Identification of morphological and stomatal characteristics of Zingiberaceae as medicinal plants in Banda Aceh, Indonesia

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Abstract. Indonesia is a tropical country that cultivates many types of herbs. Zingiberaceae commonly known as gingers family is an important herb in economic and ornamental plants as well as for traditional uses, this herb is famous for its medicinal value and distributed widely throughout the tropical regions. The research conducted in Banda Aceh province, Indonesia from June to July, 2019. The aim of research was to identify the morphological and stomatal characteristics of Zingiberaceae that cultivated in Banda Aceh for plant taxonomy. The results obtained that in Banda Aceh mostly cultivated Zingiber officinale Rosc., Curcuma domestica Val., Alpinia galanga L., and Etlingera elatior L. The parasitic type of stomata showed in all mentioned species. The highest stomatal density on adaxial (AD) was found in Alpinia galanga L. (228.82 mm⁻²), while the lowest density showed in Curcuma domestica Val. (32.30 mm⁻²). The highest stomatal density on abaxial (AB) was found in Etlingera elatior L. (1.768 mm⁻²), while the lowest density showed in Curcuma domestica Val. (600.53 mm⁻²).

1. Introduction
Zingiberaceae commonly known as gingers family, are an important monocotyledon group considerable in economic and potential ornamental plants [1]. Zingiberaceae is famous for its medicinal values and distributed widely throughout the tropical regions, particularly in Southeast Asia. Gingers are very important natural resources which provide many useful products for food, medicines, spices, perfume, dyes, and others [2, 3]. Zingiberaceae as the largest families in the plant kingdom, are distributed mainly in the tropical Asia [4].

This family consists of a large number of medicinal plants and characterized with the presence of volatile compound, oils, terpenoids and oleoresins. The fruits and rhizomes are aromatic, tonic and stimulant. Some are used for source of food as it contain large quantities of starch, various numbers of Zingiberaceae are utilized as ornamentals plants, and medicinal (vegetables and spices). The rhizome extracts are widely used in dietary intake as well as in the traditional medicine and herbs. Many terpenoids compounds with varied physiological activities in anti-microbial, anti-arthritis, antioxidant, anticancer, anti-inflammatory, cardio protective, anti-arrhythmic, antidiabetic, anti-HIV and neuroprotective have been identified in essential oils of Zingiberaceous plants [5].

Indonesia is a tropical country that grown many types of herbs. Zingiberaceae is one the flowering family known as jahe-jahean in Indonesia, widely used as spices, medicine, ornamental plants,
cosmetics, beverage, hair tonic and others. This family is the largest tribe of the *Zingiberales* order, consist of around 53 genera with more than 1,500 species throughout the world, most of that are found in tropical forests. *Zingiberaceae* is one of plants found in many tropical forests, especially Indo-Malaya, which is still not known exactly the number of species. So far, areas rich in *Zingiberaceae* species are Indonesia, Papua, Malaysia, Brunei, Singapore, Thailand, and Philippines [6]. Banda Aceh is the capital city of Aceh Province located in Sumatra Island, Indonesia. Sumatra is the fifth largest island in the world, considered rich in tropical rainforest plants variety including *Zingiberaceae*. These plants have been used by the society in Sumatra for many purposes, such as source of food, aromatic agents, natural coloring matter, insecticides, and medicines, as well as for their economic value, such as the source of a variety of woods, rattan, resin and other non-woody forest products [7].

Leaf is the most important part of the plant where the stomata located. The photosynthesis reaction strongly depends on the stomatal opening and closing which mediated by guard cell. Stomata is the way for CO$_2$ to enter the leaf and H$_2$O to be released, these chemicals are the source for the photosynthesis. Stomata in some plants mostly found on the adaxial and abaxial surface of the leaves, called as amphistomatous leaves. While some tress stomata located only on the lower surface, known as hypostomatous leaves, whereas aquatic plants like lilies found to have stomata pores on the upper surface called as epistomatous leaves [29].

Plant taxonomy generally uses the morphological characteristics to identify the plant species. However, science and technology development have increased the interest of taxonomists to find the other evidence for plant determination such as plant anatomy which is stomata characteristics. Many anatomical characteristics can be used to proof the morphological character in plant taxonomy, including size of stomata, shape of epidermal cell, type of stomata, and the density of stomata [30]. Therefore, the main objective in this study is to examine the morphological and stomatal characteristics that can be used for plant taxonomy and identification in *Zingiberaceae* family. Stomatal density proved to have a positive correlation with CO$_2$ uptake and Photosynthetic rate in some plants and will be benefit for yield [33][34], but showed a negative correlation with H$_2$O that lead to the transpiration rate [34].

### 2. Material and Methods

**2.1. Morphological Features Identification**

The study conducted in Banda Aceh area from June to July 2019. Identification of morphological features of *Zingiberaceae* using the characteristics similarity that possessed by each plant species and also using the book entitled “Plant Morphology” wrote by Tjitrosoepome (2016) as a guideline [8].

**2.2. Stomatal Type Identification**

A qualitative descriptive research method used to describe and interpreted the type of stomata on the leave base of *Zingiberaceae* and analyzed using a light microscope at Biology Department, Islamic Faculty, Muhammadiyah Aceh University. Replica method was using to prepare the slide to observe the stomatal type under microscope, the steps as follows:

1. Clean the upper and lower surfaces of the leaves
2. Apply the nail polish, left it for 10 minutes to dry
3. Dried spreads are attached with transparent tape and flattened
4. The transparent tape peeled and removed slowly from the leaves surfaces, then attached to the object glass
5. Flattened and labeled with a description of the plant type
6. Stomatal type observation using a light microscope with same magnification (40x) [9].
2.3. Stomatal Density Calculation
The stomatal density calculation conducted with three repetitions for each species with the following formulas:

\[
\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{Field of view under microscope}}
\]

Number of stomata calculated under microscope with 10x magnifications, while field of view calculated with the same formula with circle area formulas.

Field of view under microscope = \(\pi \times r^2\), \(r = 0.25\) mm\(^2\) for 10x magnifications [31].

3. Results and Discussions
Zingiberales is an herbaceous moderate sized family of relatively advanced monocotyledonous plant of Zingiberales order. Mainly found in the tropical and subtropical regions, mainly in East Asia. Several authors have mentioned different total number of genera and species but it is probably appropriate record at least 51 genera and 1500 species in the world[10]. Nature has been a source of medicinal agents for thousands of years and a lot of number of modern drugs has been isolated from natural sources that play a vital role in treatment of diseases [11]. Traditional medicinal plants usage is often cheaper, locally available and easily to consumed, raw or as simple as medicinal preparations. These simple medicinal preparations often bring out beneficial responses due to their active chemical constituents[12].

The results obtained from the fields observations; informants, herbal medicine praktioners, and interviews with the society, in Banda Aceh mainly cultivated five species of Zingiberaceae (Table 1.). These species found could recover some diseases.

| No. | Local name       | Scientific Name          | Traditional Medicine Usage                                      |
|-----|------------------|--------------------------|-----------------------------------------------------------------|
| 1   | Jahe (Ginger)    | Zingiberofficinale Rosc. | Anti-inflammatory, relieve pain, anti-bacteria [13].            |
| 2   | Kunyit (Turmeric)| Curcuma domestica Val.   | Gastritis, dyspepsia, joint pain, reduce pain during menstruation [13]. |
| 3   | Lengkuas (Galangal) | Alpinia galanga L.       | Relieve chest pain, sore throat, antifungal [13].               |
| 4   | Kecombrang (Torch Ginger) | Etlingera elatior L. | Antioxidant, anti-bacteria, lots of nutrient content            |

3.1 Morphological Features
3.1.1 Zingiberofficinale Rosc. Zingiberofficinale is well known as ginger, one of the most important members from Zingiberaceae family. William Roscoe (1753-1831), an English botanist gave this herb name Zingiberofficinale on a publication in 1807, the ginger family is one of the tropical group abundant in Indonesia-Malaysia, consist of more than 1200 species in 53 genera [14]. The morphological features of ginger shows that it is an erect perennial plants growing from one to three feet in height, the stem is about one meter in height and surrounded by the sheathing bases of the two-ranked leaves.
The leaves are 6 to 12 inches in length, elongate and the flowers are cone-like spikes about one inch thick and two to three inch long composed of overlapping green bracts. Each bract enclose single, small, yellow-green and purple flower. Unfortunately, ginger rarely flower in cultivation. The rhizome is thick with 7-15 cm in length [14, 15]. Other morphological features such as the rootstock are horizontal and tuberous. The leafy stem is elongated leaves, oblong-lanceolate, clasping the stem by their sheath. The spikes is usually radical, rarely lateral or terminal on the leafy stem peduncle short or long, persistent bracts, and usually single [15]. Zingiber officinale Rosc. has some values for medicinal, nutritional, and ethnobotanical and extensively use worldwide as a spice, flavoring agent and herbal remedy [16, 17]. In traditional way, is used in some countries to cure a variety of diseases, nausea, vomiting, asthma, cough, palpitation, inflammation, dyspepsia, loss of appetite, constipation, indigestion and pain [16], meanwhile in Banda Aceh mostly ginger used to cure cough, relieve pain, dyspepsia, anti-bacteria, additional herbs for cooking and other functions.

3.1.2 Curcuma domestica Val. Curcuma domestica Val synonym to Curcuma longa Linn. is a well-known traditional herb consist a lot of functions for health. Curcumin is an important constituent of turmeric [13]. A yellow-pigmented fraction isolated from the rhizomes of the Curcuma contains curcuminoids belonging to dicinnamoyl methane group. Curcuminoids are represented to the extent of 3-5% which is an important active ingredient responsible for Curcuma biological activity [18].

Turmeric has a pseudo-stem composed of petals or leaf midrib covering each other, the stem is wet and consists of water, round and purplish green, the high of the stem reach 0.75-1 meter. The leaves are composed of leaf midribs, leaf handles and stands leaf. The leaf length of the turmeric is between 31 – 38 cm and the leaf width is around 10-18 cm. Oval-shaped turmeric leaves lengthen with the surface is rather rough. Flat leaves and tapered end or curved to resemble a tail. The color of the leaf surface is light green, one plant consist of 6-10 leaves [19]. The inflorescence is cone-like. 10-15 cm long and is attached to a stem enclosed in a sheathing petiole. The flower has two pale green bracts 5-6 cm long and the covering bracts are whitish, often red tinged. The individual flowers have yellow color or yellowish white, with tubular, 3-lobed calyx and funnel-shaped and 3 corolla [20]. The stamen with a short filament, broad and constrict at the apex. The ovaries consist of three locules and containing two ovules for each [21, 22].

3.1.3 Alpinia galanga L. Alpinia galanga L. is a native of Indonesia and naturalized in many parts of South and South East Asia. At present, A. galanga is cultivated in all South East Asian countries such as; India, Bangladesh, Indonesia, Thailand, and Surinam also China [23]. It is a long-live herbaceous plants, perennial, tillering, and rhizomatous herb. The height of the plant reaches 3-5 m, with with an aromatic branchrhizome and grows in tight clumps. The thick of rhizome are 2.5-10.0 cm, reddish brown externally and light brown internally. The leafy stem (pseudo-stem) is erect, formed by the rolled leaf sheaths. The leaves length is 20-60 cm with 4-5 cm width, alternate, oblong-lanceolate, and glabrous.

The inflorescence is a terminal with many-flowered raceme, the flowers are fragrant, and the length is 3-4 cm, yellow-white color. The fruit is globose to ellipsoidal capsule with 1-2 cm in diameter; the color is orange-red [23, 24]. Different parts of A. galanga traditionally used for the ailments treatments including anti-fungal, anti-tumor, anti-helminthic, anti-diuretic, anti-ulcerative, heart disease, rheumatic pains, diabetes, liver burning, chest pain, dyspepsia fever, and kidney disease [25].

3.1.4 Etlingeraelator L. Etlingeraelator L. is native to Malaysia, Southern part of Thailand, Indonesia and now already cultivated throughout the tropical area [26]. This herb grows up to 5-6 m tall forming clumps [27], has aromatic rhizomes with 3-4 cm in diameter and found just under ground level with pseudo-shaped round stems enlarge on the base. When it crushed, the leaves emit a pleasant sour fragrance. Normally this plant has 15-40 leaves arranged in two rows alternately. The leaves are entirely green with a truncated base, the young leaves sometimes flushed pink with 2.5–3.5 cm in length of petioles. The flowers are compounds that form tubers with a 40-80 cm stem length. The
stamen is yellow and 7-8 cm in length, while the pistil is small and white. The crown of the flower has a shirt, hairless and pink color. The seeds are has square shape or oval in white and pink.

Torch ginger is well known for Indonesian people as vegetable, ornamental and traditional uses for medicine. The rhizome, stems, leaves and all parts of the plant are often use for daily life. This herb is rich of phenols, polyphenols, flavonoids, ant-oxidant activity and terpenoids also has a wide range of pharmacological activity as an antioxidant, antibacterial, anticancer and repellent [28]. It can be used to cure some diseases such as; diarrhea, cough, heartburn, skin diseases and help in blood circulation.

3.2 Stomatal Characteristic

The research showed that all the species has the same type of stomatal structure form both side of the leaves surface. The Parasitic type of stomata showed in Zingiber officinale Rosc., Zingiber officinale Linn. Var. Rubrum., Curcuma domestica Val., Alpinia galangal L., and Etlingera elatior L. (Fig. 1). One or more neighboring cells around stoma are parallel with the long axis of the guard cells. The same results obtained by Damayanti (2007) on the stomatal characteristics of six banana germ plasm accessions where all showed the same type of stomata [32]. The characteristics of stomata can be uses for plant identification and determination which is very important in plant taxonomy [30].

![Stomata of different species](image1.png)

**Figure 1.** Stomatal type of (a) Zingiber officinale Rosc., (b) Curcuma domestica Val., (c) Alpinia galangal L., (d) Etlingera elatior L.
Table 2. Stomatal characteristics of *Zingiberaceae* in Banda Aceh

| No | Species                  | Epidermis | Type of stomata | Epidermis cell shape | Density of stomata (mm$^{-2}$) | Repetition | Average |
|----|--------------------------|-----------|----------------|----------------------|-------------------------------|------------|---------|
| 1  | *Zingiber officinale* Rosc. | AD        | Paracytic       | Polygonal            | 192.28                        | 3          | 190.51  |
|    |                          | AB        |                | Polygonal            | 775.51                        | 2          | 794.22  |
| 2  | *Curcuma domestica* Val.  | AD        | Paracytic       | Elongated-Hexagonal  | 30.45                         | 3          | 32.30   |
|    |                          | AB        |                | Elongated-Hexagonal  | 586.73                        | 3          | 600.53  |
| 3  | *Alpinia galanga* L.      | AD        | Paracytic       | Elongated-Hexagonal  | 225.83                        | 3          | 228.82  |
|    |                          | AB        |                | Elongated-Hexagonal  | 846.93                        | 3          | 863.94  |
| 4  | *Etlingeraelatior* L.     | AD        | Paracytic       | Elongated-Hexagonal  | 120.35                        | 3          | 125.01  |
|    |                          | AB        |                | Elongated-Hexagonal  | 1.785                         | 3          | 1.768   |

The results obtained showed that the highest stomatal densities are different in each species with different shape of epidermal cell (Table 2). The highest stomatal density on adaxial (AD) was found in *Alpinia galanga* L. (228.82 mm$^{-2}$), while the lowest density showed in *Curcuma domestica* Val. (32.30 mm$^{-2}$). The highest stomatal density on abaxial (AB) was found in *Etlingeraelatior* L. (1.768 mm$^{-2}$), while the lowest density showed in *Curcuma domestica* Val. (600.53 mm$^{-2}$). The stomatal density related to the water use efficiency, gas exchange and photosynthetic rate. A research in *Arabidopsis thaliana* proved that stomatal conductance increased under ambient of CO$_2$ condition and did not show the alteration in the maximum rate of carboxylation. This phenomenon indicated that the photosynthetic rate increased because of gas exchange. Photosynthetic rate was increased under CO$_2$ condition associated with increased of stomatal density [33]. The higher stomatal density the more pore can be opened to absorb CO$_2$. Another research in Guava showed that leaf stomatal density positively correlated with stomatal conductance, net photosynthetic rate and net leaf CO$_2$ assimilation rate. Similarly, the number of stomata negatively related with transpiration rate [34].

4. Conclusion

Four species of Zingiberaceae cultivated in Banda Aceh are *Zingiber officinale* Rosc., *Curcuma domestica* Val., *Alpinia galanga* L., and *Etlingeraelatior* L. Each mentioned species can cure many diseases. These herbs mainly use for medicine, food, beverage and ornamental plants. The research obtained that the type of stomata in all species is paracytic; the shape of epidermis cell is mostly Elongated-Hexagonal. The highest stomatal density on adaxial (AD) was found in *Alpinia galanga* L. (228.82 mm$^{-2}$), while the lowest density showed in *Curcuma domestica* Val. (32.30 mm$^{-2}$). The highest stomatal density on abaxial (AB) was found in *Etlingeraelatior* L. (1.768 mm$^{-2}$), while the lowest density showed in *Curcuma domestica* Val. (600.53 mm$^{-2}$).
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