The content of chlorophyll, stomatal density and cuticle thickness of binahong (Anredera cordifolia (Ten.) steenis) accessions from Medan

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Abstract. The chlorophyll content and stomatal density in a plant vary greatly determined by plant genetic factors and plant growth environment. The research was aimed for evaluating and determining the chlorophyll content and stomata density of Medan's local binahong accessions which are lowlands. The research was conducted by survey method, by taking samples of local Medan binahong leaves, then analysed in a laboratory. Based on survey results, 8 accessions of Medan local binahongs have been obtained. The result of the research indicated that the accession of Medan 2 has the highest total chlorophyll content and stomatal density compared to other accessions. The highest of cuticle thickness was found in Medan 7 accession, while the lowest was in Medan 4 accession.

1. Introduction
Public interest in herbal medicine is currently increasing sharply. This causes the use and demand for herbal medicinal ingredients also increase. This is also supported by the rapid development of the traditional medicine industry sourced from phytopharmaca [1]. Binahong (Anredera cordifolia (Ten.) Steenis) in Indonesia is known as “gendola” and internationally well-known as Madeira vine [2].

Binahong is widely used as a medicinal plant, but has not been widely cultivated. Until now, only about 20% binahong for industrial raw materials has been obtained from cultivation, while the rest is obtained from forest. The part of binahong plant which has medicinal properties is derived from the stem, roots, leaf and flower [3].

Phytochemical compounds as secondary metabolites in binahong plants are very useful as a source of medicinal raw materials and antioxidants. Several researchers have reported that leaf of binahong contains phytochemical compounds such as flavonoids, triterpenoids, saponin, alkaloid, anthocyanin, carotenes, glucan, organic acid, mucopolysacharides such as D-galactose, L-arabinose, L-rhamnosa, aldonic acid, and also contain vitamin A, B, and C [4-5]. Binahong leaf contains flavonoid, saponin, monoterpenoid, sesquiterpenoid [6]. Phytochemical compounds contained in binahong are very useful as raw materials for medicinal plant. Binahong has multiple functions, as antioxidant, antihypercholesterolemia, anti-bacteria, anti-hyperlipidaemia, anti-obesities, anti-hypertension, wound healing, restore weak conditions after illness and prevent strokes [7-15].

Until now, previous research mostly the research of binahong reported that binahong was used as a raw material for phytopharmaca, the effect of binahong for medicinal or vegetative growth of binahong
[16-18]. The supply of raw materials mostly comes from wild plants in nature, or small-scale cultivation in the yard of the house so that the quantity and quality is inadequate.

Chlorophyll is the main pigment of plants which is widely used as food supplement. The function of the food supplement is to help for optimizing the metabolic function, the immune system, detoxification, anti-inflammatory and balance the hormonal system [18]. Chlorophyll also plays a role in stimulating blood formation because it provides the basic ingredients of forming haemoglobin. This role is due to the chlorophyll structure resembling blood haemoglobin with differences in the nucleus making up the porphyrin ring.

Until now, there are still few studies of binahong plants from various accessions as local resources related to the performance of the content of chlorophyll and the density of stomata of these plants. Therefore, this study aims to identify the performance of chlorophyll content of binahong accessions from Medan, and also examine the stomata density and cuticle thickness of the Medan binahong accession.

2. Materials and methods

2.1. Research site and materials

Binahong accessions obtained came from 8 locations from Medan. As plant materials in the research were leaf samples of binahong from Medan. Analysis of chlorophyll content, stomatal density and cuticle thickness were conducted at Laboratory of Plant Physiology and Tissue Culture, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara.

2.2. Procedures

The study was conducted by survey method by observing the location of binahong plant sampling found. The samples obtained are then taken to the laboratory for analysis. Chlorophyll analysis was carried out based on a method by Henry and Grime [19]. Determination of the content of chlorophyll was done by weighing 0.1 g of binahong fresh leaf sample from each accession, and extracted with 10 mL of 80% acetone solution. Each extract was filtered with a filter paper and analysed using a UV Vis spectrophotometer at wavelengths of 645 nm and 663 nm. The chlorophyll content was calculated by using the formula

\[
\text{Chlorophyll-a} = \left( \frac{12.7 \times A_{663} - 2.69 \times A_{645}}{10} \right)
\]

\[
\text{Chlorophyll-b} = \left( \frac{922.9 \times A_{645} - 4.68 \times A_{663}}{10} \right)
\]

\[
\text{Chlorophyll total} = \left( \frac{98.02 \times A_{663} + 20.2 \times A_{645}}{10} \right).
\]

The content of chlorophyll is expressed as mg L⁻¹.

Determination of stomatal density is conducting by printing stomata using a transparent of nail polish on the surface of binahong leaf, then drying and lifting carefully. The stomata impressed was observed under a microscope, to obtain clear visuality and note the type of magnification used (40 x 10). Stomatal density is expressed based on total number of stomata observed per area (unit.mm⁻²).

3. Results and discussions

3.1. The content of chlorophyll

The chlorophyll content in binahong accessions varies greatly. The highest of total chlorophyll, chlorophyll-a, and chlorophyll-b was found in Medan 2 accession, while the lowest of chlorophyll total, chlorophyll-a and chlorophyll-b was found in Medan 7 accession (Table 1). The differences in chlorophyll content is due to differences in the age of binahong accessions, plant genetic factors and the influence of plant growth environment.

Genes are an important factor in the chlorophyll formation. Certain genes contained in chromosomes form chlorophyll. Some researchers prove the presence of genes in plant that have the genetic ability to accumulate chlorophyll in photosynthesis [20-24]. The main difference between chlorophyll-a and b is their role in photosynthesis. Chlorophyll-a is the most important and main pigment involved in photosynthesis, which functions as the primary electron donor in the photosynthesis electron transport chain. Chlorophyll-b is an accessory pigment, collecting energy to enter chlorophyll-a [25].
The physiological status of plants can be expressed from the chlorophyll content of leaf [26-27]. The difference in chlorophyll levels in plants is due to the level of other pigments present in the leaves is more dominant or caused by the presence of adaptation factors in a plant. Binahong plants are tolerant of shade stress, so binahong have increased chlorophyll b content with low diversity between accessions in Medan. The tolerance mechanism of plants in shade stress is indicated by the high chlorophyll-b content. Chlorophyll-b is synthesized by the chlorophyll-a oxygenase (CAO) gene. In shade tolerance genotypes, it was found that partial expression of CAO genes was 3–4 higher than in shade-sensitive genotypes, both at 50% shade and dark. This proves the role of CAO gene in the mechanism of shading stress tolerance [28].

The content of total chlorophyll in dark leaves is 50% higher than light green leaves. This is because the dark green leaves contain chlorophyll which is more dominant than the light green leaves. At this stage of leaf development, a large amount of chlorophyll is synthesized from chlorophyll-a, which is followed by the development of the leaf. Chlorophyll b synthesis continues with the development of leaves which is characterized by the change in colour of light green to dark green leaves. The chlorophyll-a and chlorophyll-b are the main pigments found in thylakoid membranes. Several factors that influence the chlorophyll formation include genes, light, and the elements N, Mg, Fe as forming and catalyst in the synthesis of chlorophyll. All green plants contain chlorophyll-a and chlorophyll-b. Chlorophyll-a makes up to 75% of the total chlorophyll. The ability of leaves to photosynthesize also increases until the leaves are fully developed, and then begins to decrease slowly. Old leaves that are almost dead, turn yellow and are unable to photosynthesize due to damage to chlorophyll and loss of chloroplast function [29].

| Table 1. The content of chlorophyll of binahong accessions from Medan |
|---------------------------------|----------------|----------------|----------------|
| Accession | Chlorophyll-a (mg/L) | Chlorophyll-b (mg/L) | Total chlorophyll (mg/L) |
| Medan 1 | 12.37 ± 2.00 | 6.43 ± 2.35 | 18.80 ± 2.02 |
| Medan 2 | 17.22 ± 0.97 | 10.03 ± 1.47 | 27.25 ± 0.42 |
| Medan 3 | 10.74 ± 0.96 | 5.80 ± 0.70 | 16.55 ± 1.90 |
| Medan 4 | 14.47 ± 1.93 | 8.09 ± 1.39 | 22.56 ± 1.22 |
| Medan 5 | 9.80 ± 0.10 | 5.10 ± 1.40 | 14.90 ± 0.99 |
| Medan 6 | 15.59 ± 1.50 | 8.55 ± 2.66 | 24.15 ± 0.35 |
| Medan 7 | 3.94 ± 0.13 | 2.62 ± 1.37 | 6.56 ± 0.19 |
| Medan 8 | 9.37 ± 2.10 | 6.18 ± 0.97 | 15.55 ± 1.01 |

3.2. Stomatal density
The density of stomata in binahong accessions in Medan varies considerably. The highest stomata density at accession Medan 4, while the lowest stomata density at accession in Medan 4 (Fig. 1). The difference in stomata density of each accession is highly dependent on genetic factors. Variations in stomata competence in genotypes affect the ability of gas transportation [30].

Stomata are pores surrounded by special parenchyma cells, called guard cells. Stomata have two main function, namely allowing the exchange of gases to act as an entry point for carbon dioxide (CO₂) and release (O₂). Another main function is to regulate the movement of water vapour through transpiration. Stomata vary shape and size, able to adapt to various environmental factors, thus ensuring optimal conditions for photosynthesis process.

The previous studies reported that the physical leaf traits influence leaf function and environmental gradients. The function of leaves can be changed by stomata, because there is a change in stomatal density that can be affect CO₂ absorption, leaf cooling and the potential for air loss. Stomata can influence the function of leaf, with stomata can influence leaf function, with change in stomatal density and size that affect CO₂ uptake, cooling the leaves and the potential for water loss [30-31]. Stomata influence the photosynthesis rate. A pair guard cell is a constituent of stomata. Stomata play a role in
the exchange of gases from the atmosphere. Each leaf has its own stomatal density level. The stomatal density affects the rate of photosynthesis in plants [32].

3.3. Cuticle thickness

Based on Table 2, it can be seen that the highest of cuticle thickness was found accession 7, while the lowest of cuticle thickness was found in accession 4. The cuticle is a waxy layer on the surface of leaves and stems, serves to prevent the occurrence of dryness in plants. The layer of cuticle is located in the outer portion of epidermal cells, especially in parts of plants that grow on the ground such as stem and leaves. The cuticle as an outer coating on the body parts of organisms is strong and flexible, serves for protection so that the function of photosynthesis can run smoothly.

The difference in cuticle thickness of binahong accessions from Medan indicated that there is an adjustment to the growth environment. Previous researches reported that the mechanism of structural changes to the environment is related to the anatomical character includes the presence of a waxy layer, cuticle thickness, density and size of stomata, cell nucleus and trichomata. The cuticle has a function as a protective tissue for leaf cells. This function includes protection against sunlight penetration and the entry of CO₂ into leaf cells [33].

| Accession | Density of stomata (unit. mm⁻²) | Cuticle thickness (µm) |
|-----------|---------------------------------|------------------------|
| Medan 1   | 33.33 ± 3.86                    | 49.21 ± 4.86           |
| Medan 2   | 52.61 ± 2.67                    | 54.51 ± 1.71           |
| Medan 3   | 30.92 ± 1.16                    | 72.47 ± 2.35           |
| Medan 4   | 28.11 ± 1.06                    | 14.81 ± 2.09           |
| Medan 5   | 37.75 ± 1.06                    | 32.16 ± 1.99           |
| Medan 6   | 37.75 ± 1.81                    | 64.16 ± 3.99           |
| Medan 7   | 30.65 ± 1.41                    | 73.03 ± 2.73           |
| Medan 8   | 33.87 ± 3.15                    | 66.41 ± 2.49           |

4. Conclusion

The content chlorophyll in each accession varies greatly. Accession of Medan 2 has the highest total chlorophyll content and stomatal density compared to other accessions. The highest of cuticle thickness was found in Medan 7 accession, while the lowest was in Medan 4 accession.

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