Natural Antioxidant of Xanthosoma nigrum Stellfeld

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Abstract. The primary use of antioxidants is to break free radicals’ reaction to save the body from damage due to free radicals. Free radicals react with molecules around them to obtain electron pairs and achieve stability or molecular atoms and achieve stability or molecular atoms. This reaction would be continuously processed in the body. It would cause various diseases such as cancer, heart, cataracts, premature aging, and other degenerative diseases if it did not stop. The oxidated linoleic acid substrate determined the antioxidant of Xanthosoma nigrum Stellfeld activity. The oxidation results in the form of malondialdehyde (MDA) would be reacted with thiobarbituric acid formed products in the form of a red complex (MDA-TBA), which absorption was measured by spectrophotometer at λ 532 nm. Xanthosoma nigrum Stellfeld extract with a concentration of 100 ppm, 150 ppm, 200 ppm, and 300 ppm had the inhibition of 19.32%, 21.85%, 29.47%, and 31.05%. The positive control, with α-tocopherol 200 ppm, had inhibition of 85.14%.

1. Introduction
The modern lifestyle has become a habit in people's lives, especially in people's diets. Food, environment, and lifestyle significantly affect people’s health and the internal factors in the human body. The amount of pollution a lousy diet can trigger free radicals’ formation as a side effect of the body’s metabolic processes[1].

Free radicals are atoms or molecules unstable and highly reactive because they have one or more unpaired electrons in their outer orbitals. Free radicals will react with surrounding molecules to gain electron pairs and achieve stability or molecular atoms and achieve stability or molecular atoms. The reaction will take place continuously in the body. It will cause various diseases such as cancer, heart disease, cataracts, premature aging, and other degenerative diseases if it is not stopped. Therefore, the body needs a vital substance, namely antioxidants, to capture these free radicals to induce an infection [2].

Antioxidant compounds can be found in plants to be used as a source of natural antioxidants [3]. Chemical compounds that belong to the antioxidant group are the polyphenols, flavonoids, vitamin C, vitamin E, and carotenoids [4]. Flavonoid compounds can act as natural antioxidants because they have activity in binding oxygen radicals and inhibiting peroxidation [5]. The primary use of antioxidants is to stop or break the chain reaction of free radicals to save the body from damage due to free radicals. The examples of Synthetic antioxidants were BHA (butylated hydroxyanisole) and BHT (butylated
hydroxytoluene), which used for a long time in excessive amounts. They can provide side effects that are quite dangerous to health, such as liver damage \[6\].

\textit{Xanthosoma nigrum} Stellfeld is potential as an antioxidant. In this study, the antioxidant activity of the methanol extract of \textit{Xanthosoma nigrum} Stellfeld was determined using the TBA method. Determining the potential of a sample as an antioxidant used the inhibition of the linoleic acid oxidation process by free radicals. The purpose of this study was to determine the antioxidant activity of \textit{Xanthosoma nigrum} Stellfeld methanol extract using the thiobarbituric acid (TBA) method. The hypothesis of this study was the methanol extract of \textit{Xanthosoma nigrum} Stellfeld. It contained bioactive compounds such as flavonoids, which could be potential as antioxidants. This study's results are expected to provide scientific information to the community regarding plants' efficacy as natural antioxidants.

2. Materials and Methods

2.1. Chemicals.
Methanol, buffer phosphate, linoleic acid, ethanol, aquades, α-tocopherol, tetrametoxypropane.

2.2. Samples.
The samples were obtained from a farm in Samberejo Village, Rejang Lebong Regency, they were carefully selected and cleaned by running water.

2.3. Flavonoid test.
The sample was mashed using a mortar, then given methanol solvent Pa 99.8%. The extract obtained was dropped on a drop plate, plus 2 pieces of Mg tape and 2 drops of concentrated HCl. The formation of red color indicated the presence of flavonoid compounds \[7\].

2.4. Extraction.
79 grams of samples were put into a dark bottle and macerated using 96% methanol for 4 days. The mixture was filtered using filter paper to separate the filtrate from dregs. The filtrate obtained was concentrated using a rotary evaporator with a speed of 90 rpm at the temperature of 50°C \[7\].

2.5. Hydroperoxide Analysis from Oxidation of Linoleic Acid by Conjugated Diene Method \[8\].
Hydroperoxide analysis of linoleic acid oxidation was carried out by inserting 2 mL of 0.1 M pH 7 phosphate buffer and 2 mL of 50 mM linoleic acid in 99.8% ethanol 1 mL of distilled water into a dark bottle, then incubated at 40°C. The mixture was taken 50 μL and then put in 6 mL of 99.8% ethanol. The absorbance of the conjugated diene sample was measured directly using a UV-VIS spectrophotometer at a wavelength of 234 nm. The hydroperoxide analysis was measured every day until the maximum absorbance was obtained.

The analysis of the concentration of Malondialdehyde (MDA) used the TBA method. \textit{Xanthosoma nigrum} Stellfeld stem extract was made in 100 ppm, 200 ppm, and 300 ppm. The research took each sample was taken as much as 1 mL, then added with 2 mL of 0.1 M phosphate buffer pH 7.0 and 2 mL of 50 mM linoleic acid in 99.8% ethanol. The positive control solution used 1 mL of 200 ppm α-tocopherol, 2 mL of 0.1 M phosphate buffer pH 7.0, and 2 mL of 50 mM linoleic acid in 99.8% ethanol. In comparison, the negative control solution consisted of 1 mL of ion-free water and 2 mL of phosphate buffer 0.1 M pH 7.0 and 2 mL 50 mM linoleic acid in 99.8% ethanol. These solutions were put in a dark bottle and incubated at 40°C during the conjugated diene method’s optimum incubation time. Two days after the maximum incubation time, measurements of thiobarbituric acid reactive substances (TBARS) were carried out through the thiobarbituric acid (TBA) method by taking 1 mL from each solution. It was then added 2 mL of 20% trichloroacetate (TCA) solution and 2 mL of thiobarbituric acid (TBA) solution 1% in 50% acetic acid. The blank used was distilled water with the same treatment. Then the reaction mixture was placed in a water bath of 100°C for 10 minutes. After
cooling the solution, it was centrifuged at 3000 rpm for 15 minutes and measured at a wavelength of 532 nm.

The standard tetrametoxypropane (TMP) curve was prepared using a tetrametoxypropane solution with concentrations of 1.5, 3, 6, 9, 12, 15, and 18 μM. Each solution was piped 1 mL and added 2 mL of 20% TCA solution and 2 mL of 1% TBA solution in 50% acetic acid. The mixture is placed in a 100°C water bath for 10 minutes. After cooling, the solution was centrifuged at 3000 rpm for 15 minutes. Then the absorption was measured at a wavelength of 532 nm. The blank used was distilled water with the same treatment.

3. Results and Discussion

3.1. Flavonoid Test.
The sample's qualitative test results showed that the extract was positive for flavonoids as indicated by the formation of red color with concentrated HCl and Mg tape to reduce the benzopyrone core contained in the flavonoid structure so that the color changed to orange or red.

3.2. Hydroperoxide Analysis from Oxidation of Linoleic Acid by Conjugated Diene Method [8]

Determination of linoleic acid's incubation time with the conjugated diene method was carried out before measuring the sample's anti-oxidation activity. The absorption value curve of the conjugated diene bond during the incubation time is shown in Figure 1.

![Figure 1](image)

Figure 1. Determination of incubation time of linoleic acid

Measurement of hydroperoxide, which resulted from linoleic acid oxidation reaction, showed its maximum absorption peak on day 5. Linoleic acid, which was oxidized by oxygen at an early stage, would form hydroperoxides. This hydroperoxide level increased after reaching the maximum level. The hydroperoxide would decompose to form malondialdehyde as the end product of the lipid peroxidation reaction. Besides, the lipid peroxidation reaction decreased the curve after the 5th day. Based on the determination, the linoleic acid incubation time gave the maximum absorbance on the 5th day. The measurement of malondialdehyde (MDA) was carried out on the 7th day with the purpose that hydroperoxide had completely decomposed to form MDA.

After two days, all hydroperoxides had decomposed into MDA (the amount of MDA formed was much more than the amount of hydroperoxide formed simultaneously). The autoxidation process of linoleic acid could be inhibited in the presence of antioxidant compounds. The addition of purple taro stem extract to the mixture of linoleic acid and phosphate buffer was carried out on day 1 to produce a maximum anti-oxidizing effect in the initial stage (initiation) of linoleic oxidation acid to form hydroperoxides. Furthermore, the propagation stage occurs, in which the hydroperoxide level would continue to increase until a maximum state was formed, then. At the termination stage, the hydroperoxide was decomposed into smaller molecular compounds such as malondialdehyde. Some of the
Factors influencing linoleic acid’s autoxidation are heat, light, pH, oxygen, metal ions, and lipid radicals themselves.

3.3. Hydroperoxide Analysis from Oxidation of Linoleic Acid by Conjugated Diene Method \(^8\)

The antioxidant potential of all types of plants could be determined by comparing the absorbance values that describe the MDA concentration. A low absorbance value indicated that plants had high antioxidant potential by reducing the amount of MDA that reacted with thiobarbituric acid (TBA), which formed a red product \(^9\). MDA compounds formed from the decomposition of hydroperoxide were analyzed using the TBA method to determine their antioxidant activity. According to Leonard et al. (2004), TBA could measure the amount of MDA production in the form of thiobarbituric acid reactive substances (TBARS). The termination results were calculated as TBARS and reacted with TBA to form the MDA-TBA complex to produce a pink color complex with maximum absorption at a wavelength of 532 nm. TMP was used as the standard because it would form MDA when hydrolyzed with acid. In principle, MDA lipid peroxidation could react with TBA under acidic conditions and high temperatures with a wavelength of 532 nm \(^10\). The results of standard tetrametoxypropane (TMP) absorbance measurements are made in a linear regression graph as in Figure 2.

![Figure 2. The standard curve of tetrametoxypropane](image)

The results of the thiobarbituric acid (TBA) test showed that the methanol extract of samples was able to inhibit the oxidation of linoleic acid, which was indicated by the concentration of Malondialdehyde (MDA).

![Figure 3. Concentrations samples Vs. malondialdehyde](image)

At a concentration of 100 ppm, 150 ppm, 200 ppm, and 300 ppm could be seen in the relationship between sample concentration and [MDA]. The greater the concentration of extract, the smaller MDA concentration. In contrast, in the positive control (α-tocopherol) concentration MDA was very small compared to the extract concentration. It explained that a small MDA concentration value had a large antioxidant activity and vice versa. A large MDA concentration value explains that the antioxidant activity contained in the sample extract was smaller. In the negative control, the value of MDA concentration was more significant than α-tocopherol and sample extract. The MDA concentration
determined the antioxidant activity of the sample extract. The results obtained could be shown in Figure 4.

![Figure 4. The concentration of samples Vs. activity of antioxidant activity](image)

The concentration of samples V/s. activity of antioxidant activity is shown in Figure 4. The percentage of antioxidant activity described the potential size of each extract with various concentrations of an antioxidant. A negative control was used as a reference to determine the percentage of antioxidant activity because if the incubation was not treated so that the oxidation process run typically without any obstacles from the added extract. The relationship curve between the sample concentration and the percentage of the antioxidant activity showed that the value of [MDA] was inversely related to the percentage of its antioxidant activity. The greater the MDA, the smaller the extract's potential to inhibit linoleic acid's oxidation process. However, the amount of sample concentration was directly proportional to the percentage of the antioxidant activity. The greater the sample concentration, the greater the extract activity in inhibiting linoleic acid oxidation.

At a concentration of 300 ppm, sample extract could inhibit linoleic acid oxidation, which was greater than at the extract concentrations of 100 ppm, 150 ppm, and 200 ppm. Rohman et al. (2005) explained that the greatest antioxidant activity was in flavonoid compounds. The thought to be due to the presence of several active compounds in the extract that play a bigger role in inhibiting free radical activity. The effectiveness of antioxidants in the linoleic system and emulsion system is influenced by the polarity of the solution, lipid substrate, pH, concentration, oxidation time, and the method used to determine lipid oxidation, emulsifier, relative antioxidant stability.

4. Conclusion
Based on the research results carried out, the maximum anti-oxidation activity of the methanol extract of purple taro stems is obtained at a concentration of 300 ppm with 31.05% antioxidant activity. At a concentration of 300 ppm, it can withstand the oxidation rate of linoleic acid, which is better than the concentrations of 100 ppm, 150 ppm, and 200 ppm.

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