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SHORT COMMUNICATION

The Comparative Ability of Antioxidant Activity of *Piper crocatum* in Inhibiting Fatty Acid Oxidation and Free Radical Scavenging

MUHAMMAD ALFARABI*, MARIA BINTANG, SURYANI, MEGA SAFITHRI

[1]Department of Biochemistry, Faculty of Mathematics and Natural Science, Darmaga Campus, Bogor Agricultural University, Bogor 16680, Indonesia

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The excessive number of free radicals in human body could increase lipid peroxides and causes a variety of degenerative diseases. *Piper crocatum* is a plant containing natural chemicals that may have an antioxidant ability to inhibit fatty acid oxidation and reduce free radicals, however, there is no published scientific reports up to now. Therefore, the objective of this research is to study the ability of ethanol extract of *P. crocatum* as an antioxidant. The leaves of *P. crocatum* (25 g) were extracted with 100 ml of 70% ethanol. Antioxidant activity was measured by thiobarbituric acid method and compared with 2,2-diphenil-1-picryl hydrazyl method with α-tocopherol as the standard antioxidant. The results showed that the extract of 200 ppm inhibited fatty acids oxidation by 80.40% and IC_{50} for free radical scavenging was 85.82 ppm. There was no significant different (α = 0.05) inhibition of unsaturated fatty acids oxidation between the sample and á-tocopherol at 200 ppm. This study suggested that the leaves extract of *P. crocatum* has a potential natural antioxidant.

Key words: *Piper crocatum*, unsaturated fatty acid oxidation, free radical scavenging

INTRODUCTION

Unhealthy life style may cause xenobitic compounds entering to the body, some of them could be free radical molecules. They are reactive and unstable molecules, since they loss one or more pairs electrons (Betteridge 2000). Free radicals can be produced by biochemical processes in the body, such as reactive oxygen species (ROS), or from the environment such as ultraviolet radiation inducing the formation of free radicals. In the body, many of them cause increasing lipid peroxidation from unsaturated lipid and have a relation with degenerative diseases such as cancer, diabetes mellitus, and cardiovascular diseases. in many cases, people with diabetes mellitus has a higher level of lipid peroxides in their body than normal people have (Memisogullari *et al.* 2003). Therefore, antioxidants are important to inhibit free radical and unsaturated lipid oxidation reactions, thus reducing the occurrence of degenerative diseases (Murray *et al.* 2003).

Antioxidants can be produced in our body such as superoxide dismutase and glutathione peroxidase (Murray *et al.* 2003) or from plant secondary metabolite compounds such as flavonoid, tannin, and alkaloid (Harborne & Williams 2000). Flavonoid groups such as rutin and apigenin from *Teucrium polium* L. have antioxidant and free radical scavenging activiiesy (Sharififar *et al.* 2009). Condensed tannin from *Rosaceae* plant roots (Oszmianski *et al.* 2007) and some alkaloid groups such as linearilobin and linearilin (Kolak *et al.* 2006) also showed free radical scavenging activities in Indonesia, natural products from a variety of plants have been utilized for the treatment of various diseases, and one of them is *P. crocatum*. It is a creeping plant growing in tropical areas and previously known as an ornamental plant, then later is used as a medicinal plant. In Indonesia, the leaves of the plant was used as antidiabetic medication to lower blood glucose levels (Sudewo 2005). The leaves contained bioactive compounds such as flavonoids, tannins, and alkaloids and have antihyperglycemia activity (Safithri & Fahma 2008). Therefore, it is interesting to study the ability of *Piper crocatum* antioxidant activity in inhibiting unsaturated fatty acids oxidation and scavenging free radicals. The objective of this research is to study the possibility of using *Piper crocatum* as an antioxidant by examining its ability to inhibit unsaturated fatty acids oxidation and to scavenge free radicals.

MATERIALS AND METHODS

Plant Extracts. The fresh leaves of *Piper crocatum* were collected from Bogor, Indonesia. The leaves (25 g) were washed with water, chopped into pieces, and crushed. The samples were extracted with 100 ml of 70% ethanol as solvent for 24 hours at room temperature. The extractionwas carried out twice. The alcohol was removed by filtration and fresh alcohol was added to the samples. The combine filtrates were evaporated at 50 °C and using freeze dryer (at -50 °C, 8 mBar).

*Corresponding author. Phone/Fax: +62-251-8423267, E-mail: alfarabi17@yahoo.com

Inhibition of Unsaturated Fatty Acid Oxidation Assay. For this assay, we used TBA (Thiobarbituric acid) method (Kikuzaki & Nakatani 1993). This method was used for measuring the malondialdehyde level and antioxidant activity.

Measurement of Malondialdehyde (MDA) Level. A mixture 6 ml of 0.1 M phosphate buffer pH 7.0, 6 ml of 50 mM linoleic acid (Sigma) in absolute ethanol, and 3 ml water was placed in a vial (1 ml solution each vial) and then placed in a dark water bath at 40 °C. The level of malondialdehyde was measured at 532 nm every 24 h until three days after its absorbance reached maximum. Into 1 ml solution were added 2 ml of TCA (trichloroacetic acid) 20% and 2 ml of TBA 1% in acetic acid 50%. The mixture was placed into boiling water-bath for 10 minutes, after being cooled it was centrifuged at 15.5 g for 15 minutes.

Antioxidant Activity. The samples were prepared with different concentrations (25, 50, 75, 100, and 200 ppm). A mixture of 1 ml of each sample, 2 ml of 0.1 M phosphate buffer pH 7.0, and 2 ml of 50 mM linoleic acid in absolute ethanol was placed in dark bottle and then placed in dark water-bath at 40 °C. Antioxidant activity was measured at 532 nm on the maximum level malondialdehyde day. 1 ml solution of the sample were added with 2 ml of TCA 20% and 2 ml of TBA 1% in acetic acid 50%. The mixture was placed into boiling water-bath for 10 minutes and after being cooled, it was centrifuged at 15.5 g for 15 minutes. A mixture without sample was used as negative control and á-tocopherol (Sigma) (200 ppm) was used as positive control antioxidant. We used 1,1,3,3-tetramethoxypropane (Sigma) as malondialdehyde standard solution. The percent inhibition was calculated from this equation:

$$\% \text{ inhibition} = \frac{[\text{MDA}]_{\text{samples}} - [\text{MDA}]_{\text{negative control}}}{[\text{MDA}]_{\text{negative control}}} \times 100\%$$

Free Radical Scavenging Assay. The free radical scavenging assay used DPPH (2,2-diphenil-1-picryl hydrazyl) (Sigma) as free radical (Aqil *et al.* 2006). Two milliliters of each samples (25, 50, 75, 100, 200 ppm) were added into 2 ml of 0.1 mM DPPH in methanol solution. After 30 minutes incubation at room temperature, the reduction in the number of DPPH was measured at 517 nm. A solution DPPH without samples was used as negative control and another solution with á-tocopherol (Sigma) (1, 2.5, 5, 7.5, 10 ppm) was used as positive control antioxidant. The percent inhibition was calculated from following equation:

$$\% \text{ inhibition} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{samples}}}{\text{absorbance}_{\text{control}}} \times 100\%$$

RESULTS

Inhibition of Unsaturated Fatty Acid Oxidation Assay. Yield from ethanol 70% extract of leaves *P. crocatum* was 4.42%. These samples were used to test antioxidant activities with TBA method. The method was used to measure malondialdehyde level as the product linoleic acid oxidation. Malondialdehyde levels from day 0 to 6 has increased and decreased after 6 days. The maximum formation of malondialdehyde occurred on day 6 (Figure 1). This result showed that ethanol extract (70%) of *P. crocatum* leaves have an ability to inhibit oxidation of linoleic acid. This activity is indicated by low levels of malondialdehyde compared to the negative control (Figure 2). The increasing concentrations of samples were followed by the decreasing malondialdehyde level. The highest sample concentration (200 ppm) could inhibit oxidation linoleic acid by 80.40% and the lowest by 44.31% (Figure 3). Each sample has significant difference ($\alpha < 0.05$) inhibition with negative control. However, there was no significant difference ($\alpha > 0.05$) inhibition between the sample concentration and α-tocopherol at the same concentration meaning that antioxidant activity between sample and á-tocopherol was similar (200 ppm).

Free Radical Scavenging Assay. Besides testing the inhibition of linoleic acid oxidation, the samples were also tested for free radical scavenging. The results showed that each sample has ability to inhibited DPPH. The highest sample concentration (200 ppm) could inhibit DPPH by 73.41% and the lowest by 13.10%. But, á-tocopherol inhibited by 80.36% at 10 ppm and 37.87% at 1 ppm. IC_{50} of the samples was 85.82 ppm and á-tocopherol was 2.12 ppm (Figure 4).

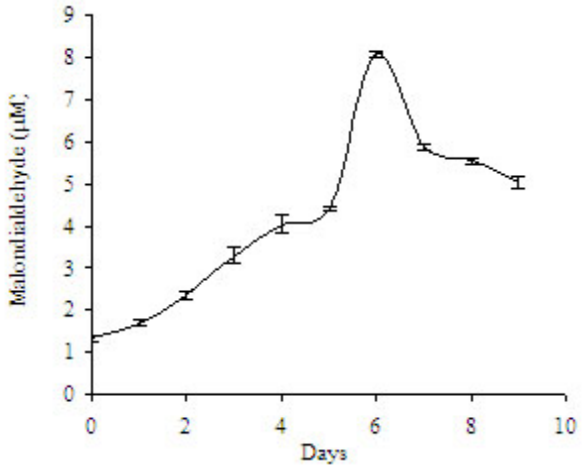

Figure 1. Malondialdehyde level each day.

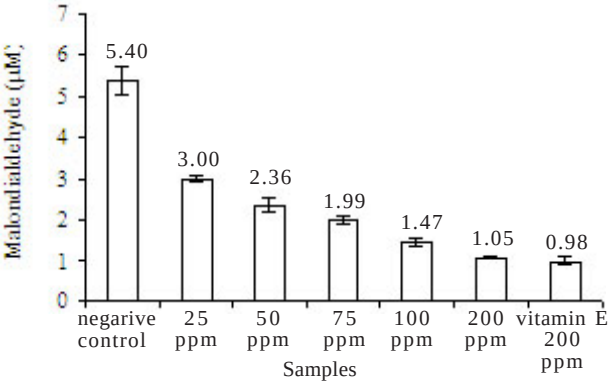

Figure 2. Malondialdehyde level in various concentration.

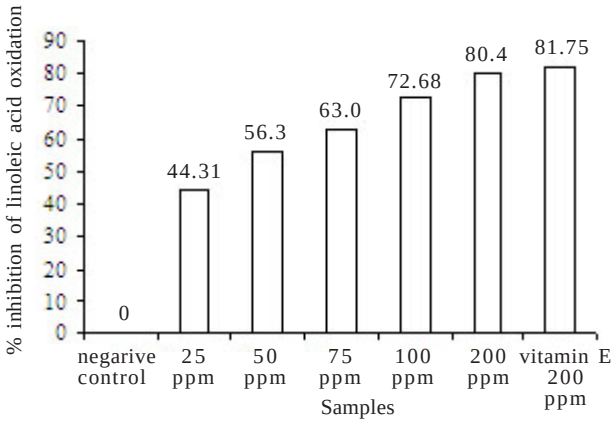

Figure 3. Inhibition of linoleic acid.

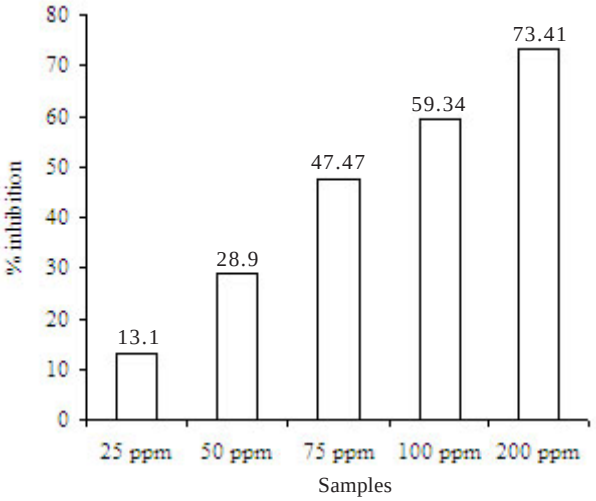

Figure 4. Extract inhibitions for DPPH.

DISCUSSION

Extraction process using 70% ethanol is in order to extract active compounds that have more antioxidant activity, such as polyphenols, flavonoids, tannins, and alkaloids. Spigno and Faveri (2007) suggested that when an extract contains more antioxidant compounds, the antioxidant activity is higher too . The polarity of ethanol is lesser than water, so make it efficient in degrading cell walls and seed which have unpolar character (Lapornik *et al.* 2005). Lapornik *et al.* (2005) found that the ethanol extract of red and black currants have more polyphenol than the water extract have.

Lipids containing unsaturated fatty acids are easily attacked by free radicals on the double bond to form lipid peroxides causing structural damaged. The primary lipid peroxidation products have hydroperoxy unsaturated fatty acyl residue (Girotti 1998), so that the hydroperoxides decompose to aldehydic product (Spiteller 2001). Several factors causing the oxidation of unsaturated fatty acids are temperature, light, metal ions, and oxygen (Sen *et al.* 2000). In this study, we used linoleic acid accelerating the oxidation rate by adjusting the temperature remained at 40 °C. Malondialdehyde is one product of aldehyde groups generated from the oxidation of unsaturated fatty acids. Other products from the oxidation could be in the forms of alcohol compounds, aldehyde, or volatile hydrocarbons (Sen *et al.* 2000). Malondialdehyde could react with TBA to produce complex malondialdehyde-TBA. If there is an antioxidant, malondialdehyde formation decreases and the formation of complex malondialdehyde-TBA also decreases. The leave extract *P. crocatum* as a natural antioxidant could inhibit the oxidation of linoleic acids indicated by low levels of malondialdehyde in the sample solution. It was because of flavonoids, alkaloids, and tannins as bioactive compounds containing in the leaves of *P. crocatum* (Safithri & Fahma 2008). Flavonoids have been shown to have strong antioxidant activity in inhibiting lipid oxidation. The compounds have ability to interact with biomembranes to protect from free radical (Saija *et al.* 1995). In addition, flavonoid could interact with human serum albumin and efficiently protecting the fraction of human serum albumin bound to linoleic acid site that is the most susceptible to oxidation (Dufour *et al.* 2007).

This sample also showed free radical scavenging activity. We used DPPH as stable free radical at room temperature. The color of DPPH is purple, when reacted with antioxidant solution the color changed to yellow. The principle of this method is to neutralize the DPPH as free radical compounds with antioxidantindicated by color changes in solution. There are two mechanisms in scavenging DPPH by antioxidant. The first is a direct transfer of electrons or H atoms from antioxidants to DPPH and the second is a proton concerted electron transfer process (Lan & Hong 2003). The free radical scavenging of the samples are lower than á-tocopherol because the sample was crude extract. There may be other compounds inhibiting the antioxidant activity. If we compared between the inhibition of oxidation linoleic acid and the free radical scavenging activity from the leaves extract *P. crocatum* at the same concentrations, the ability to inhibit linoleic acid oxidation is higher than the free radical scavenging activity. We suggested that the leave extract of *P. crocatum* is more efficient in inhibiting the oxidation of linoleic acid than the free radical scavenging activity. In conclusion, the leave extract of *P. crocatum* has potential natural antioxidant compounds that able to reduce free radicals and oxidation of fatty acids. However, the inhibition of fatty acid oxidation is higher than free radical scavenging at the same concentration.

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