Antioxidant activities of red jabon (Anthocephalus macrophyllus) ethanol extract

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Abstract. Red jabon bark extract has been traditionally used as anti-inflammatory drug for pregnant women, headache and fever medications, as well as stamina enhancer by native people of North Maluku. This study primarily aims to evaluate antioxidant activity of red jabon (Anthocephalus macrophyllus) bark using DPPH (1,1-diphenyl-2-picrylhydrazyl). Result of this study shows that concentration level of IC50 ethanol extracted red Jabon bark is about 2.545 ppm, indicating low antioxidant activity.

1. Introduction
The Red Jabon (Anthocephalus macrophyllus) is limitedly used by the community as rulers, drawings, pencils, boxes, matchsticks, composite wood, pulp, and paper. While the bark, leaves, and other parts of the tree have never been touched at all. Plants that are natively found in Indonesia have different references in several places, namely Sugi Manai (Makassar), Kahumama Merah (Banggai), Samama (Maluku), and Samama Merah (Papua) [1]. The Red Jabon tree can be found in almost all parts of North Maluku. The tree is often used by the community as a medicinal plant. The bark is used by the people of North Maluku as an anti-inflammatory drug for pregnant women, headache medicine, fever-relief drugs, and stamina enhancer. The red jabon may contain chemical compounds that can be categorized as drug. One of them is the possibility of having antioxidant activity.

Chemical compounds used as antioxidants include anthocyanin, elagic acid, flavanoids, carotenoids, and lutein [2]. Some plants have been identified undergoing antioxidant and sunscreen activities using UV-Vis instruments. Alhabsyi et al. (2014) reported that Goroho (Musa Acuminate L.) bananas had good antioxidant activity in ethanolic extract with antioxidant activity values of 75.71% with DPPH (1,1-diphenyl-2-picrylhydrazyl) method [3].

In addition, identified antioxidant and sunscreen activities on super red dragon fruit skin (Hylocereus costaricensis (F.A.C.Weber) Britton & Rose). The results of the identification showed that the super red dragon fruit skin had a low antioxidant activity with an IC50 value of 4602.740 ppm [4]. The leaves of the strawberry plant (Fragaria x ananassa A.N. Duchesne) also have antioxidant and sunscreen activities. The antioxidant activity of strawberry leaves is quite good with an IC50 value of 363,551 ppm. Considering the above descriptions, it is fair to expect that the red jabon bark (Anthocephalus macrophyllus) may undergo some antioxidant activity.
1.1. Red Jabon Tree or Samama (Anthocephalus macrophyllus)

Red Jabon Tree or Samama (Anthocephalus macrophyllus) is an endemic plant in North Maluku.

Trade name: Red Jabon, Orawa (Southeast Sulawesi), Samama (Maluku), and Red Samama (Papua).
Nama Botanis: Anthocephalus macrophyllus (Roxb.) Havil.
Family: Rubiaceae
Genus: Anthocephalus
Source: BPTH Sulawesi [1]

1.2. Morphological character
Red jabon is a type of plant that grows in the tropics with full intensity of sunlight and cannot stand with shade. Red Jabon trees can grow faster than other plants with a height reaching 45 meters with free branch stems of 30 m and stem circumference of 150 cm and diameter of 40-50 cm. Red Jabon has thinner leaves with a redish young leaves and softer compared to teak leaves, even though they look similar. High tree growth and large diameter along with growing trees, such as table 1 below [5].

| Chemical components | content (%) | Classification |
|---------------------|-------------|----------------|
| Cellulose           | 52.47       | High           |
| Pentose             | 15.23       | Low            |
| Lignin              | 26.81       | Medium         |
| Extractive          |             |                |
| Solubility in cold water | 3.39    | -              |
| Solubility in hot water   | 4.81      | -              |
| Solubility in alcohol-benzene 1:2 | 6.12    | High           |
| Solubility in NaOH 1%  | 12.83      | -              |
| Ash                 | 0.52        | Medium         |

1.3. Antioxidants
Antioxidants are chemical compounds that function as a compound that slows down lipid oxidation with the ability to capture and reduce negative effects of oxidants. An antioxidant compound was identified in a qualitative way to use color reactions. The ethanolic extract reacted with DPPH will change from purple to yellow. This change indicates that a sample is positive containing antioxidant activity [2].
Antioxidant compounds can be derived from flavanoid group compounds. Phenol compounds are known as a polar compound that can be dissolved in ethyl acetate and ethanol. The presence of hydroxyl groups in phenol compounds serves as an inhibitor of the oxidation process when reacting with radical compounds through an electron transfer mechanism [6].

Method that commonly used in determining antioxidant levels or activity is termed the DPPH method. The DPPH is a compound that captures nitrogen radicals. The hydrogen atom in a compound will be taken by DPPH in the reaction process. The antioxidants of each compound are different in terms of ability to transfer hydrogen atoms. Determination of antioxidants in vitro was carried out at a wavelength of 517 nm [6]. Determination of antioxidant levels is determined using equations:

\[
\text{IC}_{50} = \frac{\text{absorbance sample} - \text{absorbance control}}{100}
\]

The IC\(_{50}\) value is obtained from the calculation by linear regression using the equation:

\[
Y = BX + A
\]

The use of DPPH method in determining an antioxidant in the capture of a free radical by dissolving DPPH and then testing it. This is because the DPPH is a stable free radical compound [7].

2. Materials and Methods

2.1. Materials
Type 1800 Simadzu UV-Vis spectrophotometer, glassware, digital scales, rotary evaporator, red jabon bark, methanol, and ethanol.

2.2. Methods

2.2.1. Sample preparation
Red Jabon bark is cut into small pieces and then dried for several days then macerated with ethanol. The extract obtained was tested for antioxidants and sunscreen.

2.2.2. Determination of Antioxidant Activity with DPPH
5 mg DPPH was put into a 250 mL volumetric flask and dissolved in ethanol solvent to obtain a DPPH 20 ppm solution. Uptake of DPPH solution was measured by a UV-Vis spectrophotometer at a maximum absorption wavelength of 517 nm. Sample extracts (test solution) were made with concentrations of 20, 40, 50, 80 and 100 ppm. Each 0.2 mL test solution was added 3.8 mL of 20 ppm DPPH solution and then left for 30 minutes prior to measurement [2].
3. Result and Discussion

3.1. Standardization of the DPPH Method

3.1.1. Determination of the maximum wavelength (λ)
There are 3 maximum absorptions in determining the maximum wavelength, namely wavelengths of 202 nm, 327 nm, and 515 nm. Methanol solvents which have a spectrum at 205 nm wavelength also affect maximum absorption at wavelength 202. In addition, the wavelengths of 202 and 327 nm are formed due to changes in light sources in the range of 364 nm - 294 nm.

The wavelengths of 202 and 327 nm that are formed are also influenced by the intensity of the spectrum of the light source used, namely deuterium. The intensity of the deuterium light source spectrum indicates an increase in absorbance in the wavelength range of 200 nm.

DPPH wavelength is determined using UV-Vis spectrophotometer with rules. Calculation of wavelength based on Woodward-Fieser rule is 516 nm, so the wavelength of 515 nm can be used for antioxidant analysis using the DPPH method. The difference in the maximum wavelength between the results of calculations with observations usually shifts between 0-4 nm [8]. Because the wavelengths of the results of calculations and observations are still within the usual range, the wavelengths of observations can be used at a wavelength of 515 nm.

3.1.2. Curve Calibration
Making DPPH curve using DPPH solution at various concentrations, which is measured at a wavelength of 515 nm as shown in figure 2 below.

![Curve Calibration](image)

In figure 2 above, the concentration and absorbance are directly proportional. The higher the concentration obtained, the higher the absorbance so that it is in accordance with the Lambert-Beer law [9].

3.1.3. Linearity and detection range
Measurements were made at a concentration of 0.1 ppm to a concentration of 100 ppm in the previous measurements, found a linear regression curve in the concentration range of 1 ppm, 3 ppm, 5 ppm, 7 ppm, and 10 ppm, which looks in table 2. The linear regression curve in the DPPH solution forms a straight line from a concentration of 1 ppm to a concentration of 10 ppm.
The linear regression curve in Figure 3 shows that the curve generated linearly with the linear regression line equation \( y = 0.02708x + 0.00295 \) has a correlation coefficient of 0.998. A good criterion for the value of the correlation coefficient (R²) is <0.99 [10].

### Table 2. Range of linear regression concentration

| Concentration | Absorbance |
|---------------|------------|
| 1 ppm         | 0.027      |
| 3 ppm         | 0.084      |
| 5 ppm         | 0.146      |
| 7 ppm         | 0.189      |
| 10 ppm        | 0.273      |

3.1.4. Precision

Intra day and inter day is a repetition method to assess the precision to be tested. DPPH solution absorbance was analysed using the intra-day repetition method 3 times a day (morning, afternoon and evening) at a concentration of 3 ppm, 5 ppm, 7 ppm and 10 ppm. The absorbance of DPPH solution was analysed using the inter day method for 3 days at a concentration of 3 ppm, 5 ppm, 7 ppm and 10 ppm. The intra-day and inter-day repetition methods in precision tests produce relative standard deviation (RSD) values.

#### 3.1.4.1. Intra day precision test

Table 3 shows the results of the intra-day precision test at concentrations of 3 ppm, 5 ppm, 7 ppm, and 10 ppm showing the method of detailing has good precision in the morning, afternoon, and evening because the measurement results are smaller or equal to the results of% RSD located at range 0. 3 ppm concentration has repetition (0%, 0.64%, and 0%), concentration of 5 ppm (0.42%, 0%, and 0.41%), 7 ppm (0.298%, 0.297%, and 0.295% ), and concentrations of 10 ppm (0.42%, 0.42% and 0%).
### Table 3. Results precision test intra day

| No | Concentration (ppm) | Average %RSD |
|----|---------------------|--------------|
| 1  | 3                   | 0.21 %       |
| 2  | 5                   | 0.28 %       |
| 3  | 7                   | 0.0296 %     |
| 4  | 10                  | 0.028 %      |

#### 3.1.4.2. Inter day precision test

The inter-day precision test is shown in table 4 with 3 ppm, 5 ppm, 7 ppm, and 10 ppm concentrations, respectively, showing the precision method has good precision in the morning, afternoon, and evening because it is smaller or equal to the % RSD results obtained. At a concentration of 3 ppm it has repetition (1.19%, 0%, and 1.34%), a concentration of 5 ppm has repetition (0%, 0.72%, and 0.41%), a concentration of 7 ppm (0.30%, 0.30%, and 0%), and concentrations of 10 ppm (0.84%, 0.36%, and 0.21%).

### Table 4. Results precision test inter day

| No | Concentration (ppm) | Average %RSD |
|----|---------------------|--------------|
| 1  | 3                   | 0.84 %       |
| 2  | 5                   | 0.38 %       |
| 3  | 7                   | 0.02 %       |
| 4  | 10                  | 0.47 %       |

#### 3.1.5. Accuracy

The research data obtained an average% recovery DPPH solution of 99.8% from 99.3-100.1. These results indicate that the DPPH method has good accuracy. These results are in accordance with the criteria of the measured concentrations which are between 96.0% -104.0% and for the average accuracy of each concentration, namely 98.0% -102%.

#### 3.1.6. Detection Limit and Quantitative Limit

The limit of detection and quantitation limit obtained in DPPH solution were 0.021 and 0.071 obtained from the concentration of linear detection range based on the International Conference of harmonization (ICH). The approach used to determine detection limits and quantitation limits is the standard deviation approach of analyte and slope responses [11].

#### 3.2. Determination of Antioxidant Levels

Determination of antioxidant levels using the DPPH method. The DPPH method is used because this method has an antioxidant measurement procedure with a short time and easy measurement [4]. Antioxidant levels from samples can be observed by looking at the ability of samples to counteract free radicals (DPPH). The process of catching free radicals is indicated by the color change of the sample solution mixed from purple to yellow indicating that the sample has antioxidant levels.

The change in color from purple to yellow is due to the donation process of hydrogen atoms when antioxidant compounds react with DPPH radicals [6]. DPPH color reduction is also influenced by a decrease in DPPH absorbance so that antioxidant activity or free radical capture can be determined by
the ratio of DPPH absorbance decrease. The stronger an antioxidant compound in the test compound can cause the DPPH color to fade away. Antioxidant levels are characterized by the magnitude of the IC$_{50}$ value. IC$_{50}$ is an effective concentration of substances in samurai bark extract that is able to provide a percent value of antioxidant activity that can inhibit free radicals by 50%. The smaller the concentration that can cause IC$_{50}$, the better antioxidant activity of the extract. Samples with concentrations of 10 ppm, 30 ppm, 50 ppm, 80 ppm, and 100 had% inhibition values of 91, 47%, 92.15%, 97.34%, 98.70% and 98.51% respectively. The results of the assessment of antioxidant levels stated in the IC$_{50}$ values recommended in table 5 with a value of 2,544.75 ppm indicate the antioxidant levels of the sample extracts are low.

| Concentration (ppm) | Absorbance DPPH | Liquid Absorbance | %IC | Linear Regression Equations | IC$_{50}$ Value |
|---------------------|-----------------|-------------------|-----|-----------------------------|-----------------|
| 10 ppm              | 0.262           | 0.9151            |     |                             |                 |
| 30 ppm              | 0.241           | 0.9219            |     |                             |                 |
| 50 ppm              | 3.087           | 0.9734            | $y = 0.020x + 0.895$ | $r^2 = 0.850$    | 2.545           |
| 80 ppm              | 0.082           | 0.9870            |     |                             |                 |
| 100 ppm             | 0.046           | 0.9851            |     |                             |                 |

4. Conclusions
In the research carried out it can be concluded that the IC$_{50}$ value of red jabon bark extract is 2,545 ppm so it is classified as a very low antioxidant.

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