 1.2–1.4 cm width	2.5 cm height, 2.2 cm width	1.5–1.8 cm height, 2.2–2.3 cm width	1.6 cm height, 2.3 cm width	0.9–1.3 cm height, 1.8–1.9 cm width
Shape of ligule	Acute	Obtuse	Obtuse	Acute	Obtuse
Ligule margin	Entire	Entire	Entire	Entire	Lacerate
Ligule surface	Pubescent	Pubescent	Pubescent	Ciliate	Pubescent
Petiole Morphology					
Petiole length	1.8–2 cm	3–3.5 cm	1.4–1.5 cm	1.8–1.9 cm	2–2.2 cm
Petiole surface	Pubescent	Pubescent	Pubescent	Pubescent	Pubescent
Petiole color	Dark green	Strong yellow green	Dark green to dark red	Strong yellow green	Strong yellow green
Inflorescence					
Inflorescence size	8–14 cm length, pink cream to light brown	29 cm length, pink cream to white	38 cm length, yellowish green to red cream	15–17 cm, pink cream to white	37 cm length, light green to pink cream with ciliate stalk, covered by light brown bractea, dark red in inflorescences
Flower size	5.5 cm length	7 cm length	–	5.6 cm length	4.2 cm length
Flower color	Pink to yellow white in the centre	Light red and orange in the margin	Pinkish	Pinkish white	Dark red and yellow in the margin
Bracteole color	Light to deep red	White to baby pink	Pinkish white	Light brown	Dark red

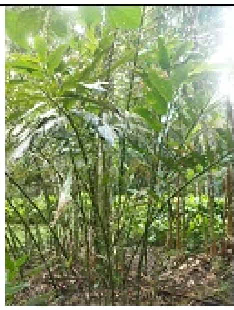
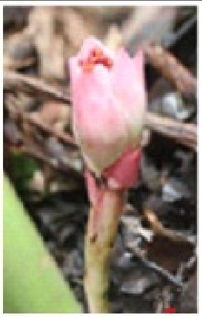
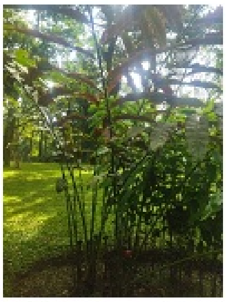
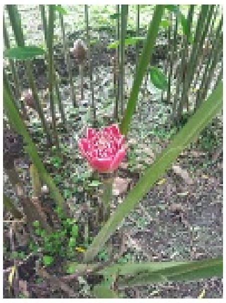
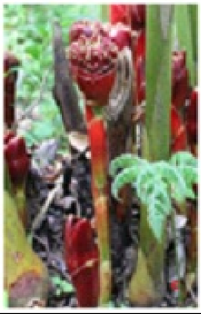
Spesies name	Habitus	Inflorescence
Etlingera elatior		
Etlingera rubloloba		
Etlingera hemispaerica		
Etlingera sp 1		
Etlingera mamasarum		

Figure 1: Habitus and inflorescence characters of Etlingera spp

Data analysis

Data analysis was conducted using descriptive comparative method with qualitative and quantitative approaches. The stomata index and stomata density are calculated by the following formula (Widianti *et al.* , 2017; Tambaru *et al.* , 2018)

The correlation analysis of stomatal and epidermal characteristics were carried out using SPSS 15.00, while the determination of mean and standard deviation was conducted using Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

Ethnobotany

Etlingera has been utilized for centuries as spices, vegetables, and medicines. Etlingera plants have been used traditionally for a variety of medicinal purposes, and recent research has confirmed many of these traditional uses (Table 1). Some of the most common uses include: treatment of coughs and colds, diarrhea and dysentery, pain and inflammation, skin infections and cancer.

Phytochemistry of Etlingera plants

Etlingera plants have been found to contain a variety of phytochemicals, including flavonoids, phenolic acids, terpenoids, steroids and alkaloids. These phytochemicals have been shown to exhibit a variety of pharmacological activities, including antioxidant activity (Juwita *et al.* 2018), antimicrobial activity (Chan *et al.* 2011), anti-inflammatory activity and anticancer activity (Krajarng *et al.* 2017). The genus Etlingera has a great potential for the development of new pharmaceuticals and other products (Table 3). The phytochemicals found in Etlingera plants have been shown to exhibit a variety of pharmacological activities, which could be used to treat a variety of diseases. Further research is needed to fully explore the potential of the genus Etlingera.

Comparison of morphological characteristics of Etlingera spp. in the Bali Botanical Garden Plants Collection.

Morphological characters: Based on the observation, the five species of Etlingera found in the studied area are herbaceous shrub, perennial, has a fibrous root system and has terminal inflorescence (figure 1). The characters of the flower show variations among five species which determine that the species are different. The leaf of Etlingera belongs to the complete type because it has a midrib, petiole, and lamina. The detailed character of each species is described below (Table 4).

Based on the analysis result, there were variations in morphological characters, both in vegetative and generative characters among five species. The vegetative variations included petiole length and color, shape of ligule, ligule margin and ligule surface. The ligule margin of *Etlingera* sp 1 showed different characters compared with others. The ligule surface of *E. mamasarum* also showed different characters compared with others. Meanwhile the generative characteristic included flower, and bracteole color shows different characters (Table 4).

Leaf Anatomy character

Stomata: Five species of Etlingera stomata have tetracytic type of stomata because the position of the guard cell was surrounded by four epidermis cells (subsidiary cells) (Figure 2). Based on Dilcher (1974) this tetracytic is explained in more detail as brachyparatetracytic which has two short cells lateral and parallel to the guard cells, two wide polar cells. The tetracytic type had already been observed in *E. elatior* in a previous study of Windarsih *et al.* (2022) and (Choon & Ding, 2015). This pattern is similar to that of Alpinia stomata (Talip *et al.*, 2005). However, this result is different with Zahara (2020) in *E. elatior* which have paracytic stomatal type.

In all species, stomata were found on both adaxial and abaxial of the epidermis layer. The number of stomata were always abundant on the abaxial epidermis but the size of stomata on abaxial is lower than on adaxial epidermis. All species had an index and density of stomata higher on the abaxial surface than adaxial surface (Table 4). This result, is according to the result obtained by Windarsih *et al.* (2022) and Zahara (2020), revealed that the densities of stomata of *E. elatior* on the abaxial surface are more than adaxial surface. *Etlingera elatior* has the highest density of stomata on abaxial surface compared with others, while *Etlingera rubroloba* has the highest index of stomata on abaxial surface (Table 5).

Epidermal cells: All species of Etlingera have hexagonal epidermis cells shape on both adaxial and abaxial surface. The difference is only on the arrangement of epidermis cells. The epidermis cells on adaxial are arranged regularly while on the abaxial surface, arranged irregularly.

Table 5: Stomatal characters of leaf in five *Etlingera* species. All stomata types observed were tetracytic. Superscript letters indicate statistically significant differences ($p < 0.05$).

Type of Stomata	Species 1	Species 2	Species 3	Species 4	Species 5
Stomata of adaxial epidermis					
Length (µm)	28–35.21	29.49–33.93	39.49–50.16	29.4–35.83	33.61–38.35
Width (µm)	18.16–21.58	15.07–21.07	15.05–26.00	20.15–25.26	13.13–17.35
Stomata index (%)	0.23 ± 0.08^{a}	0.37 ± 0.09^{a}	0.15 ± 0.10^{a}	8.47 ± 2.50^{b}	0.39 ± 0.16^{a}
Stomata density	3.44 ± 1.25^{b}	4.94 ± 1.23^{c}	1.47 ± 0.87^{b}	7.73 ± 2.14^{b}	3.56 ± 1.44^{a}
Stomata of abaxial epidermis					
Length (µm)	22.64–32.17	21.53–26.35	30.6–37.15	23.93–30.94	23.93–29.95
Width (µm)	18.33–23.18	16.05–21.13	18.23–22.84	18.8–24.99	15.6–20.07
Stomata index (%)	9.05 ± 0.42^{c}	8.68 ± 0.46^{b}	7.87 ± 0.78^{d}	8.65 ± 0.53^{b}	8.65 ± 0.53^{a}
Stomata density	193.12 ± 5.06^{b}	191 ± 19^{b}	160 ± 4.92^{a}	198.93 ± 11.31^{a}	168.27 ± 10.48^{a}

Table 6: Anatomical characters of lamina, midrib, leaf margin, and petiole in five *Etlingera* species. Superscript letters indicate statistically significant differences ($p < 0.05$).

Tissue	*E. rubroloba*	*E. mamasarum*	*E. sp. 1*	*E. elatior*	*E. hemisphaerica*
Lamina adaxial					
Shape of epidermis			Hexagonal, arranged regularly		
Length of epidermis (µm)	$55.32–68.7^{b}$	$37.12–57.47^{a}$	$32.29–58.07^{a}$	$33.37–58.82^{a}$	$62.63–80.33^{c}$
Width of epidermis (µm)	$14.21–29.3^{a}$	$14.6–25.52^{a}$	$19.05–44.42^{b}$	$16.26–30.26^{a}$	$22.21–36.33^{b}$
Hypodermis adaxial	1–(2 interrupted)	1–(2 interrupted)	0–2	1	0–1
Lamina abaxial					
Shape of epidermis			Hexagonal, arranged irregularly		
Length of epidermis (µm)	$30.11–50.18^{c}$	$29.4–40.07^{b}$	$22.9–34.98^{a}$	$25.55–39.5^{ab}$	$22.14–52.01^{b}$
Width of epidermis (µm)	$10.72–19.03^{ab}$	$14.11–22.81^{ab}$	$15.92–33.21^{c}$	$11.42–19.03^{a}$	$15.57–36.33^{bc}$
Hypodermis abaxial	1	1	1	1	1
Palisade layer (no. of layers)	1	1	1	1	1
Midrib					
Type of vascular bundles	Arc I, III, IV	Arc I, III, IV	Arc I, III, IV	Arc I, III, IV	Arc I, III, IV
Leaf Margin					
Left margin	Slightly straight	Curved up	Curved up	Curved down	Straight
Right margin	Curved down	Slightly straight	Curved up	Curved down	Straight
Petiole					
Adaxial surface	Concave	Straight	Concave	Concave	Concave
Abaxial surface	U shape	V shape	U shape	V shape	V shape
Vascular bundles	Arc I, III, IV	Arc I, III, IV	Arc I, III, IV	Arc I, III, IV	Arc I, III, IV

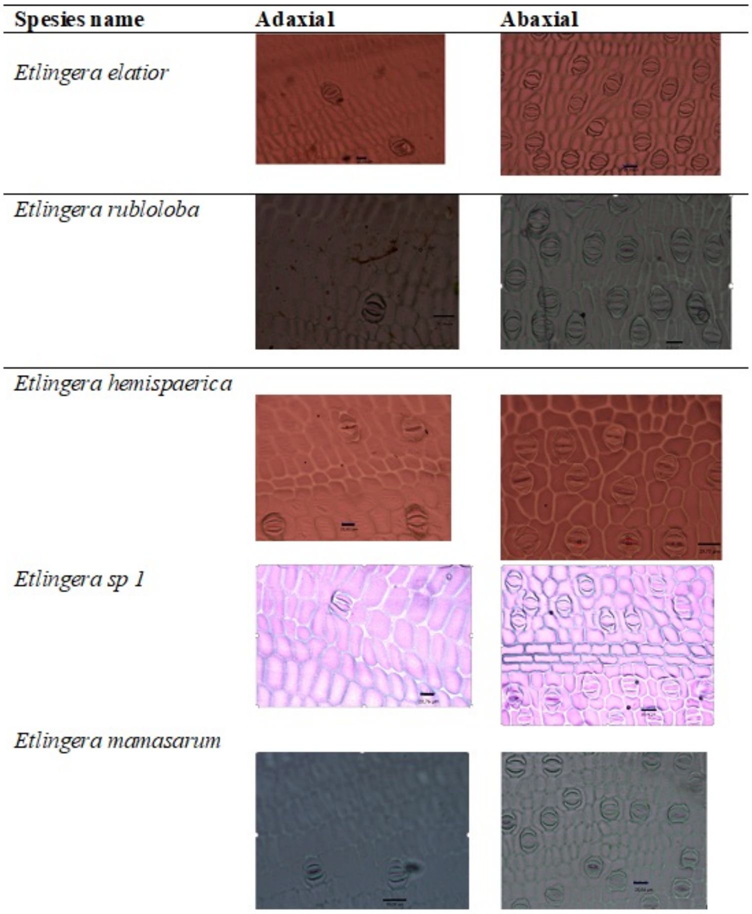


Figure 2: Leaf adaxial dan abaxial anatomical characters of Etlingera species

The fact that the result is different from Windarsih *et al* (2022) revealed *E. elatior* epidermis shape on abaxial has polygonal and irregular while the shape of adaxial surface has column and arranged regularly. However, the result is according to the study of Zahara (2020) revealed *E. elatior* has hexagonal epidermis cell both on adaxial and abaxial surface Anticlinal walls are straight in cells of adaxial surface; hexagonal shape with longer axis, usually perpendicular to veins (Fig. 2). The epidermal cell size of the abaxial surface is slightly smaller than that of the adaxial surface (Table 6). The shape of the epidermis on the adaxial side was hexagonal and arranged regularly, but the abaxial side was irregular. The longest epidermis size on adaxial surface was found on E. hemispaerica, while the longest epidermis cells on abaxial surface was found on *E. rubroloba*. (Table 6).

Hypodermis adaxial: Adaxial cells are wide and tall in both species, with the hypodermis below (Fig.3). All species of Etlingera have hypodermis layers found in abaxial and adaxial, but the number of layers varied in adaxial. *Etlingera* sp. 1 has 0–2 layers of hypodermis, while *E. rubroloba, E. mamasarum, and E. hemisphaerica* have interrupted hypodermis layers. The presence or number of hypodermis layers is not consistent for interrupted hypodermis layers, such as *E. rubroloba*, which has 1-2 hypodermis layers. Only *E. elatior* has one hypodermis layer (Table 6). In the Zingiberaceae, a hypodermis may thus be completely absent, present on both surfaces, present on the adaxial side alone, or present on the abaxial side only (Tomlinson, 1956). Adaxial hypodermis in the lamina is present in all species. E. sp. 1 shows a 0-2-layer hypodermis adaxial; the number of layers is not consistent but varies from 0–2 layers in the same lamina. While *E. rubroloba and E. mamasarum* share similar layers 1-(2 interrupted) hypodermis adaxial,

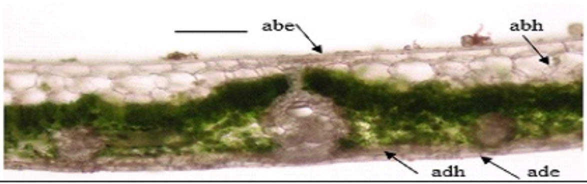
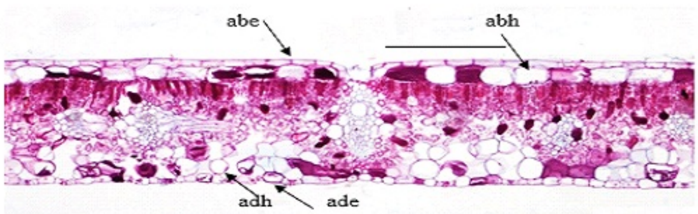
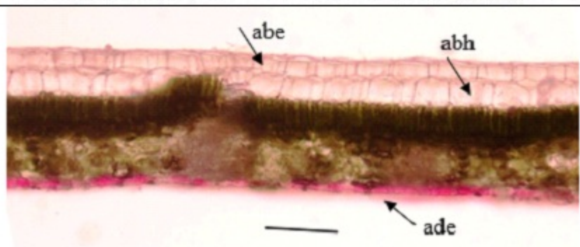
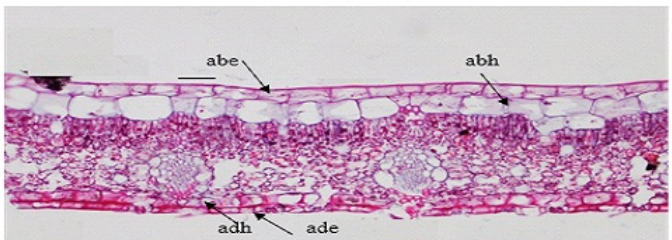
Spesies name	Transverse section of lamina and hypodermis adaxial
Etlingera elatior (scale bar 145.65µm)	
Etlingera rubloloba (scale bar 105.5 µm)	
Etlingera hemispaerica (scale bar 240 µm).	
Etlingera sp 1 (scale bar 384 µm)	
Etlingera mamasarum (scale bar 40 µm	

Figure 3: Transverse section of lamina and hypodermis adaxial of Etlingera species (ade= adaxial epidermis, adh=adaxial hypodermis, abe=abaxial epidermis, abh=abaxial hypodermis)

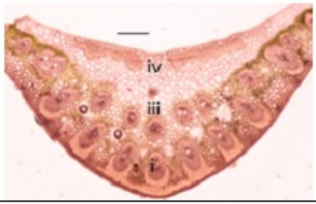
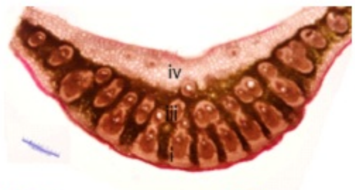
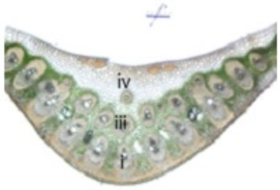
Spesies name	Transverse section of midrib
E. elatior (scale bar 142,00 µm)	
E. rubroloba (scale bar 252,3 µm	
E. hemispaerica (scale bar 422 µm)	
Etlingera sp1 (scale bar 384 µm)	
E. mamasarum (scale bar 142,1 µm)	

Figure 4: Transverse section leaf midrib of Etlingera species

Solereder & Meyer in Tomlinson (1956) suggest that the hypodermis has a water storage function.
Mesophyll (the tissue between the adaxial and abaxial epidermis) consisted of palisade and spongy tissues. The tissues consist of chloroplasts, which contain chlorophyll pigment (Sumardi & Wulandari, 2011).

Chlorenchyma: Palisade cells varied from 1 to 2 layers. The second layer is smaller than the first layer. There were two palisade layers found in *E. rubroloba. E. mamasarum* has 1-2 palisade layers, while others only have one palisade layer (Table 6).

Vascular tissue: All Etlingera species have vascular tissue consisting of arcs I, III, and IV (Table 6; Fig. 4). According to Tomlinson (1956), the vascular bundle arrangement in a plant midrib is composed of several types of arcs. Type I arcs are the main vascular bundle; Arc II is described as a vascular tissue that leads to abaxial; Arc III is a vascular tissue that leads to adaxial; and Arc IV is a vascular tissue that is very close to the adaxial epidermis. It also observed the existence of hypodermic tissue and fiber tissue (Hussin *et al.*, 2000; Talip *et al.*, 2005).

Midrib: The outline pattern of the adaxial surface is concave to all species (Fig. 4). Abaxial surface: U-shaped until V-shaped. *E. elatior and E. sp.* 1 have an abaxial surface U shape, while others are V-shaped (Table 6).

Leaf Margin: The outline of the leaf margin consists of the left and right leaf margins. The shape is rounded and tapering, with the tip facing upward, straight, or downward. In *E. rubroloba* and *E. mamasarum*, the shapes of the left and right margins are different (Fig.

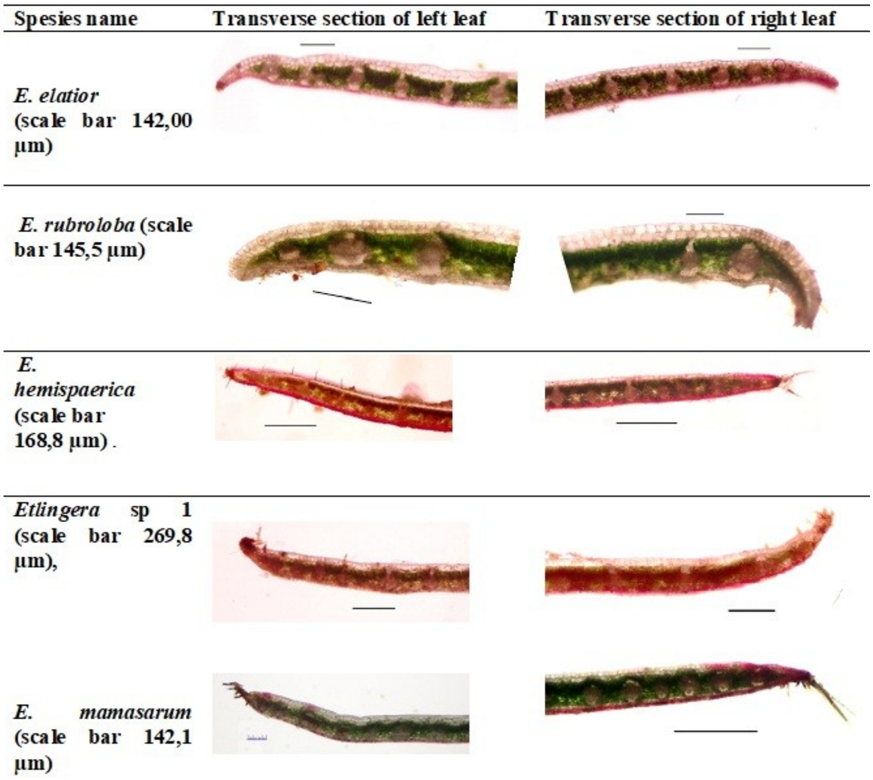


Figure 5: Transverse section left (a) and right leaf of Etlingera species (b)

5). The left and right leaf margins of *E. hemispaerica* have hair on the tip and a straight outline. The left and right leaf margins of *Etlingera sp. 1* show upwards in the tip. The left and right leaf margins of *E. elatior* show downward in the tip. The left leaf margin of *E. mamasarum* shows upwards in the tip, and the right leaf margin of *E. mamasarum* shows a straight outline and has hair in the tip. The right leaf margin of *E. rubroloba* shows a rounded beak in the tip.

Petiole: Petiole is composed of three tissue systems, i.e., the epidermis layer, parenchyma tissue, and vascular system (Sumardi & Wulandari, 2011). The outline pattern of the adaxial surface is straight to concave in most species (Fig. 6), straight in *E. mamasarum*, and concave in *E. rubroloba*, *E. elatior*, *E. hemispaerica*, and *Etlingera sp. 1*. Abaxial surface: U-shaped to wide V-shaped (Fig. 6). A special character is found in *E. rubroloba* because it has hair outside the epidermis.

Taxonomic Implications: Five species of *Etlingera* show inter specific anatomical character on stomata density, petiole, leaf margins, and hypodermis adaxial of lamina. The stomata density shows only *E. elatior* which has the highest number. The abaxial pattern of the petiole shows only *E. mamasarum* has a different feature. The pattern of the adaxial surface of the petiole of *E. mamasarum* has a slight surface, while the other is concave. A special character is also found in *E. rubroloba*, which has hairs on the abaxial surface; other species have no hairs on the abaxial surface. The pattern of the left and right leaf margins of *Etlingera* species shows a symetric pattern, except for *E. rubroloba* and *E. mamasarum*. The presence of hair at the tip of the leaf margin is also limited, only found in *E. mamasarum*.

A combination of characters such as outliners of midribs, petioles, and leaf margins and their relative sizes in transverse sections, as well as the presence of hypodermal fibers in the lamina, midribs, and petioles, can be used in the identification of species (Talip et al., 2005). Variation in leaf anatomical structures was used as a tool to study the interspecific variability of 20 *Alpinia* species. Interspecific variation in the structure of the leaf midrib and petiole was shown (Hussin *et al.*, 2000). The anatomy of vegetative organs can yield crucial taxonomic evidence. Anatomical data have proven to be valuable not only at higher levels but also, in certain cases, effectively utilized even at a specific level. Numerous subsequent investigations pertaining to the morphology of petioles have demonstrated their taxonomic and diagnostic significance across diverse taxonomic groupings (Novikoff and Mitka, 2015; Talip*et al.*, 2017). Salasiah and Meekiong (2018) said data on the size of epidermal cells and stomata may still be useful as supplementary characters for the identification of the species.

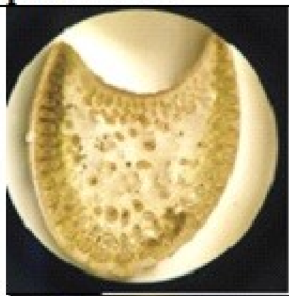
Spesies name	**Transverse section of petiole leaf**
Etlingera elatior	
Etlingera rubloloba	
E. hemispaerica	
***Etlingera* sp 1**	
Etlingera mamasarum	

Figure 6: Transverse section of petiole of Etlingera species

CONCLUSION

The outline pattern of adaxial surface of petiole, the hypodermis abaxial layer, and the outline of leaf margin show interspecific variation in Etlingera from Bali Botanical Garden collection. A combination of leaf anatomy and morphological characters is useful in distinguishing the studied species. Some of these plants have secondary metabolites that possess antibacterial, antifungal, and antioxidant characteristics, which make them highly important for a range of uses. Further, research remains to be done to uncover the content and usefulness of the genus Etlingera.

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