ANALISIS INTERAKSI KLOROFIL PANDAN (*Pandanus amaryllifolius)* DENGAN BAWANG DAYAK (*Eleutherine SP*.) MELALUI UV-VIS

Analysis of Chlorophyll-Screwpine (Pandanus amaryllifolius) Interaction with Bawang Dayak (Eleutherine sp.) via UV-Vis

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ABSTRAK

Penggunaan pigmen alami, baik pada industri pangan maupun kosmetik, semakin populer karena sifatnya yang ramah lingkungan dan minim resiko terhadap kesehatan. Salah satu pigmen alami yang sering digunakan adalah klorofil, pigmen warna yang memberikan warna hijau khas dan bermanfaat bagi kesehataann. Namun, interaksi klorofil ketika berinteraksi dengan senyawa bioaktif lain masih kurang diteliti. Pada penelitian ini bertujuan untuk menganalisis interaksi antara klorofil dari daun pandan (Pandanus amaryllifolius) dengan senyawa boaktif dari bawang dayak (Eleutherine sp.) menggunakan spektroskopi UV-Vis. melalui pencampuran ekstrak dengan variasi volume. Hasil analisi UV-Vis menunjukkan serapan pada daerah 420 nm dan 670 nm yang menunjukkan serapan khas dari klorofil. Serapan ini tidak menunjukkan perbedaan yang signifikan pada penambahan ekstrak Eleutherine sp. Dalam berbagai rasio volume. Yang menunjukkan tidak ada interaksi maupun reaksi signifikan yang mampu mendegradasi klorofil pada metabolit sekunder Eleutherine sp. Akan tetapi, analisis warna berdasarkan RGB dan CIE LAB menunjukkan perbedaan yang cukup signifikan.

Kata kunci: klorofil, pandan, bawang dayak, UV-Vis, CIE LAB

ABSTRACT

The use of natural pigments, both in the food and cosmetic industries, is gaining popularity due to their environmentally friendly nature and minimal health risks. One natural pigment that is often used is chlorophyll, a color pigment that provides a distinctive green color and is beneficial for health. However, the interaction of chlorophyll when interacting with other bioactive compounds is still poorly studied. This study aims to analyze the interaction between chlorophyll from skrewpine leaves (Pandanus amaryllifolius) with bioactive compounds from dayak onions (Eleutherine sp.) using UV-Vis spectroscopy. through mixing extracts with various volumes. The results of UV-Vis analysis showed absorption in the 420 nm and 670 nm regions which showed typical absorption of chlorophyll. These absorbances did not show significant differences in the addition of Eleutherine sp. extracts in various volume ratios. Which indicates that there are no significant interactions or reactions that can degrade chlorophyll in Eleutherine sp. secondary metabolites. However, color analysis based on RGB and CIE LAB shows significant differences.

Keyword: *chlorophyl, screpines, Bawang Dayak, UV-Vis, CIE LAB*

INTRODUCTION

Chlorophyll, green pigment found in plant, is essential for photosynthesis and responsible for the green coloration leaves, including *Pandanus amaryllifolius* (commonly known as screwpine) (Arof & Ping, 2017; Mandal & Dutta, 2020; SURYANI et al., 2020). In recognition of their vivid green clor and their qualities of essence, screwpine leaves are frequntly utilized in Southeast Asian cuisines. (INDRASTI et al., 2018; Rahmiyani et al., 2021). Moreover, chlorophyll exhibits various health benefits, especially antioxidant activity, which make valuable component in food and health-related products (Ebrahimi et al., 2023; Martins et al., 2023; Yang et al., 2024). Understanding chlorophyll's interaction with other bioactive compounds is crucial, when it comes to developing pigment-related product, whether food or cosmetic (Agustina et al., 2021; Kang et al., 2018). UV-Vis spectroscopy is an effective tool for analyzing the absorption characteristics of chlorophyll and identifying any changes that may occur due to interactions with other substances(Lichtenthaler & Buschmann, 2001; Makarska-Bialokoz & Kaczor, 2014).

Eleutherine bulbosa, commonly known as *Eleutherine sp*., is a medicinal plant known for its rich content of bioactive compounds, including flavonoids and naphthoquinones (Ieyama et al., 2011; Ifesan et al., 2009; Kamarudin et al., 2021). These compounds possess antioxidant, anti-inflammatory, and anticancer properties (Ieyama et al., 2011; Ifesan et al., 2009). When combined with chlorophyll-rich screwpine extract, these bioactive compounds may interact with chlorophyll, potentially altering its UV-Vis absorption spectrum and affecting its stability and functionality (Ashenafi et al., 2023). Investigating these interactions is important for both scientific understanding and the development of natural product formulations, particularly in industries where color and stability are key factors, such as in food, cosmetics, and nutraceuticals.

The interactions between chlorophyll from screwpine and the bioactive compounds in *Eleutherine sp*. may involve several chemical processes, including hydrogen bonding, complex formation, or redox reactions (Ebrahimi et al., 2023). These interactions could result in shifts in the UV-Vis absorption spectrum of chlorophyll, indicating changes in its structure or stability (Gorza et al., 2014; Liu, 2011). By analyzing these spectral changes, we can gain insights into how *Eleutherine sp*. influences the chemical properties of chlorophyll, including potential impacts on its color and bioactivity.

This study uses UV-Vis spectroscopy to investigate the interaction between chlorophyll from screwpine and bioactive compounds from *Eleutherine sp*.. The goal is to evaluate how these interactions affect chlorophyll's spectral properties, with a focus on understanding changes in color and stability. The findings of this research could provide valuable information for industries that utilize natural pigments, offering insights into maintaining product quality and stability when combining plant extracts rich in bioactive compounds.

METHODS

Fresh screwpine leaves (*Pandanus amaryllifolius*) were prepared by weighing 15 grams of leaves, which were then blended with 200 mL of distilled water (aquadest) to form a homogenous mixture. The mixture was filtered to separate the liquid extract containing chlorophyll and other soluble compounds. The filtrate was collected and used as the screwpine extract for further analysis.

To prepare *Eleutherine sp*.extract, 2 grams of dried *Eleutherine sp*.bulbs were added to 50 mL of distilled water. The mixture was heated using a microwave for 2 minutes to extract the bioactive compounds. After heating, the mixture was filtered, and the resulting liquid was collected as the *Eleutherine sp*. extract.

The interaction between chlorophyll from screwpine and the bioactive compounds in *Eleutherine sp*.was analyzed using UV-Vis spectroscopy. First, 1 mL of the concentrated screwpine extract and 1 mL of the concentrated *Eleutherine sp*.extract were each diluted by a factor of 10 using distilled water. After dilution, the extracts were mixed in various ratios to investigate *Eleutherine sp*. effect on chlorophyll's spectral properties. The mixtures were prepared with the following ratios of screwpine to *Eleutherine sp*.extracts: 1:0 (screwpine extract only), 1:5, 2:4, 3:3, 4:2, 5:1, and 0:1 (*Eleutherine sp*.extract only).

Each mixture was analyzed using a UV-Vis spectrophotometer, measuring the absorbance from 250 to 700 nm to capture the entire spectral range of chlorophyll and potential interactions with the bioactive compounds from *Eleutherine sp*..

To quantify chlorophyll a and b concentrations in the screwpine extract, absorbance was measured at two specific wavelengths: 663 nm for chlorophyll a and 645 nm for chlorophyll b. The concentrations of chlorophyll a and b were calculated using the following equations(Lichtenthaler & Buschmann, 2001):

$$\text{chlorophyl a} = 12{,}7 \times A_{663} - 2.69 \times A_{645} \ldots (1)$$

$$\text{chlorophyl a} = 22.9 \times A_{645} - 4.68 \times A_{663} \ldots (2)$$

$$\text{Total} = 20.2 \times A_{645} + 8.02 \times A_{663} \ldots (3)$$

RESULT AND DISCUSSION

The interaction between extracts *Eleutherine sp*.extract and screwpine (*Pandanus a.*) extract at different combination ratios is depicted in the UV-Vis spectra **figure 1**. All spectra displayed high absorption at UV wavelengths (200–400 nm), which is probably caused by the naphthoquinone or flavonoids in *Eleutherine sp.*. and the chlorophyll from *Pandanus a*. Since chlorophyll naturally absorbs red and blue light, there is a lower absorption of light in the 400–500 nm range, which is linked to chlorophyll. It is evident that the spectrum profile of the mixture can be impacted by the interaction between chlorophyll and the active ingredients from *Eleutherine sp*., particularly at larger mixture ratios.

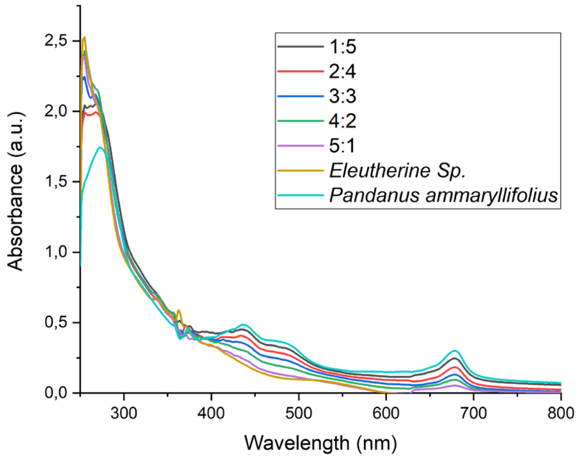

Figure 1. UV-Vis spectra of extract *Eleutherine sp* and *Pandanus a.* different combination ration

Chlorophyll, particularly chlorophyll a and b, which are known to absorb light in the blue to green range, typically absorb light in wavelengths between 400 and 500 nm, as shown in the UV-Vis spectra displayed. Additionally, chlorophyll exhibits a significant absorption of red light at wavelengths between 600 and 700 nm (Lim et al., 2015). The spectra demonstrated that the variations in absorbance that took place in these two wavelength ranges (400-500 nm and 600-700 nm) were not substantial, even though there were different addition ratios of *Eleutherine sp*. extract in the mixture with chlorophyll from *Pandanus a*.

There is a noticeable colors shift in the mixed solution even though the UV-Vis spectra data indicate that there is no discernible change in chlorophyll absorption in the wavelength areas of 400–500 nm and 600–700 nm following the addition of Eleutherine Sp. extract. RGB (Red, Green, Blue) analysis can be used to see this color shift, although UV-Vis spectroscopy cannot detect it very well. The RGB and CIE LAB analysis is available ini **table 1**.

Table 1. RGB and CIE LAB analysis of mixture of Eleutheine and *Pandanus.a* with variatin ratio

Solution (v:v)	R	G	B	L	A	B
Screw pine	160	160	62	64,1309	12,9469	49,614
1:5	122	110	38	46,2167	-4,4206	40,3628
2:4	133	114	46	48,534	-1,0366	39,3827
3:3	137	100	45	45,1123	8,8561	36,2379
4:2	141	101	52	45,9313	10,6078	33,6194
5:1	163	115	69	52,373	13,7151	32,7204

Numerous fields make extensive use of the CIE L*a*b* color scheme for quantitative analysis. In comparison to grayscale-based analysis, it provides a greater detectable range (Komatsu et al., 2016). The color change of *Pandanus.a* solution with the addition of dayak onion was analyzed using the CIE LAB color model. The pure *Pandanus.a* solution shows an L value of 64.1309, which indicates a high level of brightness. In addition, the A value of -12.9469 and B value of 49.614 indicate the dominance of green and yellow colors in the solution. However, when Dayak onions were added in varying ratios, there were significant changes in the color parameters. In the mixture of *Pandanus.a* and dayak onion at a ratio of 1:5, the L value decreased drastically to 46.2167, indicating a decrease in brightness. In addition, the near-neutral A value (-4.4206) indicates a reduction in the dominance of green, while the B value of 40.3628 still shows a tendency towards yellow. The 2:4 mixture showed an increase in the L value to 48.534, but the A value getting closer to zero (-1.0366) indicated that the green dominance was almost gone. At the same time, the B value of 39.3827 still shows a lower dominance of yellow.

When the *Pandanus.a* and dayak onion ratio became 3:3, there was a significant shift in the A value to positive (8.8561), indicating a change in color dominance from green to red. This coincides with a decrease in the B value to 36.2379, indicating that the intensity of the yellow color continues to decrease. In the 4:2 mixture, the A value further increased to 10.6078, reinforcing the red color dominance, while the B value continued to decrease to 33.6194. In the last mixture, with a ratio of 5:1, the L value increased slightly to 52.373, but the red dominance (A value = 13.7151) was the strongest among all the mixtures, while the B value remained low at 32.7204.

Overall, the addition of dayak onion to the *Pandanus.a* solution caused a decrease in brightness as well as a shift in color dominance from green-yellow to red-yellow. This phenomenon suggests *Eleutherine sp.* plays a role in changing the visual characteristics of the *Pandanus.a* solution, with the greater the proportion of *Eleutherine sp.*, the stronger the red color appears and the less intensity of the yellow color.

This study analyzed the changes in chlorophyll content in *Pandanus.a* solution after the addition of dayak onion extract in various proportions. The full data is avaliable in **table 2**. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll showed a significant decreasing trend as the proportion of dayak onion extract in the solution increased. In the first sample, which consisted of pure *Pandanus.a*, Chl a levels were recorded at 23.66 µg/mL, Chl b at 28.50 µg/mL, and total chlorophyll at 52.15 µg/mL. However, along with the addition of dayak onion extract, there was a gradual decrease until the last sample, where Chl a levels dropped to 4.02 µg/mL, Chl b to 4.66 µg/mL, and total chlorophyll to 8.68 µg/mL.

Table 2. chlorophyl a, chlorophyl b, and total chlorophyl of *Pandanus.a*

Solution	Chl a	Chl b	Total
Screw pine	23,66186	28,50387	52,14874
1:5	19,40249	23,78807	43,17644
2:4	13,88454	15,41221	29,28738
3:3	9,756313	10,39059	20,14052
4:2	7,21318	7,837596	15,04599
5:1	4,022533	4,662881	8,682609

This decrease in chlorophyll levels indicates that dayak onion extract plays a role in reducing chlorophyll content in *Pandanus.a* solution. This may be due to the reaction between the bioactive components in dayak onion with chlorophyll, which causes degradation of the photosynthetic pigment. Chlorophyll a, which is the main pigment in photosynthesis, decreased faster than chlorophyll b. In the first sample, the ratio of Chl a to Chl b was 0.83, but in the last sample, this ratio increased slightly to 0.86, which indicates that although both types of chlorophyll decreased, Chl a degraded slightly slower than Chl b.

CONCLUSSION

The relationship between the secondary metabolites in eutherine and the chlorophyll in *Pandanus.a* has been examined using UV-Vis spectroscopy. The findings of the UV analysis demonstrated that the extracts of *Pandanus amaryllifolius* and Bawang Dayak (*Eleutherine sp.*) interacted to affect the solution's chlorophyll levels and visual properties. The UV-Vis spectra revealed significant absorption in the UV region (200–400 nm), which may have been brought on by *Pandanus.a* chlorophyll and naphthoquinone and flavonoids from *Eleutherine sp*. Even though the percentage of *Eleutherine sp*. extract applied changed, there was no discernible change in absorbance at wavelengths of 400–500 nm and 600–700 nm, which are typical absorptions of chlorophyll. This implies that the secondary metabolites of Dayak onions and chlorophyll do not interact chemically. RGB and CIE LAB analysis revealed a shift in the solution's hue, despite the UV-Vis spectrum showing no discernible alteration. When *Eleutherine sp*. extract was added, the brightness (L value) decreased, and the color dominance changed from green-yellow to red-yellow. As the Dayak onion ratio rose, the red dominance became more pronounced. Despite not being visible in the UV-Vis spectral data, these visual alterations show that *Eleutherine sp*. has an impact on the solution's optical properties.

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