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# QUANTIFICATION OF CHLOROPHYLL CONTENT IN LEAVES OF *AVICENNIA GERMINANS, LAGUNCULARIA RACEMOSA, DURIO ZIBETHINUS, GARCINIA MANGOSTANA, SPINACIA OLERACEA, PIPER BETLE, PIPER SARMENTOSUM* AND *CENTELLA ASIATICA*

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**Abstract:**

This study aimed to determine chlorophyll concentrations in *Avicennia germinans*, *Laguncularia racemosa*, *Durio zibethinus*, *Garcinia mangostana*, *Spinacia oleracea*, *Piper betle*, *Piper sarmentosum*, and *Centella asiatica*. The analytical procedures required 250 mg of leaf sample to be macerated with 10 ml of 80% acetone using a pestle and mortar. The extract was centrifuged for 5 minutes at 5000 rpm. Then, the solution was transferred into a 25 ml

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volumetric flask and made up to 25 ml using 80% acetone. The steps were repeated by substituting with the rest of the leaf samples that would be tested. The colour intensity of green pigment was read at 645 and 663 nm for chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content using a spectrophotometer. The result revealed that the total chlorophyll content of all species ranged from 0.081-1.112 mg/g fresh weight. The chlorophyll-a concentration for all species ranged from 0.028-0.718 mg/g fresh weight; chlorophyll-b content ranged from 0.053-0.499 mg/g fresh weight. *Piper sarmentosum* was found to have the highest total chlorophyll content and chlorophyll-*a*, whereas *Durio zibethinus* had the highest content of chlorophyll-*b*. Chlorophyll has potential benefits, including improving health, boosting energy, and fighting illnesses. Additionally, chlorophyll has possible health benefits, including increased energy and the ability to fight infections.

**Keywords:**

Chlorophyll, Herbs, Leaves, Mangroves, Spectrophotometer

## Introduction

Chlorophyll is a compound or pigment that gives green plants their colour (Kraeutler, 2014). It aids plants in absorbing energy from the sun during the photosynthesis process. Green vegetables and other natural green organisms, such as algae, contain this component (Da Silva Ferreira & Sant'Anna, 2016). The colour of a green plant depends on the levels and composition of chlorophylls and other pigments (Limantara et al., 2015). Chlorophyll-*a* and chlorophyll-*b* are the two forms of chlorophyll found in plants, and they both have antioxidant capabilities and are fat-soluble molecules (Zhang et al., 2020). Plants contain antioxidant compounds, particularly their green leaves, stems, blossoms, and roots. Chlorophyll has been utilized as an alternative medicine for various conditions, such as blood sugar reduction, detoxification, digestion, excretion, and allergy reduction (Aderiike Grace, 2019). It is also utilized as a green color since it's non-toxic to people and has a higher level of cleanliness than manufactured synthetic pigments (Tran et al., 2019).

Photosynthesis, which allows plants to absorb energy from light, requires chlorophyll. Photosystems are embedded in the thylakoid membranes of chloroplasts, and chlorophyll molecules are distributed in and around them (Kirchhoff, 2014; Rumak et al., 2012). Chlorophyll has three roles in these complexes. Most of the chlorophyll (up to several hundred molecules per photosystem) has the function of light absorption (Ma et al., 2013). After that, these identical centres perform their second function by transferring the light energy to a particular chlorophyll pair in the photosystem's reaction centre via resonance energy transfer (Fassioli et al., 2014). This pair impacts chlorophylls' last function, charge separation, which leads to biosynthesis (Jassim, 2020).

Chlorophyll concentration is a good indicator of plant nutrient stress, photosynthesis, and growing periods; the chlorophyll content in plant leaves indicates the growth status of the crops. It is also essential for exchanging mass and energy with the outside world. Thus, real-time monitoring of chlorophyll content is a crucial step in completing crop monitoring and yield estimation (Muñoz-Huerta et al., 2013; Weiss et al., 2020). Chlorophyll quantification provides vital information on the impact of various environments on plant development (Kalaji et al., 2016). As the chlorophyll content is an indicator of crop growth and development, it is critical to determine and measure chlorophyll concentration precisely (Sonobe et al., 2020).

Local herbs and plants such as *Avicennia germinans, Laguncularia racemosa, Durio zibethinus, Garcinia mangostana, Spinacia oleracea, Piper betle, Piper sarmentosum*, and *Centella asiatica* are widely consumed, particularly in Southeast Asia, or used in traditional remedies (Hussain et al., 2012; Timkhum & Terdwongworakul, 2012). However, there is a paucity of data on their chlorophyll contents. The objective of this study was to quantify the chlorophyll concentrations of *Avicennia germinans, Laguncularia racemosa, Durio zibethinus, Garcinia mangostana, Spinacia oleracea, Piper betle, Piper sarmentosum,* and *Centella asiatica.*

## Literature Review

Photosynthesis, the process through which plants harness energy from light, relies on chlorophyll as a vital component. Within chloroplasts, photosystems are integrated into thylakoid membranes, surrounded by chlorophyll molecules that play distinct roles in these complexes (Kirchhoff, 2014; Rumak et al., 2012). The primary role of many chlorophyll molecules (up to several hundred per photosystem) is light absorption, capturing energy from incoming light (Ma et al., 2013). Subsequently, these identical centres execute their second function by transferring the captured light energy to a specific chlorophyll pair in the photosystem's reaction centre via resonance energy transfer (Fassioli et al., 2014). This chlorophyll pair then catalyses the charge separation process, which is pivotal for biosynthesis (Jassim, 2020).

Previous studies reveal different parameters that affect the chlorophyll content of a species, for example, the seasonal change effect on mangrove species such as *Avicennia germinans* (Flores-de-Santiago et al., 2012). Other than that, different light conditions also differentiate a species' photosynthetic response and chlorophyll content, for instance, *Durio zibethinus* (Kondo et al., 2017). Photosystem II and Photosystem I are the two currently approved photosystem units, each with its reaction centre (P680 and P700, respectively) (Müh & Zouni, 2020). The wavelength of their red-peak absorption maximum gives these centres their names. The varieties of chlorophyll in each photosystem have individual identities, functions, and spectral qualities defined by each other and the protein structure surrounding them (Sirohiwal et al., 2020). These chlorophyll pigments may be separated into chlorophyll a and b using organic solvents such as acetone, methanol, or ethanol (Eghbali Babadi et al., 2020; Tran et al., 2019).

The concentration of chlorophyll serves as an informative gauge for plant nutrient stress, photosynthesis, and growth stages, reflecting the developmental status of crops. Moreover, chlorophyll content plays a critical role in the exchange of mass and energy with the external environment. Consequently, the real-time assessment of chlorophyll content emerges as a pivotal stride in comprehensive crop monitoring and yield prediction (Muñoz-Huerta et al., 2013; Weiss et al., 2020). The quantification of chlorophyll furnishes essential insights into the repercussions of diverse environmental conditions on plant maturation (Kalaji et al., 2016). Given that chlorophyll content is a dependable indicator of crop advancement and health, the accurate determination and measurement of chlorophyll concentration hold profound significance (Sonobe et al., 2020).

## Methodology

### *Samples Collection*

The leaves samples of *Avicennia germinans* (Black mangrove) and *Laguncularia racemosa* (White mangrove) were taken from the mangrove area at Kuala Perlis. Meanwhile, *Durio zibethinus* (Durian), *Garcinia mangostana* (Mangosteen), *Spinacia oleracea* (Spinach), *Piper betle* (Betel), *Piper sarmentosum* (Wild betel), and *Centella asiatica* (Pennywort) leaves samples were harvested from a local garden located near Beseri, Perlis. Precautions were taken to avoid mechanical damage before the materials were preserved in vacuumed plastic bags and chilled for two days.

### *Analytical Procedures*

250 mg of leaf sample was macerated with 10 ml of 80% acetone using a pestle and mortar. The extract was centrifuged for 5 minutes at 5000 rpm. Then, the solution was transferred into a 25 ml volumetric flask and made up to 25 ml using 80% acetone. The steps were repeated by substituting with the rest of the tested leaf samples. The colour intensity of green pigment was read using a spectrophotometer at 645, 663, and 652 nm for chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content. The concentrations of chlorophylls were determined using the following formula/equation in Table 1. Table 1 shows the formula to determine the concentration of chlorophylls.

**Table 1: Formula To Determine The Concentration Of Chlorophylls**

| Solvent | Formula / Equation | |
|---|---|---|
| 80% Acetone | Chlorophyll-*a* mg/g tissue | $=\frac{12.7(AR_{645}R)-2.69(AR_{663}R)\times V}{1000}\times W$ |
| | Chlorophyll-*b* mg/g tissue | $=\frac{20.2(AR_{645}R)+8.02(AR_{663}R)\times V}{1000}\times W$ |
| | Total chlorophyll mg/g tissue | $=\frac{22.9(AR_{645}R)-4.68(AR_{663}R)\times V}{1000}\times W$ |

where,
A = Absorbance at specific wavelengths
V = Final volume of chlorophyll extract in 80% acetone
W = Fresh weight of tissue extracted.

## Results and Discussion

In this comparative study, eight species of plants: *Avicennia germinans*, *Laguncularia racemosa*, *Durio zibethinus*, *Garcinia mangostana*, *Spinacia oleracea*, *Piper betle*, *Piper sarmentosum*, and *Centella asiatica* were chosen to be quantified for their chlorophyll content. The chlorophyll from the plants were extracted using 80% acetone before being measured by a spectrophotometer. The results were calculated to estimate all species' chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content. It was found that *P. sarmentosum* had the highest chlorophyll content, which was 1.112 mg/g fresh weight, followed by *D. zibethinus*, *S. oleracea*, and *A. germinans* had relatively high chlorophyll content. Meanwhile, *P. betle* was found to have the lowest chlorophyll content of all species. *P. sarmentosum* and *P. betle* both come from the same family, Piperaceae. However, each species showed a significant difference in terms of chlorophyll content. Based on the colour observation, the deeper the green colour of the extract, the higher the chlorophyll content. Table 2 shows the spectrophotometric determination of absorbance for chlorophylls of eight different plant species. Figure 1 on the other hand, shows the chlorophyll content of eight different species.

**Table 2: Spectrophotometric Determination Of Absorbance For Chlorophylls Of Eight Different Plant Species.**

| Plant Species | Chlorophyll-*a* (mg/g) Fresh Weight | Chlorophyll-*b* (mg/g) Fresh Weight | Total chlorophyll (mg/g) Fresh Weight |
|---|---|---|---|
| *Avicennia germinans* | 0.619 | 0.297 | 0.916 |
| *Laguncularia racemosa* | 0.140 | 0.095 | 0.235 |
| *Durio zibethinus* | 0.523 | 0.499 | 1.022 |
| *Garcinia mangostana* | 0.207 | 0.287 | 0.494 |
| *Spinacia oleracea* | 0.631 | 0.319 | 0.950 |
| *Piper betle* | 0.028 | 0.053 | 0.081 |
| *Piper sarmentosum* | 0.718 | 0.394 | 1.112 |
| *Centella asiatica* | 0.380 | 0.219 | 0.599 |

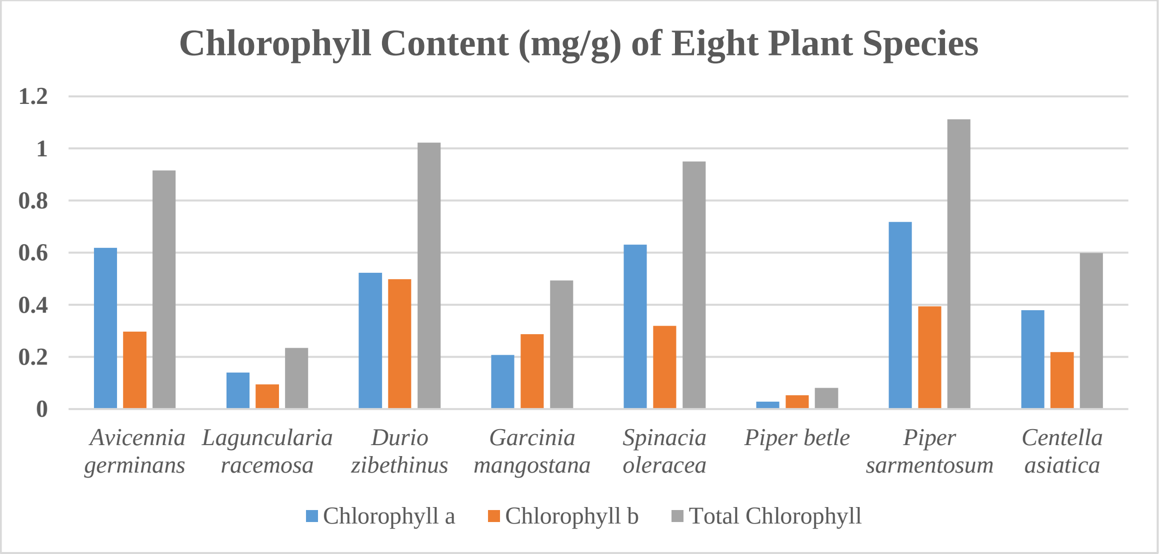

**Figure 1: Chlorophyll Content Of Eight Different Species**

The chlorophyll-*a* content of *P. sarmentosum* was the highest which was 0.718 mg/g fresh weight, followed by *S. oleracea*, *A. germinans*, *D. zibethinus*, *C. asiatica*, *G. mangostana*, *L. racemosa*, and *P. betle* which had chlorophyll-*a* content of 0.631, 0.619, 0.523, 0.380, 0.207, 0.140 and 0.028 mg/g fresh weight respectively. Most of the species were found to possess more chlorophyll-*a* compared to chlorophyll-*b*. For chlorophyll-*b* content, *D. zibethinus* showed the highest content, which was 0.499 mg/g fresh weight, followed by *P. sarmentosum*, *S. oleracea*, *A. germinans*, *G. mangostana*, *C. asiatica*, *L. racemosa* and *P. betle* which had chlorophyll-*b* content of 0.394, 0.319, 0.297, 0.287, 0.219, 0.095 and 0.053 mg/g fresh weight respectively.

It can be observed that the chlorophyll extraction from *L. racemosa* and *P. betle* leaves gave a yellow colour extraction. Meanwhile, other leaves gave a green colour extraction. It was also found that *L. racemosa* and *P. betle* leaves' chlorophyll content was the lowest among all species. The primary pigments in green plants are chlorophylls, represented by chlorophyll-*a* and chlorophyll-*b*, which appear green. The other two pigments are types of carotenoids, which appear yellow, orange, or brown. Since *L. racemosa* and *P. betle* leaves were found to have low chlorophyll content and produced yellow extractions, the extractions probably had higher carotenoid content than chlorophyll content. Figure 2 shows the extracted chlorophyll of eight selected species.

**Figure 2: Extracted Chlorophyll Of Eight Selected Species**

## Conclusion

This study has successfully examined variation of chlorophyll content in selected plant species, with *P. sarmentosum* had the highest chlorophyll content. Notably, *D. zibethinus*, *S. oleracea*, and *A. germinans* also exhibited relatively high total chlorophyll content, enriching our comprehension of chlorophyll distribution among species. Conversely, *P. betle* displayed the lowest total chlorophyll content across all species. Furthermore, these findings collectively provide significant insights into species-specific chlorophyll variations, contributing to our understanding of plant physiology and offering implications for ecological and agricultural contexts. Thus, it is recommended that further investigations delve into the underlying factors driving these variations, thereby deepening our comprehension of plant biology and ecosystem dynamics and potentially informing strategies for enhancing crop productivity and ecological sustainability. The findings of this research are aligned with aspiration of the United Nation's Sustainable Development Goals 15 – Life on Land.

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