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THE MORPHOLOGICAL AND ANATOMICAL STUDIES OF THE AERIAL PARTS OF *Abroma augusta* L. FROM SEMARANG

Rita Ariyana Nur Khasanah[1*], Niken Kusumarini[2]

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[1,2]Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Walisongo. Jl. Prof. Hamka, Tambakaji, Ngaliyan, Semarang, Central Java, Indonesia 50185

e-mail:
*[1]*ritaariyana@walisongo.ac.id*
[2]*kusumarini.niken@walisongo.ac.id*

**Corresponding author*

Abstract. *Abroma augusta* L. *known as Devil's cotton belongs to Malvaceae. The exploratory study aimed to study the morphological and anatomical characteristics of the aerial parts of A. augusta* L. *from Semarang. The transverse section of the aerial parts was made by a simple method (fresh preparation) and then observed under a binocular microscope with an optilab. All characteristics were observed and then compared with the references. The collected data were analyzed descriptively and quantitatively. In summary, the results showed that A. augusta* L. *was an evergreen shrub (small tree) with orthotropic and plagiotropic branches and polymorphous leaves. The inflorenscence was found in the terminal and axillar plagiotropic branching with bisex, actinomorphic, and pentamerous flowers. The fruit was unique (obconical capsule with a rounded base and truncate-tip with 5 angled wings) including cotton fibers and numerous black seeds. The petiole was composed of epidermis, collenchyma, cortical parenchyma, sclerenchyma, vascular bundle, mucilaginous ducts, and pith. The dorsiventral leaf was composed of upper and lower epidermis, palisade, and spongy parenchyma. The stomata type was ranunculaceous (anomocytic) while the guard cell was kidney-shaped. The stomata density on the abaxial leaf was higher than that of the adaxial leaf. The stellate and unicellular non-glandular trichomes, and capitate glandular trichomes were found abundantly on the petiole and leaf blade. These morphological and anatomical studies are important to support the identification as a part of the conservation effort of the plant. Further studies are recommended to investigate the root morphology and anatomy and also biochemical characteristics of each part of the plant in order to obtain complete plant identification.*

Keywords*: Abroma augusta* L., *aerial parts, anatomical characteristics, morphological characteristics*

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INTRODUCTION

Abroma augusta L. known as 'Ulat Kambal' (Hindi) or Devil's cotton (English) belongs to Malvaceae (previously under family Sterculiaceae). This plant is popular especially in India and mainly used for the treatment of various diseases and disorders in traditional medicine, such as diabetes mellitus in type 2, infection, wound healing, rheumatic pain, etc. and used as a uterine tonic in some uterine and menstrual disorders (Ahmad et al.,

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2020; Chowdhury et al., 2019). Many studies have reported about biological activities of this plant such as anti-diabetic (Ahmad et al., 2020; Khanra et al., 2015), anti-inflammatory (Das et al., 2012; Latief et al., 2020), analgesic, antioxidant, thrombolytic, and hypolipidemic activities (Chowdhury et al., 2019). According to Saikot et al. (2012), the acetone extract of its leaves also has antimicrobial and cytotoxic activity. Some phytochemicals, such as steroids, triterpenes, flavonoids, alkaloids, abromines, sterols, friedelin, abromasterol, taroxerylacetate, taraxerol, β-sitosterol, etc. are related to its biological activities and found and distributed in almost all body parts of the plant (Chowdhury et al., 2019; Gupta et al., 2011). Hence, the barks, leaves, and roots of *A. augusta* L. can be used in disease/ disorder treatment (Chowdhury et al., 2019). According to Miah et al. (2020), some phytochemicals such as alkaloids, steroids, terpenoids, flavonoids, reducing sugars, and glycosides have been found in the methanolic extract of the *A. augusta* L. bark showing the highest antioxidant capacity.

A. augusta L. is an evergreen shrub (small tree) with many branches and is usually found in tropical Asia, South and Eastern Africa, and Australia (Das et al., 2012; Parkash et al., 2019). This plant naturally grows in some regions in North-East India, such as in Assam, Arunachal Pradesh, Meghalaya, and Tripura with various edaphic factors (Parkash et al., 2019). In Indonesia, especially at Universitas Indonesia, Depok City, West Java, this woody plant grows wild with rare in status (Mustaqim & Nisyawati, 2020). This plant is also found in Muaro Jambi District, Mendalo Darat Village, and the community has used it is to treat the inflammation of the joints and fractures (Latief et al., 2020). As a medicinal plant, it is important for conservation as has been studied by Parkash & Saikia (2015) who examined the association between some abiotic soil properties and rhizospheric fungal biota as an ecological indicator for the conservation of *A. augusta* L.

A. augusta L. also grows wild around UIN Walisongo Semarang, Ngaliyan, Semarang, Indonesia. The morphological and anatomical studies of the medicinal plant are important for identification and as a part of the conservation practice of the species (Trias-Blasi & Vorontsova, 2015). Leaf anatomical characteristics including stomata and trichomes are important tools for identification in Plant Systematic studies (Chachad & Vaidya, 2016). The morphological and anatomical characteristics of *A. augusta* have been studied in North-East India with edaphological variability in the region (Parkash et al., 2019; Rani & Datta, 2020). We know that in addition to genetic factors, the morphological and anatomical characteristics of plant are influenced by edaphic and environmental factors. Therefore, the study aimed to study the morphological and anatomical characteristics of the aerial parts of *A augusta* L. from Semarang.

MATERIALS AND METHODS

The study was an exploration conducted from October to November 2020. The sample was collected from *A. augusta* L. growing wild around Science Laboratory of Faculty of Science and Technology, UIN Walisongo Semarang, Prof. Dr. Hamka Street, Tambakaji, Ngaliyan, Semarang City, Central Java, Indonesia (6° 59' 23.946" S, 110° 21' 12.0132" E). The identification of anatomical structures was carried out in the Structure Laboratory of UIN Walisongo Semarang. Leaf samples (the 5th leaf from the branch tip) and their petioles were taken in the morning at 08.00 am. The environmental conditions consisted of the air

temperature of 30°C, the air humidity of 72%, the elevation of 99 above sea level, the air pressure of 1000.1 hPa, the light intensity of 18,860 lux, and the soil pH of 6.8.

Morphological Observation of Aerial Parts of *A. augusta* L.

The aerial parts of *A. augusta* L. were observed as a whole. The stem characteristics observed were the plant habit, shape, bark color, branching, and the presence of trichomes in the branch. The observed leaf characteristics were the leaf blade shape, leaf edge, leaf tip, leaf base, leaf surface, and leaf venation. Furthermore, the length and width of the leaves and the length of the petioles were measured. The characteristics of the inflorescence and the flower were also observed as a whole, consisting of the type, shape, color, and size of the perianths and the sex organs. The sex organs were observed under a stereomicroscope (EM-32 Meiji Techno). The observed characteristics of young and ripe fruits were the shape and color, while the observed seed characteristics were the number, size, shape, and color of the seeds.

Anatomical and Micro-morphological Observations of Aerial Parts of *A. augusta* L.

The sample preparation was carried out using a simple method (fresh preparation with free-hand sectioning method) (Yeung, 1998). The petiole (2 cm from the base) and the leaf blade were made a transverse section as thin as possible using a sharp razor blade. The incision was placed on a glass object having been dropped with tap water and then closed with a deck glass. Some trichomes on the leaf blade were carefully collected by sharp razor and then placed on a glass object having been dropped with tap water, and then closed with a deck glass. Parademal preparations were made by boiling the leaves (1x1 cm^2) in a solution of HNO_3 25% to separate the epidermis (Cutler, 1978). The epidermis was stained using safranin 25%, and then placed on a glass object to be observed. Paradermal preparations also were made by the replica technique (Sari and Harlita, 2018) with alteco glue and plastic mica. All samples were observed using a binocular microscope (MT-30 Meiji Tecno) with magnifications of 100x and 400x.

The measured anatomical parameters were the organ tissues and the tissue thickness. The observed parameter in the paradermal incision consisted of 1) the stomata type based on the position and number of neighboring cells around them; 2) guard cell shape; 3) the length and width of the stomata; 4) the distance between two stomata; and 5) the number and density of stomata. The obtained data was the mean value from the measurement of 3 randomly selected fields of views, each with 3 replications. The determination of stomata density is calculated by the following formula 1 (Paul et al., 2017).

$$Stomata\ density = \frac{Number\ of\ stomata}{Wide\ field\ of\ view\ (mm^2)} \quad (1)$$

Data analysis

The results were analyzed by referring to ssome references such as Parkash et al. (2019) who examined the morphological and the anatomical characteristics of aerial part of the plant from some regions in North-East India based on the influence of some edaphic factors; Rani & Datta (2020) who studied seed and seedling morphology of some medicinal plants of Malvaceae, including *A.augusta* L. in Tripura, North-East India; Bayer & Kubitzki (2003) who studied morphological characteristics in Malvaceae, including in *A. augusta* L. Generally, the books about plant

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morphology by Tjitrosoepomo (2007) and plant anatomy by Fahn (1995) and Simpson (2010) were also used as references. All of the collected morphological and anatomical data of aerial parts of *A. augusta* L were analyzed descriptively and quantitatively by SPSS 16 to provide comprehensive information to the readers.

RESULTS AND DISCUSSION

Morphological Characteristics of The Aerial Parts of *A. augusta* L.

The observed morphological characteristics of the aerial parts *of A. augusta* L. included the stem and its branching, petiole, leaf, flower, fruit, and seed.

Morphological Characteristics of The Stems and Branches

A. augusta L. was a perennial plant and an evergreen shrub (small tree) with a height of up to 2.5 meters and many velvety branches (Figure 1a,b). This plant had two types of branching, namely vertical or orthotropic branching maintaining its vegetative nature (Figure 1d) and horizontal or plagiotropic branching maintaining its generative nature (Figure 1e). The stem was rounded with dark brownish-gray in color, wrinkled bark (Figure 1b), and green to purplish young branches. There were many trichomes on the surface of the branches and some axillary stipules in the axillar (Figure 1c).

Morphological Characteristics of The Leaves

The leaf of *A. augusta* L. was simple and incomplete, consisting of a petiole and a leaf blade. This plant had polymorphous leaves namely palmate (lobed leaves) and cordate (unlobed leaves) (Figure 1f, 1h, 1i). The palmate leaves appeared in orthotropic branches, while cordate leaves appeared in plagiotropic branches. The arrangement of leaves on the stem (phyllotaxis) was alternate (Figure 1g). Several leaf morphological parameters (5^{th} leaf) of *A. augusta* L. were observed (Table 1). Young leaf was greenish in color, cordate-ovate or lanceolate in shape, denticulate, fine hairy, with a short petiole. Additionally, there was purplish-green stipule, (2-4) mm, with linear veins and acute tips in the axillar (Figure 1c).

Table 1. Comparison of the morphology of the 2 leaf types on *A. augusta* L. **(5^{th} leaf sample)**

Parameters	Palmate Leaves	Cordate Leaves
Leaf blade	Cordate-ovate to orbicular	Cordate-lanceolate
Lobes	Lobed	Unlobed
Leaf tip	Acuminate	Acuminate
Leaf edge	Denticulate	Denticulate
Leaf base	Cordate	Cordate
Leaf venation	Palmate usually 5-7 nerved base	Palmate-pinnate usually 5 nerved base
Adaxial/dorsal surface	Pubescent (hairy)	Pubescent (hairy)
Abaxial/ventral surface	Pubescent (hairy)	Pubescent (hairy)
Mature leaf color	Dark green	Dark green
Leaf length (cm)	13.5-16.5	13.5-15.5
Leaf width (cm)	15.5-21.5	7.8-9
Petiole length (cm)	14.5-16.5	2-2.5
Petiole color	Purplish green	Purplish green

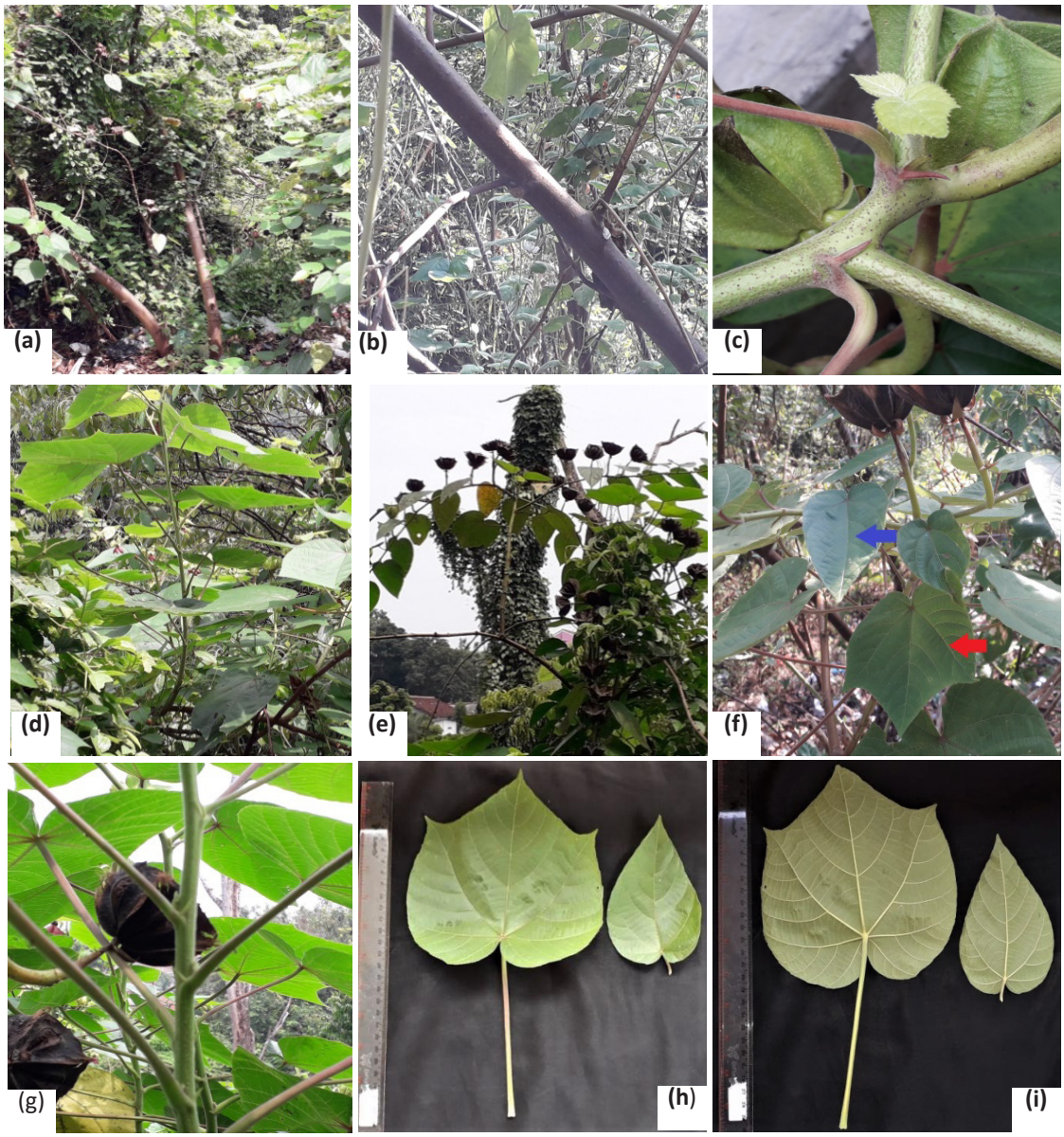

Figure 1. Morphological characteristic of *A. augusta* L.: (a-b) habitus-shrub, round, dark brownish gray and wrinkled stem; (c) branch surface (green to purplish in color with many trichomes (d) orthotropic branches; (e) plagiotropic branches; (f) polymorphous leaves, cordate (blue arrow), palmate (red arrow); (g) phyllotaxis (alternate); (h) leaf adaxial surface (pubescent, hairy); (i) leaf abaxial surface (pubescens, hairy).

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Morphological Characteristics of The Inflorescences and The Flowers

Abroma augusta L. had inflorescence in racemose type, usually 4 in number with different development stages located at the terminal and the axillary leaf of the plagiotropic branches or reduced in solitary flowers located in the axillary leaf of the branches (Figure 2a, b). The Inflorescence had pedunculus of 2.3-2.5 cm in length and had the bractea. The flower was bisex, symmetric (actinomorph) with a diameter of 3-5 cm, and pentamerous (Figure 2a, b, c). It had pedicelus of 2.7-3.5 cm in length. The calyx was synsepalous (lobed or free nearly to the base), typically *5*-merous, each sepal was lanceolate-triangular ((2-2.2) cm x 0.8 cm), light green color with reddish pigmentation and persistent. The corolla was apopetalous, typically *5*-merous, each petal was spoon-shaped (2.5 cm x 1 cm), dark red (maroon) in color with white color in the base, and deciduous (Figure 2c, d). The androecium consisted of a short stamen tube in which five anthers alternate with five staminodes (Figure 2d). The gynoecium consisted of a pistil with five lobes and a superior ovary (Figure 2e). The number of ovules in this recent study was not observed, but the seed number produced was numerous, so that the number of ovules was also numerous. Parkash et al. (2019) reported that the number of ovules in the gynoecium of this species flower was numerous with axil placentation.

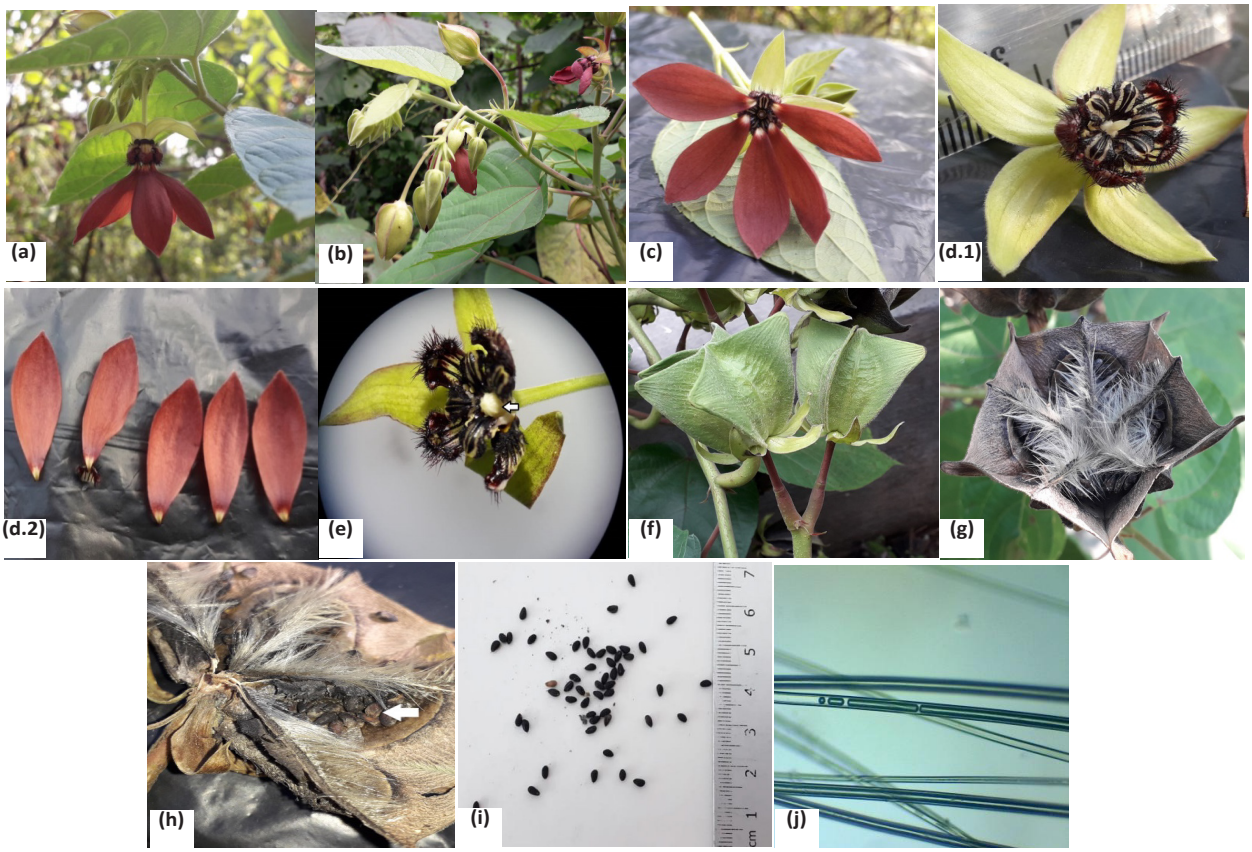

Figure 2. Flower and fruit of *Abroma augusta* L. (a-b) inflorescence; (c) a whole flower; (d.1-d.2) flower parts; (e) calyx showing the structure of androecium and gynacieum with superior ovary (arrow) under stereo microscope; (f) young fruit; (g) and (h) ripe fruit; (i) seeds; (j) cotton fiber under binocular microscope (M: 100x; scale bar 100 µm).

Morphological Characteristic of The Fruits and The Seeds

The fruit of *Abroma augusta* L. was an obconical capsule (4 cm x (3-4) cm), rounded base and flat tip (truncate) with 5 "wings" and formed an angle. The young fruit was greenish in color while the ripe fruit was dark brown in color (Figure 2f, g). The top of the ripe fruit was opened up space (Figure 2g). Inside the ripe fruit, there were light wooly cotton fibers (main characteristic of Malvaceae, hence it was called 'Devil's cotton', Figure 2g, h) and black seeds with a cylindrical-obovoid shape ((3-4) mm x (1-2) mm) in numerous quantities (Figure 2g, h, i). This seed morphology has been checked with reference (Rani & Datta, 2020). They reported that *A. augusta* L. seeds have black and ovate seeds in numerous quantities with 0.55-0.65 g in weight, 0.25-0.32 cm in length and 0.15-0.2 cm in width, rounded apex, acute base, rough, basal and cream hilum. Propagation using these seeds resulted in epigeal germination (Rani & Datta, 2020). The calyx was persistent during fruit development and turned to blackishbrown color when the fruit was ripe (Figure 2h).

Anatomical Characteristics of The Aerial Parts of *A. augusta* L.

The observed anatomical characteristics of the aerial parts *of A. augusta* L. included the petiole and leaf blade. We also observed the stomata and trichomes on the surface of the aerial parts.

Anatomical Characteristics of The Petioles

The transverse-section of petiole of *A. augusta* L. showed various tissues, namely epidermis, collenchyma, cortical parenchyma, sclerenchyma, vascular bundle, and pith (Figure 3a, b). Each cell of the single-layered epidermis was cubical and externally covered by a thin cuticle, stellate and unicellular non glandular trichomes, and capitate glandular trichomes (Figure 3a, b, c). The epidermis was followed by the cortex consisting of some layer of collenchyma (Figure 3c) and polyhedral to isodiametric cortical parenchyma. Pericycle was represented by sclerenchyma fibers (thick-walled, lignified cells with pointed ends and narrow lumen) (Figure 3d). The vascular bundle consisted of the phloem, procambium, and xylem, with a collateral type (Figure 3d). The collateral bundle is a vascular bundle in which a phloem strand is present outside the xylem strand (Simpson, 2010). The stellar type was eustele, an apomorphic type in seed plants (Spermatophyta) (Simpson, 2010). The central zone (pith) consisted of isodiametric parenchyma with intercellular spaces. Mucilaginous ducts were present in the cortical parenchyma and periphery of pith parenchyma (Figure 3a, b). They may provide lubrication and protection against desiccation during early stages of organ development (Garcia et al., 2014). In addition, there were numerous clusters of ergastic compounds in the form of druse calcium oxalate crystals scattered in the collenchyma, parenchyma toward pith (Figure 3a, b, e). Ergastic compounds are cellular compounds that are not actively metabolized and only function as storage or waste (Simpson, 2010). The features of mucilaginous ducts, trichomes, and druse calcium oxalate crystals are commonly found in the petiole of Malvaceae, such as in *Theobroma grandiflorum, T. speciosum,* and *T. subincanum* (Garcia et al., 2014) and in *Abutilon hirtum* (Lam.) Sweet (Gomaa et al., 2016). Measurement of tissue thickness of *A. augusta* L. petiole is presented in Table 2.

Anatomical Characteristics of The Leaf Blade

Transverse section of *A. augusta* L. leaf showed various tissues such as upper and lower

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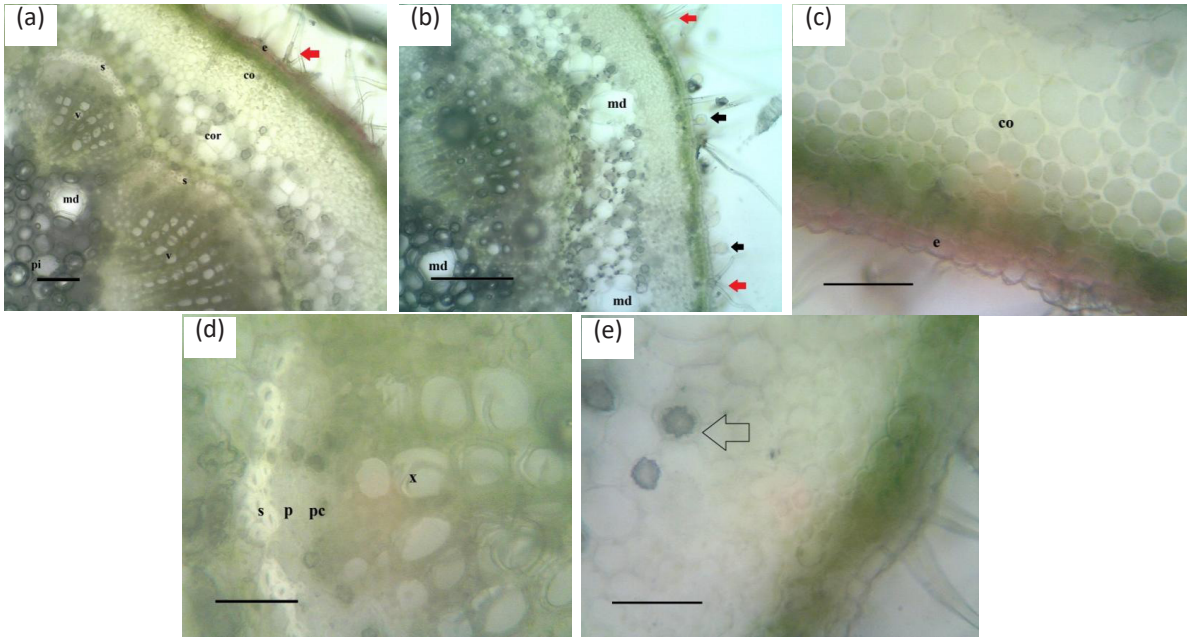

Figure 3. Anatomical characteristics of *A. augusta* L. petiole: (a,b) transverse section of the petiole (c) detail of epidermal and collenchyma tissue; (d) detail of the sclerenchyma-vascular bundle; and (e) druse Ca oxalate crystals shown by transparent arrows. (a,b) M: 100x; scale bar: 100 µm; (c,d,e) M: 400x; scale bar: 50 µm. Description: e (epidermis); co (collenchyma); cor (cortical parenchyma); s (sclerenchyma); v (vascular bundle); pi (pith); md (mucilaginous duct) p (phloem); pc (pro cambium); x (xylem). Red arrow shows stellate trichome, black arrow shows capitate glandular trichome.

Table 2. Tissue thickness of *A. augusta* L. petiole (N = 3)

Tissue	Thickness (µm)
Epidermis	24.15 ± 1.17
Chollenchyma	137.42 ± 3.63
Cortical parenchyma	190.44 ± 10.20
Vascular bundles	280.54 ± 48.38

epidermis, palisade, and spongy parenchyma (Figure 4a). The epidermis was composed of cubical and tangentially elongated cells covered with cuticles. Palisade and spongy parenchyma tissue were clearly differentiated in which the palisade parenchyma consisted of one layer and was arranged on the upper side while spongy parenchyma was arranged on the lower side. The bundle sheaths were not seen and maybe they were embedded in the mesophyll. The tissue thickness is presented in Table 3. The leaf was dorsiventral. The transverse section of the midrib showed epidermis, collenchyma, cortex, and vascular bundle (figure 4b). The epidermal cells were thick-walled and externally covered by striated cuticles and several types of trichomes. Numerous clusters of druse calcium oxalate crystals were scattered in the cortical parenchyma toward the pith of the midrib and spongy parenchyma.

Stomata and Trichomes

Stomata are holes in the adaxial and abaxial surface of leaves surrounded by two guard cells and some neighboring cells (Fahn,

1995; Simpson, 2010). The stomata can be found either on both surfaces (amphistomatic leaf) or on only one, either the upper surface (epistomatic leaf) or more commonly on the lower surface (hypostomatic leaf) (Chachad & Vaidya, 2016). The stomata developments and features (size, number, shape, density, and distribution) are influenced by genotype and environmental factors (Casson & Gray, 2008; Driesen et al., 2020). The study showed that stomata were found both on the adaxial and abaxial sides of the leaves of *A. augusta* L and distributed irregularly (Figure 5a-d). The stomata type was ranunculaceous (anomocytic) while the guard cells were kidney-shaped (Figure 5e). The stomata features are as presented in Table 4. The stomata density on the abaxial side of the leaves of *A. augusta* L. was higher than that of the adaxial side of the leaves (Table 4), the same as in other species of Malvaceae, such as *Urena lobata, Sida glutinosa, and Sida rhombifoliaa* (Dorly et al., 2016).

Another epidermal derivative is the trichome. Trichomes have a degree of diversity in each plant in type, shape, size, density, and have a function as plant protection against UV rays or herbivore attacks (Simpson, 2010). There were abundant stellate trichomes with three and some arms and unicellular non-glandular trichomes, and also capitate glandular trichomes both on the adaxial and abaxial leaf surfaces as well as on the petiole (Figure 5f, g). Capitate trichome consists of 1-2 unicellular bases (stalk) with a unicellular head (Jia et al., 2013).

Stellate trichome is a composed of considerable number of unicellular ray cells fused together in the center and this type is a specific characteristic in Malvaceae (Garcia

Table 3. Tissue thickness of *A. Augusta* L. Leaf (N = 3)

Tissue	Thickness (µm)
Upper epidermis	21.12 ± 0.97
Palisade parenchyma	66.40 ± 3.54
Spongy parenchyma	43.75 ± 3.47
Lower epidermis	22.53 ± 2.38

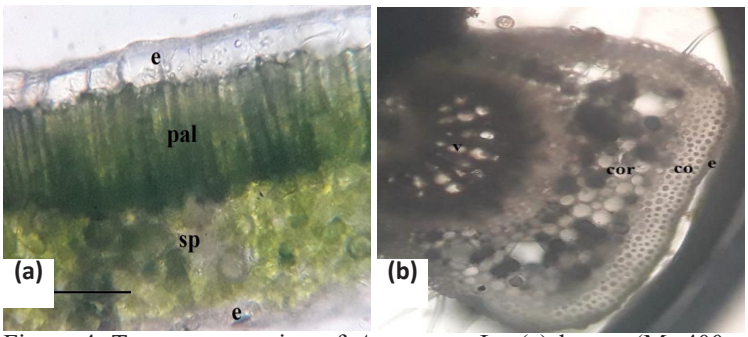

Figure 4. Transverse section of *A. augusta* L.: (a) leaves (M: 400x; scale bar: 50 µm); (b) mid rib (M: 100x with zoom in). Description: e (epidermis); pal (palisade parenchyma); sp (spongy parenchyma); co (collenchyma); cor (cortex); v

Table 4. Mean value of stomata variable of *A. augusta* L. leaves (N=3)

Variable	Adaxial Leaf	Abaxial Leaf
Stomata length (µm)	24.35 ± 1.14	21.77 ± 0.44
Stomata width (µm)	21.85 ± 0.69	14.84 ± 0.54
The distance between two stomata (µm)	44.37 ± 6.70	9.87 ± 2.70
Stomata number (M: 400x)	4-6	35-38
Stomata density (stomata number/mm^2) (M: 400x)	111.11 ± 22.21	800 ± 22.22

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et al., 2014; Gomaa et al., 2016; Shaheen, et al., 2009a). Trichome of *A. augusta* L. observed separately from the organs (leaves and/or petiole) was also shown (Figure 5g). The variation in type, morphology and distribution of trichomes are unique and thus, they can easily be used as an important tool in taxonomic character, especially in Malvaceae (Shaheen, et al., 2009a; Shaheen et al., 2009b).

Based on the morphological observation of the aerial parts of *A. augusta* L., our findings were the similar as those of previous studies (Parkash et al. (2019); Rani & Datta (2020), although the quantitative data sizes showed

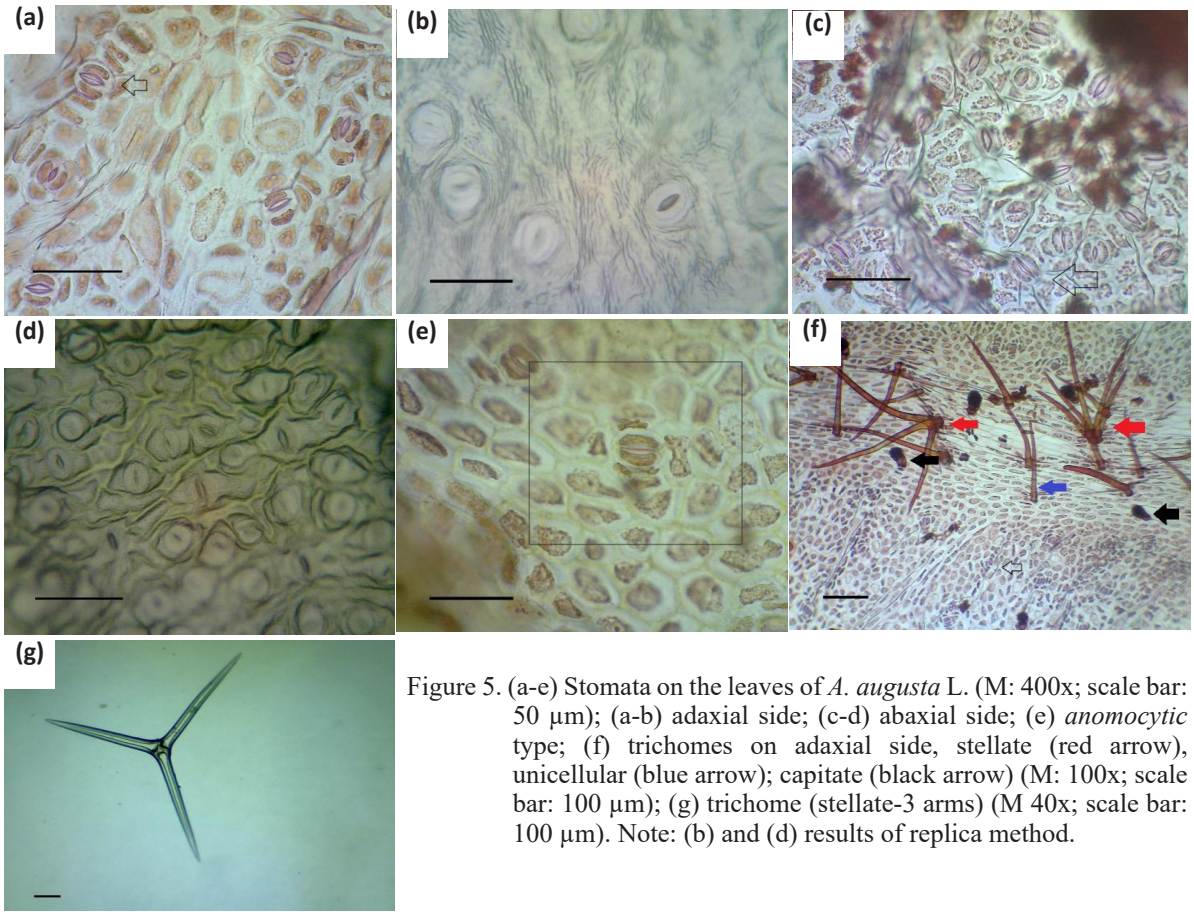

Figure 5. (a-e) Stomata on the leaves of *A. augusta* L. (M: 400x; scale bar: 50 µm); (a-b) adaxial side; (c-d) abaxial side; (e) *anomocytic* type; (f) trichomes on adaxial side, stellate (red arrow), unicellular (blue arrow); capitate (black arrow) (M: 100x; scale bar: 100 µm); (g) trichome (stellate-3 arms) (M 40x; scale bar: 100 µm). Note: (b) and (d) results of replica method.

the differences under influences of plant age, genetic, and environmental factors. As far as we know, we have found limited research discussed about the anatomy of the aerial parts (petioles and leaves) of *A. augusta* L. Parkash et al. (2019) have presented the anatomical characteristics of the stem of *A. augusta* L. which appropriated with anatomical characteristics of the petiole of *A. augusta* L, consisted of the epidermis with fine hair, collenchyma, sclerenchyma, phloem, pre-cambium strip, and vascular ray. We have compared the characteristics of *A. augusta* L. with other species of Malvaceae. We found mucilaginous ducts in the petiole of *A. augusta* L., the same as mucilage cavity in the stem of *Abutilon hirtum* (Lam.) Sweet (Gomaa et al., 2016). Some types of trichomes and druse calcium oxalate crystals were also found in the petiole of *A. augusta* L., the same as those in *Abutilon hirtum* (Lam.) Sweet (Gomaa et al., 2016). We also found many stellate

trichomes (sessile and stalked) in the petiole of *A. augusta* L., the same as in *Theobroma grandiflorum* and *Theobroma subincanum* (Garcia et al., 2014). The stomata type of *A. augusta* L. was anomocytic, the same with stomata type in *Abutilon hirtum* (Lam.) Sweet (Gomaa et al., 2016). The stomata distribution of *A. augusta* L. was on two sides (adaxial and abaxial leaves) in which the stomata density on the abaxial side was higher than that of the adaxial side, the same as those in other species of Malvaceae, such as *Urena lobata, Sida glutinosa,* and *Sida rhombifoliaa* (Dorly et al., 2016).

A species is grouped in a family because it has an apomorph feature as well as in *A. augusta* L. This species has some features including cotton fibers in the fruit, mucilaginous ducts in the petiole, several types of trichomes and druse calcium oxalate crystals in the petiole and the leaf which are specific features in the Malvaceae. These morphological and anatomical studies of this plant are quite important to support the plant identification as a part of conservation effort of the plant. Then, further studies are recommended to investigate the root morphology and anatomy and also biochemical characteristics of each part of the plant in order to obtain the complete plant identification.

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