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A COMPARATIVE STUDY OF MORPHO-ANATOMY, THE CONTENT OF CHLOROPHYLL AND ASCORBIC ACID ON ARDISIA HUMILIS THUNBERG IN THE AREA WITH DIFFERENT LIGHT INTENSITY AT THE NATURE PRESERVE OF PANANJUNG PANGANDARAN, WEST JAVA, INDONESIA

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Abstract

This study was conducted in order to seek out different morpho-anatomy responses, the content of chlorophyll and ascorbic acid in Ardisia humilis Thunberg in the area of different light intensity in the nature preserve of Pangandaran. The determination of the location was based on the existence of Ardisia humilis Thunberg in the area both with direct exposure of sun light and not. The location to obtain the open area-sample was located behind the office of Conservation of Natural Resources (BKSDA) with the light intensity of 922.000 Lux. The light intensity of the area without direct sun light exposure was 7906.6 Lux and the location was in Ciborok. Three leaves of three different plants were obtained as the samples. The observed parameter was the width and thickness of the leaves, the density of stomatal, the thickness of palisade, the content of

chlorophyll and ascorbic acid. Ascorbic acid test used the method of iodometric titration. The result of the study showed that the average of the leaf thickness, leaf area, stomatal density, palisade thickness, chlorophyll content, and ascorbic acid on sun leaves were 0.25 mm, 46.032 cm^2, 132.48 cells/mm^2, 54.89 µm, 10.88 CCI, and 0.0077 mg/g, respectively; while, for shade leaves were 0.23 mm, 57.159 cm^2, 116.63 cells/mm^2, 47.66 µm, 32.41 CCI, and 0.0107 mg/g, respectively. In the sun area, it has higher average of leaf thickness, lower level of surface, higher level of stomata density and palisade thickness, and lower average of chlorophyll and ascorbic acid content compared to the one in the shade area.

Keywords

Light Intensity, Morpho-Anatomy, Chlorophyll, Ascorbic Acid, *Ardisia Humilis*

1. Introduction

Plants growing in Indonesia are used as a source of carbohydrates and protein; they are also rich in fiber, antioxidants and micronutrients, such as vitamins and minerals which benefit human beings (Purwono & Purnamawati, 2007). One of the vitamins needed by the body of human in large amount is vitamin C. Pacier & Danik (2015) assert that vitamin C or ascorbic acid is an important component on human health and is considered a functional food as it is an bioactive compound with its antioxidant nature.

One of the plants growing at the Nature Preserve of Pananjung Pangandaran is Lampeni (*Ardisia humilis* Thunberg). According to Abdullah & Dewi (2010) People use Lampeni (*Ardisia humilis* Thunberg) for various purposes including food supply obtained from its fruit and young leaves. Fruit and leaves of Lampeni (*Ardisia humilis* Thunberg) are sour in terms of taste. That such sour taste in plants is possibly caused by the presence of vitamin C (Aak, 1991).

Lampeni (*Ardisia humilis* Thunberg) is found easily in the wild. This species is a tolerant plant under the shade and usually grows under a tree canopy (Dominguez, Edwards, & Subler, 2002). However, based on the field survey, Lampeni (*Ardisia humilis* Thunberg) is also found growing under direct sunlight. Light intensity is one the main factors affecting plant growth. Each plant species owns different light intensity requirement for its optimum growth; under low or high intensity of light, plants respond differently as a form of adaptation including morphology, anatomy, and physiology (Kong, Li, Wang, & Ban, 2016).

Morphological responses are observable from the width and thickness of the leaves, while anatomical responses are observable from the stomata density and the thickness of palisade layer of the leaf as well as the chlorophyll content as a physiological response. According to Murchie & Horton (1997), The shade leaves have wider area and serve to capture more sunlight, while the sun leaves are smaller and thicker. Kaufman (1989) mentions that when the shade leaves and the sun leaves are compared, the thick palisade anatomy of the sun leaves is thicker due to its layered palisade mesophyll, the weight of the leaves is heavier than the shade leaves, it also has more chlorophyll in terms of dry weight. In addition, light intensity affects the synthesis of vitamin C through the process of metabolism. Harris & Karnas (1989), The diversity of light intensity indirectly alters the rate of precursor formation and does not affect the conversion of precursor to ascorbic acid (vitamin C) or the amount formed in the process of plant metabolism.

Within this study, the comparison between morpho-anatomy response, chlorophyll and ascorbic acid content in Lampeni (*Ardisia humilis* Thunberg) leaves in the areas of different light intensity is conducted.

2. Materials and Methods

2.1 Leaf Thickness and Leaf Area Measurement

Fresh and turgid samples were used. For leaf thickness, samples were measured with mircometer screw. For leaf area, leaf samples was replicated in milimeter block paper. With gravimetri method, the replicas were weighed to measure leaf area. The result was compared with average standard paper weight in 1 cm^2. Leaf area was measured with this equation

$$\text{Leaf area } (1\ cm^2) = \frac{\text{Leaf replica weight (gram)}}{\text{Weight of standard paper (gram)}} \text{ x Standard paper area (1cm)} \quad (1)$$

2.2 Somatal Density

Replica method was used in stomata density observation. First, leaf was polished with clear nail polish and let it dry. Once the polish dried, leaf was taped with clear tape. The tape was removed and stomata density was observed with microscope in 400x magnification. The result was calculated with this equation

$$\text{Stomata density (sel/mm2)} = \frac{\text{amount of stomata (cell)}}{\text{microscope wide field of view (mm2)}} \quad (2)$$

$$\text{Microscope wide field of view in 400x magnification} = \tfrac{1}{4}\,\pi\, d^2 \quad (3)$$

$$= \tfrac{1}{4} \text{ x } 3.14 \text{ x } (0.5)^2$$

$= 0.19625 \text{ mm}^2$

2.3 Chlorophyll Content Measurement

Chlorophyll content was measured with chlorophyll meter. Leaf sample was taken as many as 3 strands of 3 different individuals in shaded area and sun area. Measurement on each leaf was taken as many as 5 repetition to got an accurate data.

2.4 Environmental Parameter Measurement

There were many environmental parameter that had to measured, such as light intensity, temprature, humidity, and pH. Lux meter was used to measure light intensity, thermohigrometer was used to measure temprature and humidity, and soil tester was used to measure soil pH. Each environmental parameters were measure at sun area and shaded area.

2.5 Ascorbic Acid Content Analysis

Analysis of ascorbic acid content is conducted using iodometric titration method. The leaf sample is grounded using mortar. The grounded material (slurry) is taken 3 grams and put in the 100 mL beaker glass. The distilled water is added up to 40 mL, then filtered using filter paper. The filtrate is taken 2 mL and put in the 100 mL Erlenmeyer flask, then 20 mL distilled water and 1 mL of 1% starch solution are added. The next stage is titration using standard iodine solution of 0.01 M made of KI and iodine until both forming blue solution. FAO (2015) states that in 1 mL of 0.01 N iodine solution used is equivalent to 0.88 mg of ascorbic acid, so the calculation of ascorbic acid content is done by multiplying the volume of iodine solution used in the titration process by 0.88 mg. Then, the calculation of the content is obtained from the following formula

$$\text{Ascorbic acid content } (\%\ {}^{b}/_{b}) = \frac{V\ I_2 \times N\ I_2 \times 8.808}{\text{mg sampel} \times 0.1} \times 100\% \qquad (4)$$

3. Result and Discussion

Lampeni (*Ardisia humilis* Thunberg) leaf sampling is carried out in two areas of different light intensity: sun area (behind the office of Nature Conservation Agency Pananjung Pangandaran) and shaded area (Ciborok). The light intensity shown in the sun area is 922.000 Lux, while the shaded area shows lower light intensity of 7906.6 Lux. Different light intensity in both areas also result in higher temperature behind the office of Nature Conversation Agency (BKSDA) of 28^{o}C compared to Ciborok area of 26.2^{o}C. This is in accordance with the

description of Croxdale (2000) stating that if the light intensity in an area is high, the temperature will be high as well. Environmental parameters, namely light intensity, can affect the growth of plants. Low or high level of light intensity in plants brings different response such as morphology, anatomy, and physiology (Kong et al., 2016).

3.1 The Effect of Light Intensity on Leaf Morphological Response

Observation of morphological response in a plant can be seen from the width and thickness of leaves. After measuring the two parameters on the sample of Lampeni (*Ardisia humilis* Thunberg) leaves in two areas of different light intensity, the results are obtained as shown in Figure 1.

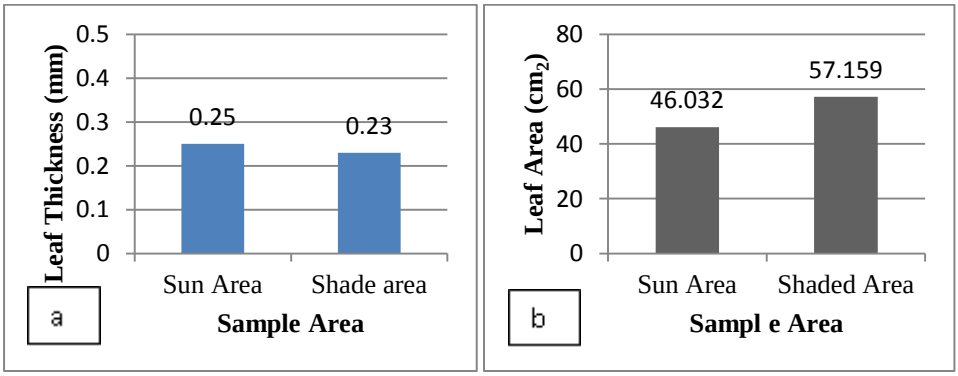

Figure 1: *Average graph of Ardisia humilis Leaf thickness (a) and Leaf Area (b) in sun area and shaded area*

In Figure 1 (a), it is seen that the average measurement result in terms of thickness in *Ardisia humilis* leaves in the sun area and shade areas are not significantly different. In the shade area, the average leaf thickness is 0.23 mm, while the average leaf thickness in the sun area is 0.25 mm. Leaf thickness is determined by the level of light intensity received by each plant and it is related to the diffusion process in the mesophyll layer. The obtained results are in accordance with the statements by Terashima, Miyazawa & Hanba (2001) and Terashima, Miyazawa, Yano, Hanba, & Kogami (2006) stating that thicker leaves have higher obstacle diffusion of CO_2 in the intercellular space and require more energy in forming and maintaining leaf cells, but the thicker leaves have higher level of surface area in which the chloroplast facing the intercellular space (Sc). Sc increase shows an increase in CO_2 diffusion area, so that it increases the assimilation of CO_2. At high level of light intensity, the plant has sufficient energy in order to form cell and increase Sc, whereas in low level of light intensity, the plant reduces the intercellular space and the formation of mesophyll cells which result in decreased leaf thickness.

The average leaf area obtained shows an inverse result with the leaf thickness. The sun leaves have narrower surface of 46.032 cm^2, while the shade leaves have wider surface of 57.159 cm^2 (Figure 1.b). The shade leaves have wider surface which serves in order to enlarge or maximize the absorption of sunlight, and the sun leaves have narrower surface in order to reduce evaporation. Levitt (1980) asserts that increased leaf area under low level of light intensity is one of the adaptation mechanisms in order to obtain optimal light. Avoidance in terms of light deficit is achievable by increasing the interception of light capture efficiency, namely by increasing the area of light capture through increasing the leaf area per plant tissue unit. Musyarofah, Susanto, Aziz, & Kartosoewarno (2007) mentions that shade brings impact to the leaf area which means that the higher the level of shade is the wider the leaf area.

Figure 2: *Ardisia humilis Thunberg Leaves in Sun Area (Left) and shaded Area (Right) (Personal Doc)*

A rather observable difference in the leaf sample of sun and shade areas is in terms of color (Figure 2). The shade leaves are greener than the sun leaves. This corresponds to the study conducted by Ardhie (2006) on *Hoya diversifolia* leaf. In lower level of light intensity, the leaf color is greener than in higher light intensity. The leaf color is greener as the decrease in terms of light intensity is due to higher chlorophyll content in leaves of low light intensity.

3.2 The Effect of Light Intensity on Leaf Anatomical Response

Observation in terms of leaf anatomical structure in sun and shade areas is seen from two characteristics: the thickness of palisade and the density of stomata. The results of the measurements of these characteristics are shown in Figure 3.

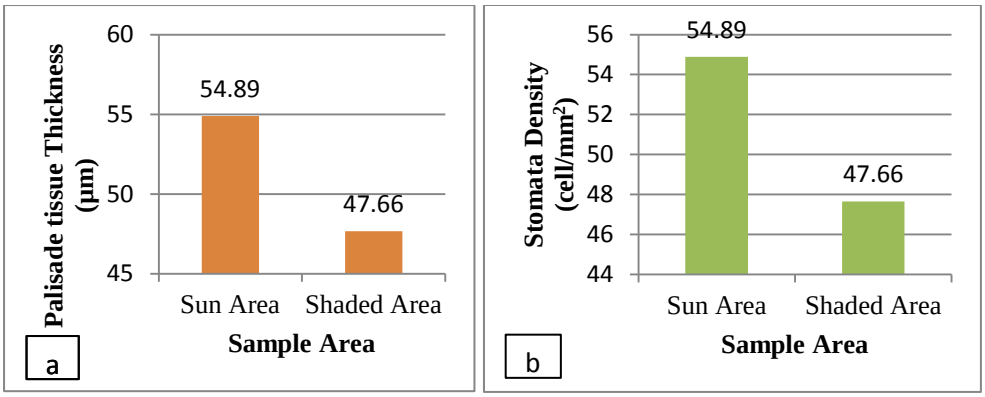

Figure 3: *Average graph of Ardisia humilis Palisade tissue thickness (a) and stomata density (b) in sun area and shaded area*

In Figure 3 (a), it appears that the average palisade thickness of the leaf in the sun area is higher at 54.89 µm, while the palisade thickness of the leaf in the shade area is 47.66 µm. Changes in the anatomical structure of the leaf-forming tissue are inseparable from its morphological structure of the leaf. If the leaf is thin, it will form a thin tissue, as well, and vice versa. In Figure 4, the leaf in the shade area (right) has thinner palisade than the one in the sun area (left). Simms, Seeman, & Luo (1998) mention that increased leaf thickness in high level of light intensity is due to the increased number of layers and mesophyll cell size, both spongy and palisade mesophyll. Widiastuti, Tohari, & Sulistyaningsih (2004) also state that in low level of light intensity, plants produce larger and thinner leaves with thin epidermal layer, little palisade tissue, wider intercellular space and more stomata.

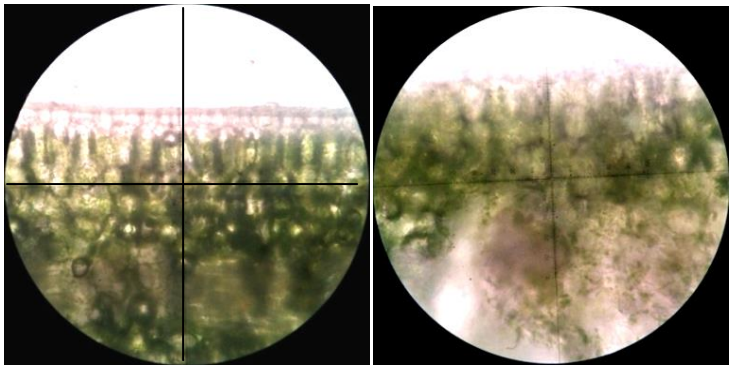

Figure 4: *Palisade tissue thickness of Ardisia humilis Thunberg Leaf in sun Area (Left) and shaded Area (Right) with 100x magnification (Personal Doc)*

Figure 5 shows the distribution of stomata on the paradermal incision of *Ardisia humilis* Thunberg leaf in two different areas. The type of stomata in *A. humilis* is parasitic/ Rubiaceous.

Mulyani (2006) opines that parasitic stomata is recognizable easily with the characteristic of each guard cell accompanied by one neighbor cell or more with its long axis parallel to the axis of the guard cell. Fahn (1991) states that the parasitic type has its longitudinal axis parallel to the axis of the neighbor cell, found in Rubiaceae and Magnoliaceae

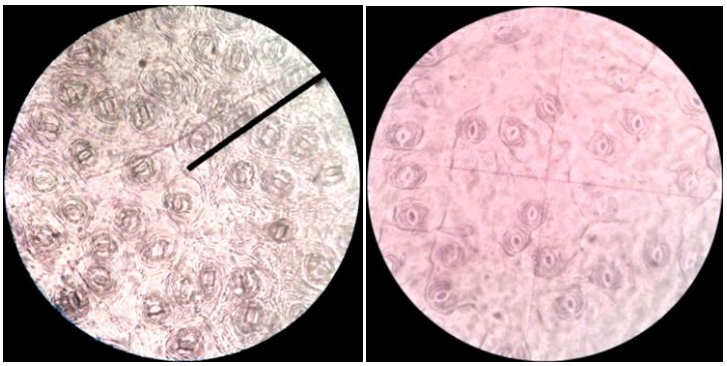

Figure 5: *Comparison of stomatal distribution pattern on lower epidermis surface Ardisia humilis Thunberg leaf in Sun Area (Left) and shaded Area (Right) with 400x magnification (Personal Doc.)*

The average number of stomata on the leaf surface of the sun area is 26 cells, while the average number of stomata on the leaf surface of the shade area is 23 cells. Stomata density can be seen from the average number of stomata obtained from the observation. The average of stomata density in *Ardisia humilis* leaf of the sun area is higher (132.48 cells/mm^2) than in the shade area (116.63 cells/mm^2). It is glaring that the higher the light intensity is the higher the stomata density. This aligns to the statement by Paluvi, Mukarlina, & Linda (2015) mentioning that the higher the light intensity is, the higher the stomata density on both surface. The density and the large number of stomata are the adaptation process of the plant towards the environmental condition. Light intensity affects the ambient temperature which means that the higher the light intensity is the higher the temperature.

The observation of stomata preparation in high level of light intensity (sun area) shows large size of closed stomata cells. This is in contrast to the stomata preparation in the shade area which has smaller size of opened cells.

3.3 The Effect of Light Intensity on Chlorophyll and Vitamin C Content

The total chlorophyll content is significantly influenced by the amount of sunlight received by the plants. It is proven on the result of leaf chlorophyll content in the sun and shade area shown in Figure 6.

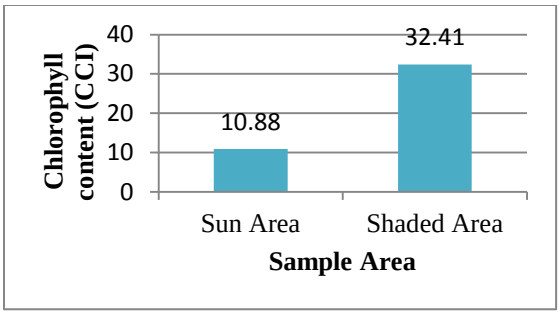

Figure 6: *Average graph of chlorophyll content Ardisia humilis Thunberg leaf in sun area and shaded area*

Figure 6 shows that the sun leaves have significantly lower chlorophyll content than the shade leaves. The average chlorophyll content in the sun area is 10.88 CCI, while the leaf in the shade area has the average chlorophyll content of 32.41 CCI. It shows that the higher the shade is around the area where the plant is growing, the chlorophyll content increases. Juhaeti, on her study regarding *Thyponium flageliforme* leaves (2001), states that shade increases the content of both chlorophyll a and b, such that the total chlorophyll increases. Shade treatment significantly affects chlorophyll a content and total chlorophyll. High level of chlorophyll content is obtained from 75% shade which shows a significantly large value of 130.31%

If seen from the leaf morphology, the leaf in the shade area has darker tint of green. The Leaf Color Chart can help determine the amount of chlorophyll content within the leaf. According to Ardhie (2006), leaf color is greener along with decreased light intensity for it is caused by the higher chlorophyll content within the leaf in the area of low light intensity. The leaf in the shade area can maximize the absorption of light and use such energy in order to produce chlorophyll which enables it in order to use the limited amount of light received.

Besides chlorophyll content, the physiological response of *Ardisia humilis* leaves influenced by light intensity is the content of ascorbic acid. Figure 7 displays the result of iodometric titration in order to determine the amount of ascorbic acid content within the leaf of two different areas.

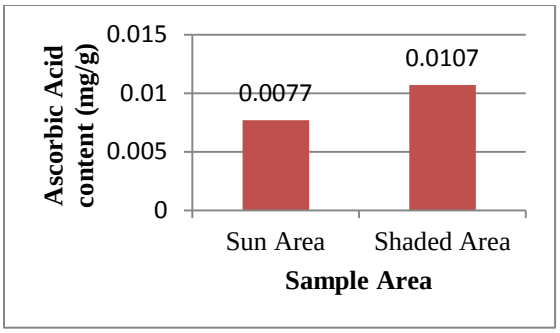

Figure 7: *Average graph Ascobic acid content Ardisia humilis Thunberg leaf in sun area and shaded area*

Based on the result shown in Figure 7, it can be seen that *Ardisia humilis* leaf in the shade area has higher content of ascorbic acid of 28.125% compared to the one in the sun area. The average content of ascorbic acid in *Ardisia humilis* leaf in the shade area is 0.0101 mg/g and 0.0077 mg/g for *Ardisia humilis* leaf in the sun area. Low level of ascorbic acid in the sun area is caused by low content of water within the leaf due to the excessive exposure to sunlight. Water content plays an important role in the determination of ascorbic acid content due to the water-soluble nature of ascorbic acid. High level of light intensity causes the leaf to evaporate excessively which leads to decreased ascorbic acid content. This is in accordance with the statement of Nasution on his study regarding the content of ascorbic acid in spinach (2010) which states that sunlight is the main energy for the process of photosynthesis in green plants, the water content in spinach undergoes evaporation, so that the content of glucose as the raw material of synthesis affects the content of ascorbic acid (reduced). In this case, water plays the role of raw material as well as reaction medium in spinach.

Environmental conditions greatly affect the secondary metabolite and nutrient in a plant. The role of environmental factors includes light intensity, temperature, altitude, humidity, etc. According to Fatchurrozak, Suranto, & Sugiyarto (2013) on their study regarding *Carica pubescens*, the higher the height of the place is the higher the environmental stress, namely lower temperature, higher humidity, lower level of light intensity, and shorter duration of irradiation. Temperature, light, humidity and other stress affect the production of plant secondary metabolites. When plants experience stress, secondary metabolite production including the production of ascorbic acid increases, too. This is the effort in order to fight environmental stress. The lower the height of the place is the light intensity and the temperature get higher

meaning that the content of ascorbic acid is easily oxidized, such that the ascorbic acid content at 1400 m amsl (above mean sea level) is lower than at the height of 1900 and 2400 m amsl.

4. Conclusion

Based on the conducted study, it can be concluded that light intensity affect the morpho-anatomy response, chlorophyll and ascorbic acid content on *Ardisia humilis* leaf. In the sun area, it has higher average of leaf thickness, lower level of surface, higher level of stomata density and palisade thickness, and lower average of chlorophyll and ascorbic acid content compared to the one in the shade area. Hope that this research can be used as an advanced study material on plant physiology such as plant secondary metabolites or tissue culture.

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