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Anatomical Structure and Terpenoid Content of Zodia (*Evodia suaveolens* Scheff) Leaves

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Abstract. Zodia (*Evodia suaveolens* Scheff) is a member of Rutaceae contain terpenoids, triterpenoids, alkaloids, flavonoids, and xanthones which have anti-mosquito activity. This research aimed to observe the anatomical structure, the location, and distribution of terpenoid based on the leaves' age. Anatomical slides preparation of leaves were made using the paraffin embedding method with safranin staining. The distribution of terpenoid was analyzed by the histochemical assay. Leaf anatomical structure shows that the 3rd and 6th leaf bifacial (dorsiventral) consisted of the upper epidermis, mesophyll (palisade and sponge), collateral vascular bundle, parenchyma midrib, abaxial epidermis and oil glands in mesophyll that is underneath both epidermises. The diameter of oil glands with larger sizes was on the 6th leaf, whereas the density is not different in the 3rd and 6th leaves. The histochemical test showed that terpenoid was observed in the leaf vascular bundles, oil glands, and epidermis.

1 Introduction

Zodia (*Evodia suaveolens* Scheff) family Rutaceae, contain anti-mosquito terpenoids [1], triterpenoids [2], alkaloids [3], flavonoids, and xanthon [4]. Anti-mosquito compounds include essential oils Linalool [5], as well as alkaloid evodiamine [6] and rutaecarpine [7]. One of the mosquitoes types that must be controlled is *Aedes aegypti.* Linalool compounds in zodia plants as a mosquito repellent. [7,8,9,4].

Essential oils have benefits for cosmetic raw materials, for example, perfume and lotion [10,11]. The Indonesian Ministry of Agriculture (2006) has determined that *E. suaveolens* is included in the list of target plants which is classified as biopharma commodities (useful for medicines, health, and cosmetics purposes).

E. suaveolens woody plants (shrubs) can also be as an ornamental plant, which is originally from Papua. Indigenous Papuans have used leaves as a mosquito repellent by rubbing on the hands and feet [9].

E. suaveolens leaf has a distinctive aroma and is used by the community in the forest as an anti-mosquito, therefore it is necessary to observe the anatomical structure of the leaves of *E. suaveolens* to see the oil glands that contain essential oil compounds.

2 Materials and Methods

2.1 Plant materials

The materials used were the 3rd (young leave) and 6th (old leave) leaves from the tip stem of Zodia collected from our collection. Three replicate samples of leaves were taken from three different individual plants. The sample plant identification was done by Herbarium Bogoriense, Indonesian Institute of Sciences, Bogor, Indonesia.

2.2 Anatomical slides preparation

For permanent anatomical slides, the leaves transverse sections were prepared using paraffin embedding method, and safranin staining, according to the procedure of [12] with slight modifications. The leaves were fixed in FAA (40% Formalin: Glacial acetic acid: 70% Alcohol; 5:5:90) solution for 24 hours. Dehydration was done by soaking the samples in the alcohol with 70%, 80%, 95% concentrations, each for 30 minutes. De-alcoholization was done by immersing the samples in alcohol: xylol with a ratio of 3:1, 1:1, 1:3, and xylol 100% I and xylol 100% II, each for 30 min. The sample was immersed in a solution of xylol II, an infiltration process was then performed with paraffin: xylol (9:1) at 57°C temperature for 24 hours. The mixture of paraffin: xylol was replaced with pure paraffin at a fixed 57°C temperature for 24 hours. The sample was blocked in pure paraffin. The paraffin which contains a sample was then sliced using a rotary microtome with 20 µm thickness. The sample sections were stained using safranin.

2.3 Histochemical assay

The histochemical assay enables the identification and localization of specific substances within tissues. The methods depend on chemical reactions between the substance identified and its localization in a tissue section, and one or more reagents in which the tissue section is incubated. The histochemist tries to arrange matters so that the end product of the chemical reaction is both colored and insoluble, and therefore easily visible on microscopy [13].

The cellular distribution of terpenoid in plants can be observed using a histochemical method according to [14] with slight modifications. The leaves samples were thinly sliced using a hand microtome and razor blades. The thin transverse sections of leaves were then immersed for 24 hours in 5% copper acetate. After that,

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the sections of the sample were then covered with glycerol and observed under a microscope. Brownish-yellow color formation indicated a positive result for the terpenoid compound.

2.4 Data analysis

The qualitative anatomical and terpenoid content data obtained were analyzed descriptively. The quantitative data of the glandular cells density, in the 3rd and 6th leaves, were analyzed by t-test.

3 Result and discussion

3.1 Anatomical structure

Anatomical studies are critical to know the structure of the organ, the cells, and the tissues that are possible to synthesize secondary metabolite compounds [15].

The cross-section structures of the 3rd and 6th leaves showed almost the same in the anatomical structure. They consisted of upper (adaxial) and lower (abaxial) epidermises, as well as. The parasitic type of stomata. The number of stomata in the lower epidermal is relatively more than the upper one.

The leaf mesophyll of *E. suaveolens* is the dorsiventral or bifacial type, consists of palisade and sponge. Palisade parenchymal tissue is located one layer below the upper epidermis with long shape. Sponge tissue 5-6 hollow layers, irregular hexagonal shape, located under the palisade.

The oil glands in mesophyll are globular and underneath both epidermis (Figure 1A and B). This structure is larger than mesophylls, but the size is very prominent and almost half of the leaf cross-section. The shape of the leaf oil glands of *E. suaveolens* is similar to the leaves of *Eucalyptus polybractea* [16].

According to [17], the structure of secretion glands is also called an idioblast. The secretion cells have an elongated or larger size, so it is called a tube or sac. The secretion cell is larger than the parenchyma, located in the base parenchyma, as well as the vascular bundles of leaves and stems.

Vascular bundles consist of xylem and phloem of the collateral type. Sclerenchyma is on the outside of the vascular bundles. The oil glands in the upper and lower sections of the midrib (Figure 2).

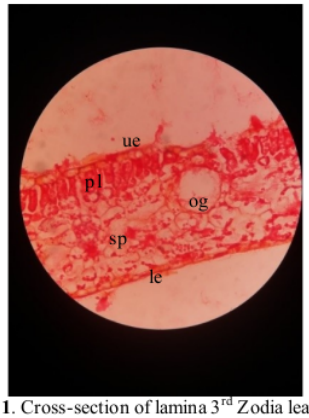

Figure 1. Cross-section of lamina 3rd Zodia leaves, at a magnification of x 400. Notes: upper epidermis (ue), palisade (pl), sponge (sp), abaxial epidermis (le), Oil gland (og).

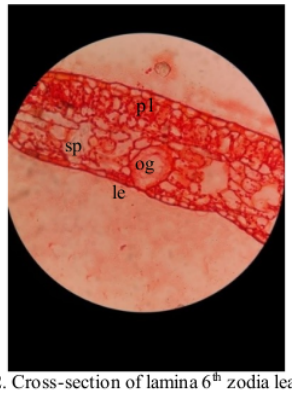

Figure 2. Cross-section of lamina 6th zodia leaves, at a magnification of x 400. Notes: upper epidermis (ue), palisade (pl), sponge (sp), abaxial epidermis (le), Oil gland (og).

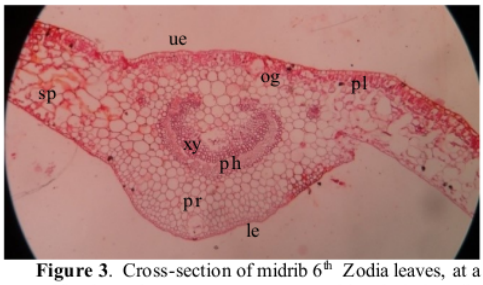

Figure 3. Cross-section of midrib 6th Zodia leaves, at a magnification of x 100. Note : upper epidermis (ue), palisade pl), sponge (sp), phloem (ph), xylem (xy), abaxial epidermis (le), parenchyma (pr), oil gland (og).

Table 1. Diameter and density of oil glands in the 3rd and 6th leaves of *E. suaveolens*

Leaf Sample	Diameter (µm)	Density (mm^2)
3rd	67.5	1.3
6th	77.5	1.2

The diameter of the oil glands in the 3rd leaf (67.5 µm) is smaller than the 6th (77.5 µm). Oil gland density has almost the same value on the 3rd leaf (1.3 / mm^2) and the 6th (1.2 / mm^2) (Table 1).

According to [18], plant growth is shown by increasing size, because multicellular organisms grow from zygotes. Consequently, it increases in volume, weight, number of cells, number of protoplasms, and level of complexity. The process of growth and development, both are the resulting in 3 simple stages at the cellular level. First, the cell division stage, adult cells divide into two separate cells, which are not always similar to each other. Second, the cell enlargement, one or both of the daughter cells enlarges in volume. The last is differentiation, where cells that have reached volume eventually become specialized in a certain way. Following these stages, in which cells divide, enlarge, and specialize have generated various types of plant tissues and organs, as well as many forms of plants.

The cell enlargement itself is the absorption process of water into the expanding vacuole, then stretching the cell wall. In this process, the driving force for growth is turgor pressure. The pressure inside the cell is due to the mechanical resistance of the cell wall to stretching. If this resistance is reduced then the wall is relaxed. The water potential decreases and the potential gradient of the water will increase, then the water will move into the cell causing an increase in the cell [18].

3.2 Terpenoid content

Histochemical assay of the 3rd and 6th leaf terpenoid compounds showed positive in the presence of brownish-yellow color in the vascular bundles, epidermis, and oil glands. (Figures 4 and 5).

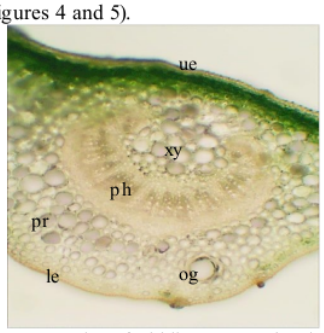

Figure 4. Cross-section of midrib *E. suaveolens* leaves before staining, at a magnification of x 520. Note : upper epidermis (ua), phloem (ph), xylem (xy), abaxial epidermis (le), parenchyma (pr), oil gland (og).

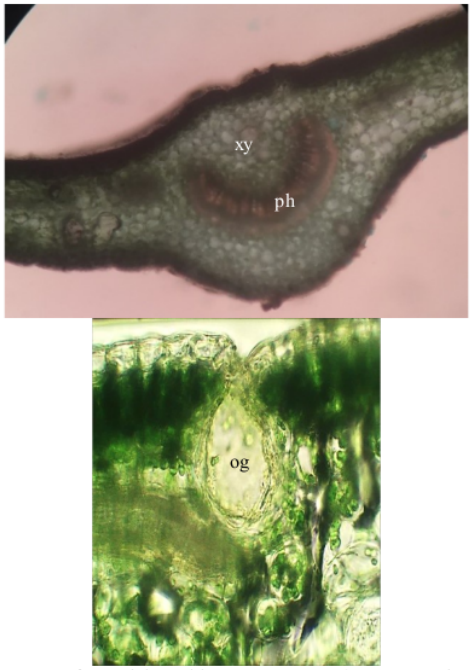

Figure 4. After staining with copper acetate A). Cross-section of midrib 3rd *E. suaveolens* Leaves, terpenoid is a vascular bundle, oil gland and epidermis showed brownish yellow color, at a magnification of x 100. B). Terpenoid in the oil gland showed brownish-yellow color, at a magnification of x 400. Oil gland (og).

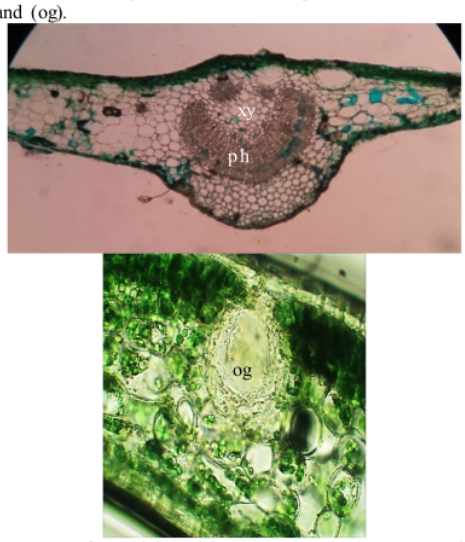

Figure 5. After staining with copper acetate A). Cross-section of midrib 6th *E. suaveolens* Leaves, terpenoid is in a vascular bundle, oil gland, and epidermis showed brownish yellow color, at a magnification of x 100. B). Terpenoid in the oil gland showed brownish yellow color, at a magnification of x 400. Oil gland (og).

The leaves of *E. suaveolens* contain essential oils, with a very characteristic aroma. According to [19], this substance is terpenoids found in the vaporized fraction, commonly referred to as « atsiri » (Indonesian). This substance causes the release of a special aroma in various plants as well, and anatomically produced in glandular cells in plant tissues Zodia has an oil gland shape that is similar to the oil glands in the leaf petioles of Citrus and the fruit skin of *Citrus liriodendron* [20].

4 Conclusion

1. Cross-section of zodia leaves composed of upper and lower epidermis, where stomata are located in both epidermises. Mesophyll consisting of palisade and sponges. The diameter of the oil gland is greater in the 6th leaf, while the density does not differ in the 3rd and 6th leaves. The oil glands in mesophyll are underneath both epidermises.
2. Terpenoids on the 3rd and 6th leaves are positive in the vascular bundles, oil glands, and epidermis.

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