Estimation of leaf antioxidant activity using image processing

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Abstract. Physiologically, the influence of inundated conditions on many plants varies. The eco-physiological response of most plantations to flooding is wilt and stomata closure, root damage, reduction of root biomass. This results in a decrease in the production of bioactive compounds, which have an effect on decreasing antioxidant activity. Various methods of measuring antioxidant activity still use chemicals through extraction processes that require a long time. So, it is necessary to examine alternative methods that are cheap, easy and fast. This method is digital processing. The leaf samples were then photographed for image analysis in the Telkom School Banjarbaru Multimedia Laboratory. Antioxidant activity was analyzed through the hydroxyl scavenging method at the laboratory of Biochemistry and Biomolecular, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru. The results of image analysis showed that the antioxidant activity model of RGB values was $AA = -54.9592 + 1.0295 R + 0.3757 G + 2.006 B - 0.0004 R*G - 0.0012 R*B + 0.004 G*B$ ($AA =$ antioxidant activity; $R =$ red; $G =$ green; $B =$ blue) with $p = 0.001$ ($p < 0.005$) and $r = 0.76$. In this model, the $R$ value has a significant effect on antioxidant activity. Image analysis using RGB can be used to measure leaf antioxidant activity. However, further research is needed.

1. Introduction
Indonesia's peat swamp forests have been degraded due to more drainage and conversion of forests to agricultural land, and irreversible environments, and large-scale fires [1]. The condition of post-combustion peat swamp forests is not beneficial for plants. Site conditions that have become open areas, physically and chemically damaged peat, burning peat forms a lower surface which is flooded in the rainy season. This condition is a barrier to the success of restoration. Physiologically, the influence of inundated conditions on many plants varies. The eco-physiological response of most plantations to flooding is wilt and stomata closure, root damage, reduction of root biomass [2].

Research using the image method continues to increase in development. In classifying plant diseases, the last 25 years continue to improve the ability to detect and classify accuracy and accuracy of image analysis [3]. Some reports show increasing use of image processing in plant research. Study on plant recognition based on image processing has been rising, and more and more
popular because this method is rapid and objective, is not dependent on the person’s subjective judgment, effectively and improves work efficiency [4].

Digital images processing techniques to analyze and detect, classify, quantify plant leaf diseases [5] and help to support in the identification of diseases at an early or initial stage and provide useful information for its control [6]. Various methods of measuring antioxidant activity still use chemicals through extraction processes that require a long time. So, it is necessary to examine alternative methods that are cheap, easy and fast. This method is digital processing. Therefore, the digital image processing should be useful as a simplified method for estimating antioxidant activity of plantations.

2. Materials and method
2.1. Study design
This research is an experimental in-vitro study for one week in May 2019.

2.2. Experimental section
The taking of leaves samples was done on Notaphoebe sp. Fresh Notaphoebe sp leaves were collected from plants in the Tumbang Nusa Research Station, Central Kalimantan, Indonesia, at 39 months age, during rainy season in April 2019. The taking of leaves samples was carried out under conditions of raised and inundated plants. Extraction of leaves samples was carried out on plants randomly. All leaves sample then out in paper bag and taken to the laboratory. Handle samples must reach the laboratory in good condition and clean [7].

2.3. Measurement of RGB
The leaves samples were then photographed for image analysis in the Telkom School Banjarbaru Multimedia Laboratory, Banjarbaru, South Kalimantan. Fresh leaves are then cleaned. To get a picture, the leaves are scanned with Canon LiDE 110. Nikai RGB is determined with imageJ the free software.

2.4. Measurement of antioxidant activity
Gemor leaf obtained in maceration for 3 days with methanol as a solvent. The extract obtained was determined antioxidant activity using the method by Purwanto et al [8]. Antioxidant activity was analyzed through the hydroxyl scavenging method at the laboratory of Biochemistry and Biomolecular, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan.

3. Results and discussion
Wet and dry leaves obtained are scanned. The scanned image is seen in Figure 1.

![Figure 1. Scanned image of wet (a) and dry (b) leaves.](image)

Measurement of antioxidant activity with RGB technique is based on phytochemical compound producing a certain color spectrum. Changes in the color spectrum reflect changes in phytochemical structure and antioxidant activity. RGB measurement results for plants are presented in figure 1. Looking at figure 2, there is a change in the RGB value in (a) and (b).

The results of image analysis showed that the antioxidant activity model of RGB values was $AA = -54.9592 + 1.0295 R + 0.3757 G + 2.006 B -0.0004 R*G - 0.0012 R*B + 0.004 G*B$ (AA =
antioxidant activity; \( R = \text{red}; \, G = \text{green}; \, B = \text{blue} \) with \( p = 0.001 \) (\( p < 0.005 \)) and \( r = 0.76 \). In this model, the \( R \) value has a significant effect on antioxidant activity.

![Figure 2. RGB value of wet (a) and dry (b) leaves.](image)

Figures 1 and 2 show that in wet leaves the value of Red (R) and Green (G) is higher than that of dry leaves. Changes in the value of RGB indicate changes in chemical structure such as covalent bonds or groups of molecules that are bound. This structural change causes the molecule to release \( H^+ \) ions to be donated to other molecules. This molecule is called an antioxidant.

Antioxidants in plants have long been used by people for herbal medicine. Antioxidants are believed to inhibit aging, prevent coronary heart disease, hypercholesterolemia, enhance immunity and others. Antioxidants work by hydroxyl radical scavenging, peroxide scavenging, and metal chelating. A high green color is assumed to be a leaf green or chlorophyll or anthocyanin compound which gives green pigment (figure 3).

![Figure 3. Chlorophyll or anthocyanin structure.](image)
Many studies reveal the antioxidant activity of anthocinins. In that study it was found that anthocinin is also an anti-inflammatory [9-10].

4. Conclusion
Image analysis using RGB can be used to measure leaf activity. However, further research is needed.

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