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Secretory Structures, Histochemistry, and Antibacterial Activity of *Macaranga gigantea*

Evi Muliyah[1], Yohana C. Sulistyaningsih[2*], Sulistijorini[3], Mohamad Rafi[3]

[1]Department of Biology Education, Faculty of Educational Sciences, Universitas Islam Negeri Syarif Hidayatullah Jakarta, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Indonesia.
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Indonesia.

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Corresponding Author:
Yohana C. Sulistyaningsih
yohanasu@apps.ipb.ac.id

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Abstract: *Macaranga gigantea* is a member of the family Euphorbiaceae. Anak Dalam tribe, an indigenous people that live in Bukit Duabelas National Park Jambi province, used to utilize the bark of *M. gigantea* to treat diarrhea. This study aimed to identify the secretory structure, histochemical aspects, and antibacterial potency of *M. gigantea* bark. Microscopic preparations have been made for the observation of secretory structures. Histochemical testing uses Wagner reagent, cupri acetate, ferric trichloride, and aluminum trichloride. Antibacterial activity was tested using the well diffusion method. The type of secretory structure was carried out. *M. gigantea* bark has idioblast cells as a secretory structure. The idioblast cells are distributed in the cortex. The idioblast cells contain phenolic compounds, alkaloids, and terpenoids. The well diffusion method was used to test different concentrations of bark extracts. According to the findings, *M. gigantea* bark extracts at 100 mg/mL had the best inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*, with a maximal inhibition zone.

Keywords: Antibacterial Activity; Histochemistry; Idioblast Cells; *Macaranga Gigantea.*

Introduction

Macaranga is one of the largest genera in the *Euphorbiaceae* family and contains about 250 species. Macaranga plants can be found in Indonesia, Africa, Madagascar, Asia, the east coast of Australia, and parts of the Pacific Islands. Macaranga gigantea is a significant pioneer plant species in the tropical secondary forest of Kalimantan, although the appealing wood species has yet to be economically cultivated (Susanto et al., 2016). Phytochemical studies have shown that this genus is a rich source of phenolic compounds, especially isoprenylated and geranylated flavonoids and stilbenes, terpenoids, tannins, coumarins, and other types of compounds (Magadula, 2014).

Macaranga gigantea is known by Anak Dalam tribe as sangkarubungon. *M. gigantea* is also known by the local names as merkubung, mahang, and kayu kubung (Muhaimin et al., 2018; Sutedjo et al., 2021). The tribe of Anak Dalam uses this plant to treat diarrhea by drinking boiled water from the bark. Another part that is used from the *M. gigantea* plant is the leaves. *M. gigantea* leaves have antiplasmodial activity, which helps cure malaria (Muhaimin et al., 2019). *M. gigantea* leaves are also helpful as antioxidants and have cytotoxicity against cancer cells (Aminah et al., 2014; Arung et al., 2018). Investigating the active compounds responsible for these effects can validate and promote traditional knowledge and practices.

Plants have a unique structure that can produce secondary metabolites called secretory structures. Secretory structures can be secretory cavities, idioblast cells, glandular trichomes, non-glandular trichomes, laticifers, resin ducts, secretory cavities, and oil glands (Kuster & Vale, 2016; Almeida et al., 2020). Exploring the secretory structures in plants and their role in producing secondary metabolites provides insights into plant physiology and their ecological interactions. This knowledge enhances understanding of how plants have

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evolved to protect themselves and interact with other organisms, which is critical for ecological research.

Secretory structures in the form of ducts and cavities in the bark of *Larix gmelinii* and *L. kaempferi* contain secondary metabolites in the form of terpenoids (Seki et al., 2019). In the bark of *Cinchona ledgeriana* Moens, idioblasts were found in the cortex and secondary phloem, which include alkaloid, terpenoid, phenolic, and lipophilic compounds (Pratiwi et al., 2020). There have been many studies examining the leaves of *M. gigantea*, but studies on the bark of the stem still need to be made available. Therefore, this study aimed to identify the secretory structure, histochemical aspects, and antibacterial activity of the bark of *M. gigantea* used by the Anak Dalam tribe to treat diarrhea.

Method

Sample collection

The bark of *M. gigantea* was collected in Bukit Duabelas National Park, Jambi, Sumatra, Indonesia. Fresh samples are used for histochemical testing. In the antibacterial test, the sample was dried in the sun for three days and then in an oven at 50°C for five days.

Secretory Structures Observation

A frozen microtome was used to create a cross-section of the bark to observe the presence of the secretory structure of the bark. The secretory structure was observed by observing the sample preparations under an Olympus BX51 microscope equipped with an Optilab camera. The secretory structure of each sample was observed for its type, location, shape, size, and density. Idioblast cell density was determined by the following Equation 1 (Willmer & Fricker, 1996).

$$\text{Idioblast cell density} = \frac{The\ number\ of\ idioblast\ cells}{The\ area\ of\ field\ of\ view\ (mm2)} \quad (1)$$

Histochemical Tests

A dual-purpose microtome (Yamato RV240) was used to cut fresh bark at 20-25 µm for histochemical analysis. The bark sections were then treated with various reagents and examined under a light microscope to confirm the presence of alkaloids, phenols, terpenoids, and flavonoids. The presence of alkaloids was tested by immersing the sections in Wagner's reagent. Reddish-brown or yellow deposits indicate positive results. In the phenol test, sample sections were soaked in 10% trichloride, treated with several flakes of sodium carbonate, and then incubated at room temperature for 15 minutes. Positive results are indicated by a dark green or black appearance (Mazzoni-Viveiros & de Moraes Castro, 2016). Terpenoids were identified by immersing the sections in a 5% copper acetate solution (Demarco, 2017). A yellow or brownish-yellow appearance indicates positive results. The sample section was treated with 5% aluminum trichloride in 85% ethanol for flavonoid content and observed by fluorescence microscope. The positive result is indicated by the appearance of yellow, greenish yellow, or blue color (Guerin et al., 1971).

Plant Extraction

The dried sample was cut into small pieces and crushed into powder. The powder was extracted using the maceration method using methanol as the solvent. Next, the extraction result was evaporated by a rotary evaporator. The extract was then diluted with 10% dimethyl sulfoxide (DMSO) in 25, 50, 75, and 100 mg/mL concentrations.

Antibacterial Analysis

Antibacterial activity was tested using a well-diffused method (Gonelimali et al., 2018). *Staphylococcus aureus* and *Escherichia coli* were cultured in a sterile nutrient agar medium and suspended in a sterile nutrient broth medium. The cultures were incubated at 37°C for 24 hours and resuspended on a 1% liquid nutrient agar medium.

The agar well was created using a sterile 7 mm diameter corn borer. Different concentrations of 50 ml *M. gigantea* bark extract (100, 75, 50, and 25 mg/ml) were added to the plate wells. In addition, 50 µg/ml of tetracycline antibiotic was used as a positive control, and 10% DMSO was used as a negative control. The culture was incubated for 24 hours. Antibacterial activity was measured as the diameter (mm) of the apparent growth inhibition zone. All experiments were performed three times.

Result and Discussion

Secretory Structures

Secretory structures are crucial to plants because they attract animals, emit dissolved compounds, attract pollinators by giving nectar, and aid herbivore defense. They may also build up in a cell's vacuole as amorphous inclusions (as in tannin and oil cells) or crystals (as in idioblasts that include crystals) (Crang et al., 2018). There are various types of secretory structures. In this study, the secretory structures found in the bark of *M. gigantea* were idioblast cells. Idioblast cells can be found scattered in plant organs. The results showed that in the bark of *M. gigantea*, idioblast cells were found in the cortex (Figure 1). Some Solanum species have idioblast cells containing crystals scattered in the periderm, cortex, and axial parenchyma layers of the root (Matias et al., 2016). *Tontela micrantha* (Celastraceae) idioblast cells were found in the bark scattered in the periderm

and cortex (Mercadante-Simões et al., 2014). Idioblast cells in Brassicales, known as myrosine cells, were scattered in the transport tissue (Shirakawa et al., 2022). A plant can have several secretory structures. For example, Catharantus plants have secretory structures in the form of idioblast cells and laticifer cells (Uzaki et al., 2022).

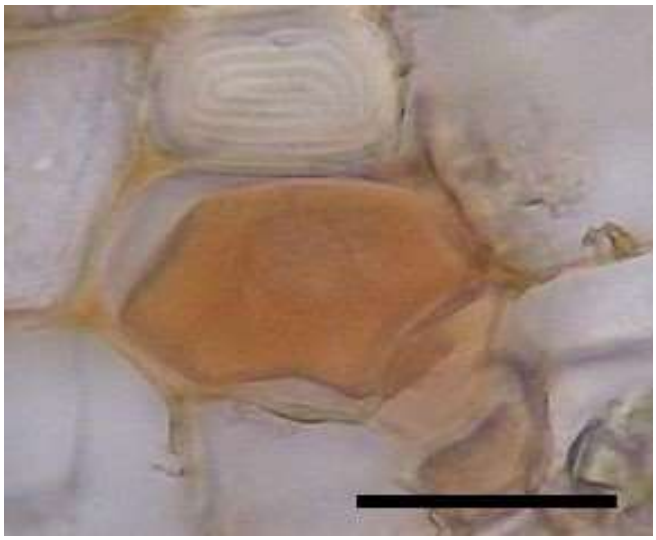

Figure 1. Idioblast cells of *M. gigantea* bark. Bar: 30 µm

Idioblast cells are specialized cells whose form and contents fluctuate from encompassing homogeneous cells (Hara et al., 2015). Idioblast cells have different shapes, sizes, and contents (Muliyah et al., 2018). In the bark of *M. gigantea*, the idioblast cells are cylindrical with a length of 68.3 ± 9.1 µm and a width of 27.3 ± 2.0 µm (Table 1). Cylindrical idioblast cells are also found in Chamaenerion plants, while circular ones are found in Epilobium taxa (Güven et al., 2021). The shape of idioblast cells in *Amorphophallus konjac* varies from circular to elongated. It is 15 times larger than the surrounding parenchyma cells. This idioblast has a diameter ranging from 150 to 600 µm (Chua et al., 2013).

Table 1. Size and density of idioblast cells

Density (mm^{-2})	Length (µm)	Width (µm)
2.30 ± 0.20	68.30 ± 9.10	27.30 ± 2.00

The density of idioblast cells in the bark of *M. gigantea* was 2.3 ± 0.2 mm-2. The size of the cells themselves influences the density of idioblast cells. The larger the cell size, the lower the density. On the other hand, if the size of the idioblast is small, the density will be higher.

Histochemistry of Idioblast Cells

Histochemical procedures determine chemical substances' presence, distribution, and density within biological cells and tissues in various organs. These approaches use the color-stain reaction technique and photographic documentation. These involve fixing specimens with various stains and examining them using microscopic tools. It is successfully used for the detection and localization of active cell constituents like proteins, carbohydrates, lipids, nucleic acids, and a variety of ionic elements present in cell solutions, as well as for characterizing secretory structures and determining the chemical makeup of secreted compounds (Badria & Aboelmaaty, 2019). The histochemical technique is a fast and inexpensive method that can be used to identify groups of bioactive compounds in tissue and cell compartments. In four medicinal plants, namely *Byrsonima verbascifolia*, *Campomanesia adamantium*, *Roupala montana,* and *Solanum lycocarpum*, most of the secondary metabolites were found in idioblast cells. Secondary metabolites are found in the form of alkaloids, flavonoids, essential oils, and lipids (Kuster & Vale, 2016)

The bark extract of *M. gigantea* contains phenolics, tannins, saponins, terpenoids, flavonoids, and alkaloids (Hidayat et al., 2019). However, the results of histochemical tests showed that the bark of *M. gigantea* contained only terpenoids and phenols (Table 2).

Table 2. Histochemistry result of secretory structures of *M. gigantea* bark

Reagent	Target compounds	Observed color	The presence of metabolites
Wagner reagent	Alkaloids	Reddish brown or yellow	-
Ferric trichloride	Phenols	Dark-green	+
Cupric acetate	Terpenoids	Yellow-Brownish yellow	+
Aluminum trichloride	Flavonoids	Yellow-green yellow or blue	-

Information:
(+) : positive result
(-) : negative result

The histochemical test of the idioblast cells showed a positive result for terpenoids, confirmed by the brownish-yellow color with cupric acetate reagent. Idioblast cells positively containing phenols showed the formation of dark color when the samples were added with Ferric trichloride reagent (Figure 2). Histochemical analysis has been widely studied on idioblast cells. Idioblast cells containing tannins were found in the lianas of Paullinieae (*Sapindaceae*) (Neto et al., 2017).

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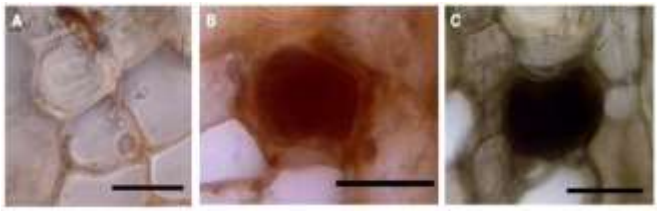

Figure 2. Light micrographs showing the response of idioblast cells to histochemical tests A). Control; B) cupric acetate for terpenoid; C). ferric trichloride for phenolic compounds. Bar: 30 µm

Antibacterial Activity

The extract of *M. gigantea* bark has inhibition activity against *S. aureus* and *E. coli,* shown by the appearance of an inhibition zone (Figure 3). A metabolite compound is considered to have inhibition activity against bacterial growth once the size of its inhibition zone is more significant than the excellent diameter (Aneja et al., 2012). The test results showed the maximum zone of inhibition against both bacteria at 100 mg/ml extract concentration of 15.00 ± 0.58 mm and 13.67 ± 1.20 mm, respectively (the well diameter was 7 mm) (Table 3). This result follows the research of Hidayat et al. (2019) that *M. gigantea* bark extracts with a concentration of 100 mg/ml had the best ability to inhibit the growth of *Enterococcus faecalis*.

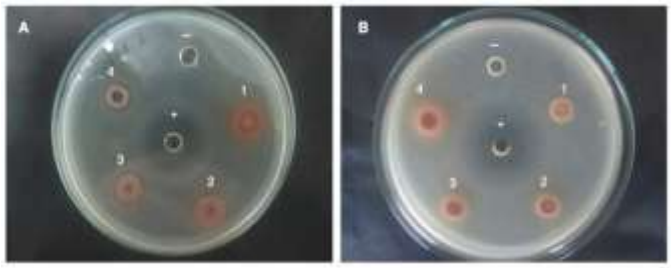

Figure 3. Antibacterial activity of *M. gigantea* bark extraction to *S. aureus* (A) and *E. coli* (B). The extract concentrations are 25 mg/mL (1); 50 mg/mL (2); 75 mg/mL (3); 100 mg/mL (4); tetracyclin 30 µg/mL (+); DMSO 10% (-)

At the lowest concentration (25 mg/ml), the bark extract still showed inhibition activity against both bacteria with a maximum zone of inhibition, 11,50±0,29 mm, and 9.67 ± 0.33 mm, respectively. Based on the lowest concentration of extract used, which was 25 mg/ml, the inhibitory activity against gram-positive bacteria was found to be more effective than against gram-negative bacteria. Differences in the composition of the bacterial cell wall cause the difference in the sensitivity of bacteria to plant extracts. The cell wall of gram-positive bacteria is composed of a thick layer of peptidoglycan. The coating is not effective as a barrier against hydrophilic molecules. The cell wall of gram-negative bacteria is composed of a thin layer of peptidoglycan and has an outer membrane of lipopolysaccharide which is toxic and more resistant to antibiotics. The membrane is impermeable to hydrophilic molecules.

Table 3. Antibacterial activity of *M. gigantea* bark extract against *S. aureus* and *E. coli*

Concentration (mg/ml)	Diameter of the inhibition zone *S. aureus* (mm)	Diameter of the inhibition zone *E. coli* (mm)
25	11.50 ± 0.29	9.67 ± 0.33
50	12.17 ± 0.17	12.00 ± 1.00
75	13.67 ± 0.88	13.00 ± 1.00
100	15.00 ± 0.58	13.67 ± 1.20
50 µg/ml Tetracyclin *	20.33 ± 0.33	16.67 ± 1.86
10% DMSO **	-	-

Information:
(*) : as a positive control
(**) : as a negative control

The criteria for determining the strength of an antibacterial effect are as follows, according to Febriani et al., (2019): an inhibition zone diameter of 5 mm or less is classified as weak, an inhibition zone of 5–10 mm is classified as moderate, an inhibition zone of 10–20 mm is classified as strong, and an inhibition zone of 20 mm or more is classified as very strong. Based on these criteria, *M. gigantea* has a strong inhibition against *S. aureus* and *E. coli.*

Conclusion

The *M. gigantea* bark has idioblast cells as a secretory structure. The histochemical test in *M. gigantea* shows that the idioblast cells mainly contain phenols and terpenoids. The extract at a concentration of 100 mg/ml showed the highest inhibitory activity and had a strong inhibition against *S. aureus* and *E. coli.*

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Author Contributions
Conceptualization, E.M. and Y.S.; methodology, Y.S.; validation, S.S., and M.R.; formal analysis, E.M and Y.S.; resources, Y.S.; writing—original draft preparation, E.M and Y..; writing—review and editing, S.S. and M.R.; supervision, Y.S.; project administration, E.M.; funding acquisition, Y.S.

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Conflicts of Interest
The authors declare no conflict of interest.

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