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# Study on The Potency of Methanol Extracts From *Xanthosoma nigrum* Stellfeld As Natural Anti Oxidant by Thiobarbituric Acid Method

**[1*]Devi Ratnawati, [2]Lusiana, [3]Agus Martono and [4]Wahidah Fitriyanti**
Chemistry Department, University of Bengkulu, Bengkulu, Indonesia.
*Corresponding email: devi_r64@yahoo.com

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**Abstract**- In this research *Xanthosoma nigrum* Stellfeld (the Purple yam) was selected as experimental material. This plant was collected from Rejang Lebong region, Bengkulu Province. Methanol extract 96% from stem of purple yam was studied its anti-oxidant activity in various concentrations with α-tocopherol (200 ppm) as standard of antioxidant. Antioxidant activity was determined using Thiobarbituric Acid (TBA) method. Linoleic acid was oxidized at 40 ºC for seven days with or without extract and the final product malondialdehyde (MDA) was reacted with thiostembituric acid to be of red colored complex (MDA-TBA) and was then measured by UV-VIS spectrophotometer at λ 532 nm. Stem extract of purple yam with concentration of 100 ppm, 150 ppm, 200 ppm and 300 ppm respectively had the inhibition of 19.32%, 21.85%, 29.47%, and 31.05%. α-Tocopherol as positive control which showed inhibition ability of 85.14% at 200 ppm. Based on the result obtained in this study, the stem's extract of Purpel yam plant showed that antioxidant activity was lower than α-tocopherol.

**Keywords** : *Xanthosoma nigrum*; Stellfeld; MDA; Antioxidant.

## Introduction

Discussion about the beneficial effects of saturated versus unsaturated fatty acids has been a topic of research among the world's leading nutritional experts. A diet containing fats of the unsaturated moeity has shown to be beneficial in the prevention of atherosclerosis and coronary heart disease (Wolfram, 2003). Long-term diets containing monounsaturated fatty acids have been reported to reduce platelet aggregation and decrease plasma LDL-cholesterol levels (Smith *et al.*, 2003). Saturated fatty acids undergo less peroxidation than their unsaturated counterparts. Indeed, supplementation with polyunsaturated as opposed to saturated fatty acids results in a statistically significant increase in lipid peroxidation in the plasma and liver (Song *et al.*, 2000; Song and Miyazawa, 2001; Shin, 2003). The formation of hydroperoxides, cholesterol hydroperoxides, and endoperoxides in biological system leads to a serious disesase such as coronary arteriosclerosis and diabetes mellitus as well as being associated with aging and carcinogenesis (Gaziano, 1996; Gerber *et al.*, 1996; Halliwel, 1997; Haraguchi *et al.*, 1997; Abd El-Bakry *et al.*, 2002). Lipid peroxidation has attracted much attention in relation to oxidative damage of biological molecules due to the formation of lipid peroxides which in presence of cellular ion containing compounds, can break down to yield oxygen radicals (Pryor, 1987).

One of means that should be done in order to preventing cardiovascular disesase is to consume of antioxidant which neutralize free radical (Schwenke *et al.,* 2002). It can functionize as modulator of apoptosis and fasten metal ion in forming of ROS (Reactive Oxygen Substrate) species which finally generate degenerative problem specially disesase, i.e cardiovascular (Ziesel, 2004). One of plants with potential antioxidant is *Xanthosoma nigrum* Stellfeld. *Xanthosoma nigrum* Stellfeld has been classified into family of Araceae and the Araceae contain crystal of calcium oxalate, which are often cited as causing the intense irritation experienced when handling or consuming the raw plant tissue or many genera in the family (Arditty and Rodriguez, 1982). It is also contain flavonoid as generate of phenol which can be function as antioxidant (Ratnawati and Lusiana, 2013). Despite the toxic effects of Araceae, species of several genera are also cultivated as both food and medicinal plant (Plowman, 1969).

Picerno *et al.* (2003) has been done the research about phenolic compound and antioxidant characteristif of *X. violaceum* leaves. Activity of antioxidant measured by DPPH test and peroxidation was induced by *2,2' azobis(2-amidinopropane) hydrochloride* as inisiator of radical in linoleic acid mixture. Antioxidant activity of *X. sagittifolium* Schoot has been measured. Total phenol was analyze by the use of Folin-Ciocalteu method, DPPH was used to determine the activity of antioxidant and ascorbic acid is used as standard. In this research, activity of antioxidant is determine by Thiostembituric Acid (TBA) method. The oxidation products of unsaturated fatty acids reacted with TBA to form a red-colored compound, the reaction with TBA has been widely adopted as a sensitive assay method for lipid

peroxidation in animal (Bernheim *et al*., 1948; Wilbur *et al*., 1949). This method is sensitive and precise in determining peroxides of linoleic acid, linolenic acid, and arachidonic acid (Kenaston *et al*., 1955).

The thiobarbituric acid (TBA) test is an easy and quick assay for the assessment of lipid peroxidation in which malondialdehyde (MDA) is derivatized. The rationale and methodology have been discussed in detail elsewhere (Esterbauer, 1996; Moore and Roberts, 1998) and have rightfully been criticized for low specificity and artifact formation because only a fraction of the MDA measured was generated in vivo (Halliwell and Gutteridge*, 1999;* Moore and Roberts, 1998). Furthermore, the TBA derivatization procedure itself leads to the formation of several MDA-unrelated ultraviolet (UV)- absorbing and fluorescent species. Despite this fact, the method remains one of the most useful and commonly used measurements of oxidative damage because of its simplicity. In recent years, several HPLC-based TBA assays have evolved with increased specificity (Londero and Lo, 1996; Suttnar *et al*., 2001), but nevertheless the spectrophotometric methods remain commonly used. However, there have been limited studies on *X. nigrum* Stellfeld. Hence, the principal objective of this study was to determine wheather methanol extract of *X. nigrum* Stellfeld have potency as natural antioxidant.

## Materials and Methods

Natural *X. nigrum* Stellfeld was collected from Desa Samberjo Kecamatan Selupu Rejang Kabupaten Rejang Lebong, Indonesia. Methanol P.a, ethanol P.a, acetic acid, and hydrochloric acid (HCL) were purchased from Merck, 1.1.2-trimethoxypropane (TMP), trichloroacetic (TCA), α-tocopherol and thiostembituric acid (TBA) from Sigma-Aldrich, linoleic acid from Fluka were used as received. Destilled water, phosphate buffer pH 7 and Mg ribbon are available at Chemistry Laboratory, Faculty of Mathematics and Natural Science, University of Bengkulu, Indonesia.

**Preparation of sample and extraction**

The collected sample was cleaned by using water, dried over the sun shine and crushed into powder and was then macerated by using technical methanol 96% for 3 days. Mixture was filtered by using filter paper to dissociate filtrate with stem residue of purple yam. Filtrate condensed by using rotary evaporator with speed 90 rpm at temperature 50 $^{o}$C. It was stored in refigerator till it used.

**Qualitative test of flavonoid**

Extract was dripped on drip plate, then given of methanol 96%, added 2 cutting-ribbon of Mg and 2 mL drip of HCL condensed 98%. The red colour was formed by addition of concentrate HCL showing the existence of flavonoids. As comparator used betelnut with the same procedure ( Sundaryono, 2011).

**Analysis of hydroperoxide from oxidation of linoleic acid by method of diene conjugation (Kikuzaki and Nakatani, 1993)**

Briefly, analysis of hydroperoxide from oxidation of linoleic acid done by added 2 mL phosphate buffer 0.1 M pH 7, 2 mL of linoleic acid 50 mM in etanol 99.8%, and 1 mL destilled water into dark bottle, then incubated at temperature 40$^{o}$C. Mixture of sampel taken by 50 μL, and then added into 6 mL ethanol 75%. Absorbance of diene conjugation of sampel measured by used of UV-VIS Spectrophotometer at wavelength 234 nm. Analyze this hydroperoxide measured every day till got maximum absorbance.

**Concentration analysis of malonadialdehida (MDA) with method of TBA**

Stem extract of purple yam made in variety concentration (100 ppm, 200 ppm and 300 ppm). Each sampel taken 1 mL, it was added 2 mL phosphate buffer 0.1 M pH 7.0 and 2 mL linoleic acid 50 mM in etanol 99.8%. As positive control used 1 mL α-tocopherol, 2 mL phosphate buffer mL 0.1 M pH 7.0 and 2 mL linoleic acid 50 mM in etanol 99.8%, while for negative control 1 mL α-tocopherol replaced by 1 mL destilled water (Kikuzaki and Nakatani, 1993). All the mixture packed into dark bottle and incubated at temperature 40 °C during optimum incubation of method of diene conjugation. Two days after time of maximum incubation, measurement of TBARS (*Thiobarbituric Acid Reactive Substance*) through method of TBA by taking 1 mL from each samples was added 2 mL of TCA 20% and 2 mL of TBA 1% in acetate acid 50%. As blank is used destilled water with same treatment. The mixture put down in bath at 100°C during 10 minutes. After chilled, it was centrifuge 3000 rpm during 15 minutes and then solution is measured at λ 532 nm (Kikuzaki and Nakatani, 1993)

Standard curve have been made by using 1.1.2-trimetoxypropane (TMP) with variety concentration, such as 1.5, 3, 6, 9, 12, 15, and 18 μM, every solution was taken 1 mL and added 2 mL TCA 20% and 2 mL of TBA 1% in acetate acid 50%. The mixture put down in bath at 100 °C during 10 minutes. After chilled, it was centrifuge 3000 rpm during 15 minutes and then solution is measured at λ 532 nm. As blank is used destilled water with same treatment.

## Results and Discussion

Based on qualitative analysis of flavonoid, it showed that stem extract of purple yam contained of flavonoid (++++), which means that sample contain plenty of flavonoid compared to betelnut (+++). According to Ukieyanna (2012), more content of flavonoid in samples, more effective activity of antioksidan yielded. Flavonoid is chemical compound which included into group of antioxidant could be used to lessen continuous reaction related to free radical (Syaefudin, 2008). Compound of flavonoid was assumed to component of bioactive could be function as natural antioksidan capable to inhibition produce of peroxide lipid at system of linoleic ( Gulcin, 2010).

Determination of linoleic acid incubation time by the use of diena conjugation method is conducted before measurement of activity of sample oxidation. Analyze the hydroperoxide measured every day till got maximum absorbance.

Table 1. Absorbance of diene conjugation

| Days | Absorbance | | | Average absorbance |
|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 3 | |
| 1 | 0.0906 | 0.0622 | 0.0562 | 0.0696 |
| 2 | 0.1275 | 0.0944 | 0.0890 | 0.1036 |
| 3 | 0.2264 | 0.1170 | 0.0090 | 0.1174 |
| 4 | 0.1329 | 0.1290 | 0.1236 | 0.1285 |
| 5 | 0.1622 | 0.1454 | 0.2018 | 0.1698 |
| 6 | 0.1271 | 0.1231 | 0.1315 | 0.1272 |
| 7 | 0.1374 | 0.1138 | 0.1351 | 0.1287 |

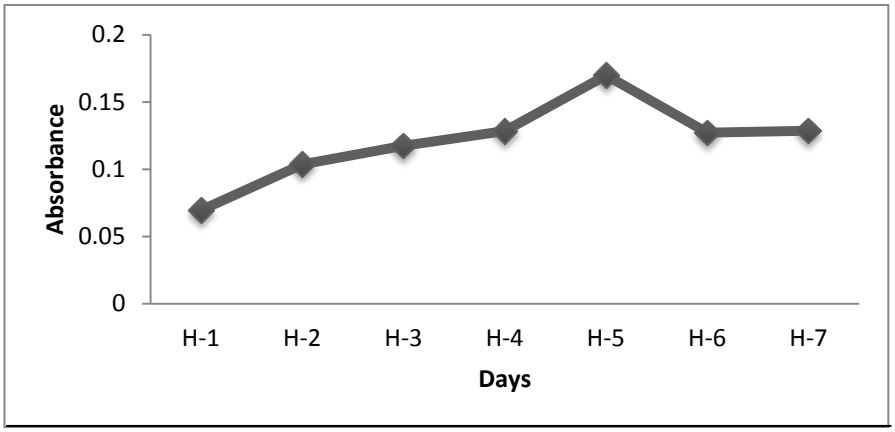


Figure 1. Maximum absorbance of diene conjugation

Analysis of diene conjugation give result of measurement which reach from first day and down after 5 days. Figure 1 showed the maximum absorbance is 0.1698 on days to 5 which caused by the optimum product of hydroperoxide (Bernheim *et al.*, 1948; Wilbur *et al.,* 1949). Linoleic acid was oxidized by oxidation agent at early stage to form hydroperoxide. Hydroperoxide will be decomposition form malondialdehida (MDA) as the final product reaction of lipid peroxidation. The purpose of measurement of MDA after 7 days was to give the chance of hydroperoxide have perfect decomposition. Determination of linoleic acid incubation time by the use of diene conjugation method, influenced by some factor could be assign value maximum absorbance which was different each other, such as the quality of linoleic acid, temperature of incubation which is not constant, and also existence of oxygen (Kenaston *et al.*,1955).

**Analysis potency of antioxidant in methanol extract of stem purple yam.**

Compound of MDA was formed from hydroperoxide decomposition analysed with method of TBA to determine its antioxidant activity which measured as *tiostembituric acid reactive substance* (TSTEMS) and react with TBA form red coloured complex of MDA-TBA with maximum absorption at λ 532 nm ( Kikuzaki & Nakatani, 1993). As its standard curve is used 1.1.2-trimethoxypropane (TMP) at various concentration such as: 1.5, 3, 6, 9, 12, 15, and 18 μM. TMP used as standard because could produced MDA if hydrolysis with acid (Xiong *et al.*, 1993). Equation of linier regretion was calculated that is $y = 0.1718x + 0.0181$, $R^2 = 0.9993$ was used to determine the concentration of MDA and inhibition stem extract of purple yam (Figure 2).

Based on Figure 3, it showed that addition of methanol extract of stem purpel yam could inhibit oxidation of linoleic acid, marked with concentration of MDA measured smaller than negative control. Negative control has highest concentration of MDA comparing to other treatment, that was 5.6935 μM. It was caused by inexistence compound which can be function as antioxidant, so that linoleic acid continue to oxidation form much of MDA (Falakh, 2008). α-tocopherol 200 ppm have concentration of MDA equal to 0.8463 μM. It is indicate that α-tocopherol as antioxidant could inhibit produce of MDA. α- tocopherol as proton donor can change radical of peroxyl (product of lipid peroxidation) becoming radical of tocopherol which was less reactive, so that was unable to destroy chain of linoleic acid (Moncheva *et al.*, 2004). Autooxidation of linoleic acid inhibit in existence of antioxidant compound (Tuminah, 2000). Addition of methanol stem purpel yam extract to linoleic mixture was done in early stage (days 0) to result antioxidant maximum effect. In existence of antioxidant, concentration of malonaldehide (MDA) which produce from

autooxidation smaller than without addition antioxidant. Absorbance value is proportional to concentration of MDA and inversely with activity of antioxidant (Wong *et al.*, 1993).

Table 2. Absorbance of standard 1.1.2-trimethoxypropane (TMP)

| TMP (µM) | Absorbance |
|---|---|
| 0 | 0.0001 |
| 1,5 | 0.3267 |
| 3 | 0.5221 |
| 6 | 1.0307 |
| 9 | 1.5608 |
| 12 | 2.0411 |
| 15 | 2.6361 |
| 18 | 3.106 |

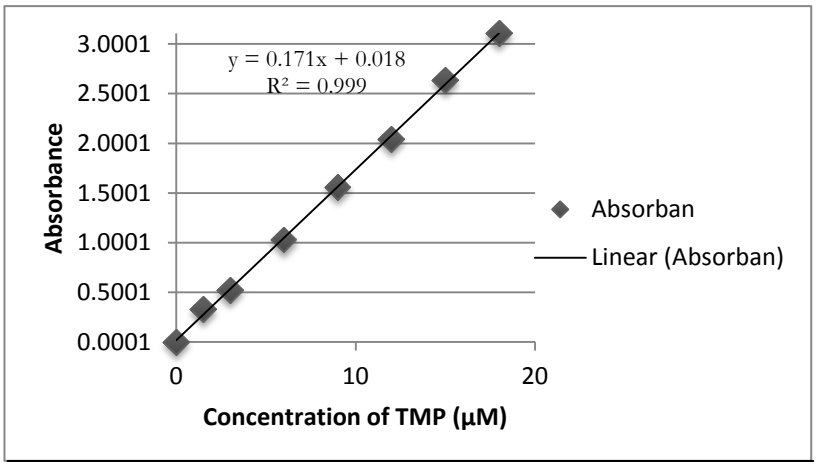


Figure 2. Curve of standard 1.1.2-trimethoxypropane (TMP)

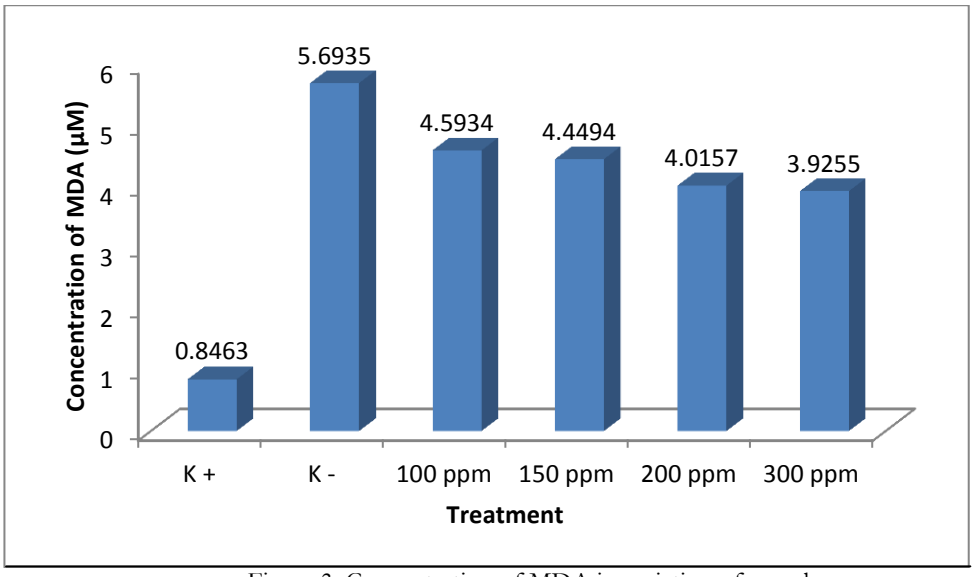


Figure 3. Concentration of MDA in variation of sample

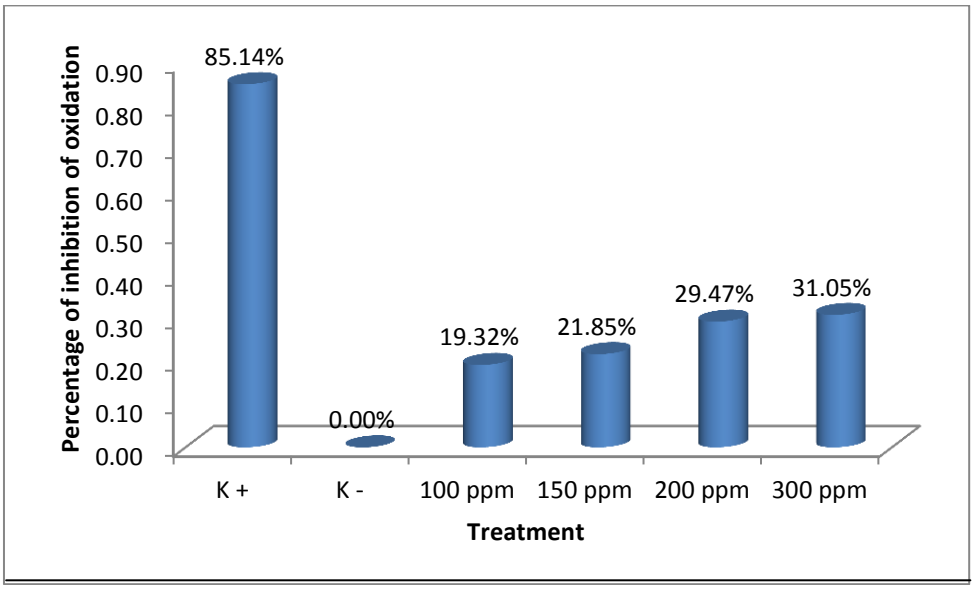


Figure 4. Percentage of inhibition of oxidation purpel yam stem extract

Inhibition activity forming of MDA of each treatment calculated from rate of MDA obtained (done by tree repetition) showed at Figure 4. Percentage of inhibition show the level of potency each extract as antioxidant. Negative control used as reference to determine percentage of resistivity because no treatment on it, so that process oxidation is normal without existence of extract. α-tocopherol 200 ppm as positive control having inhibition ability for 85.14%. At positive control was used 200 ppm concentration, 200 ppm is the limit of existence antioxidant in our body (Bermond, 1990). Purpel yam stem extract with concentration 100, 150, 200 and 300 ppm each has inhibition ability 19.32%, 21.85%, 29.47% and 31.05%. According to the research, in general stem extract of purple yam have activity of antioxidant but still below α-tocopherol as positive control.

## Conclusions

Qualitative test of flavonoid showed that stem extract of purple yam contained of higher flavonoid compared to betelnut. Purpel yam stem extract which contained flavonoid at concentration 100, 150, 200 and 300 ppm each had inhibition ability 19.32%, 21.85%, 29.47% and 31.05%. In general stem extract of purple yam showed activity of antioxidant but it was still below α-tocopherol as positive control. In this research, activity of antioxidant was determined by calculating inhibition ability of the extract to inhibit oxidation of linoleic acid.

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