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Antioxidant Potential of Ethanol Extract from Mango Parasite (*Dendrophthoe petandra*) to Maintain Crude Palm Oil (CPO) Quality

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Abstract. The present study aimed to determine the effectiveness of adding natural antioxidants from ethanolic extract of mango parasite (*Dendrophthoe petandra*) to oxidation stability of crude palm oil (CPO) during storage. The parameters tested were levels of free fatty acid (FFA), acid values, and deterioration of bleachability index (DOBI). The antioxidant activity of *D. petandra* extract obtained was IC_{50} of 6.369 ppm. Concentrations of *D. petandra* extract range from 200 to 1000 ppm. Based on the results, the ethanolic extract of *D. petandra* was able to reduce the FFA and acid value and increase the DOBI compared to the negative control. The lowest FFA levels and acid numbers were obtained from samples with addition of 1000 ppm natural antioxidants with FFA of 4.2% and acid value of 7.4 mg KOH/g, while the DOBI value increased to 1.300.

Keywords: *Dendrophthoe petandra, crude palm oil, free fatty acid, acid value, deterioration of bleachability index*

Abstrak. Penelitian ini bertujuan untuk mengetahui efektivitas penambahan antioksidan alami ekstrak etanol benalu mangga (*Dendrophthoe petandra*) terhadap stabilitas oksidasi minyak sawit mentah (CPO) selama penyimpanan. Parameter yang diuji adalah kadar asam lemak bebas (FFA), bilangan asam dan *deterioration of bleachability index* (DOBI). Aktivitas antioksidan ekstrak *D. petandra* yang diperoleh adalah IC_{50} 6,369 ppm. Konsentrasi ekstrak *D. petandra* yang diukur dari 200 hingga 1000 ppm. Berdasarkan hasil penelitian, ekstrak etanol *D. petandra* mampu menurunkan FFA dan Bilangan asam serta meningkatkan DOBI dibandingkan dengan kontrol negatif. Kadar FFA dan bilangan asam terendah diperoleh dari sampel dengan penambahan 1000 ppm antioksidan alami, dengan kadar FFA yaitu 4,2% dan bilangan asam yaitu 7,4 mg KOH/g, sedangkan nilai DOBI meningkat menjadi 1,300.

Kata Kunci: *Dendrophthoe petandra, minyak sawit, asam lemak bebas, bilangan asam, deterioration of bleachability index*

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INTRODUCTION

Generally, palm oil (about 80%) is used as a raw material for food products (Hasibuan, 2021). More than 99% the composition of CPO is lipid, which 95% are triglycerides and the rest are free fatty acids. While, the other 1% composition is non-oil components, like a water, carotene, phosphatides, aldehydes and other components in small amounts (Nugroho, 2019). However, during storage, CPO often undergoes a decreasing in quality both of organoleptic and nutritional aspects. These deterioration depends on several factors such as the influence of enzymes, increasing

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temperature, light, oxidation, the presence of metals and microbial activity (Frank *et al.,* 2013). Since CPO is mainly used for food applications, the quality of the CPO produced must meet a number of quality parameters. The PORAM specification is the most commonly used quality standard for the palm oil trading (Chew *et al*., 2021). CPO with higher quality will increase the selling price. CPO quality standards generally cover hydrolytic stability through the measurement of free fatty acids (FFA) and acid value, moisture and impurities as well as Deterioration of Blachability Index (DOBI) (Chong, 2012; PORAM, 2000). One of the most important factor that causes a decrease in the quality of CPO is oxidative stability. The oxidation process can increase the levels of FFA, acid value and decrease DOBI.

An alternative way to slow or minimize oil oxidation is adding of antioxidants. Antioxidants consist of compounds which can inactivate free radicals by donating hydrogen atoms or electrons to these molecules (Brewer, 2011). In food industry, the most commonly used is synthetic antioxidant are phenolic compound, such as butylhydroxyanisol (BHA), butylhydroxytoluene (BHT), tertbutylhydroquinone (TBHQ) and propyl gallate (PGa), which can regenerate acylglycerol and interfere the oxidation mechanism by proton donation (de Melo *et al.,* 2020). But recently, the use of synthetic antioxidants is starting to get a negative response because can be precursor of cancer in the body. Therefore, replacing an additive antioxidant with natural antioxidant is an alternative way to be safer for health (Holil & Griana, 2020). One of the plants that can be used as natural antioxidants is mango parasite *(Dendrophthoe petandra).* The compounds contained in the *D. petandra* extract are flavonoids, amino acids, carbohydrates, tannins, alkaloids and saponins (Hasan *et al*., 2018). In Artanti's 2009 study, ethanol extract of *D. petandra* showed varying antioxidant activity with IC_{50} of 6.4 to 38.7 g/ml. The results of the Least Significant Different (LSD) test showed that the ethanol extract of *D. petandra* did not show any toxic effects (LD50>1000µg/mL). The quercetin level of *D. petandra* was higher than the level of *Scurulla atropurpurea* (tea parasite). The level of quercetin in the *D. petandra* was 39.8 mg/g while the *S. atropurpurea* was only 9.6 mg/g (Endharti *et al.,* 2013). It is necessary to conduct study to test the quality of CPO samples with the addition of ethanol extract of *D. petandra* with a various concentrations and length time of homogenization. Furthermore, the quality was tested by the parameters of free fatty acids, acid values and DOBI.

MATERIAL AND METHODS

Materials

CPO samples from Banjarmasin, *D. petandra* from Pandeglang, methanol 96%, ethanol 96%, DPPH (1,1-diphenyl-2-picrylhydrazyl), $AlCl_3$ 2%, CH_3CO_2K, NaOH KOH, Quercetin standard and Phenolphthalein all materials obtained from Sigma-Aldrich, USA.

Instrumentation

The equipment used in this study was rotary evaporator (B-one, China), spektrophotometer UV-Vis (Optima SP 300, Japan), glassware (Iwaki, Japan).

Procedure

Sample extraction

Dried simplicia powder of *D. petandra* as much as 4.5 kg was macerated using ethanol

2.5 L for 3x72 h. Then, the filtrate was was concentrated by vacuum evaporator at 50 ℃ to obtain a crude extract of *D. petandra* (Ajileye *et al.,* 2015).

Antioxidant activity test

The antioxidant activity test was carried by the using of DPPH method (1,1-diphenyl-2-picrylhydrazyl). 0.05 g of *D. petandra* ethanolic extract was dissolved in 50 mL of methanol (1000 ppm), then extract was diluted into 0, 50, 100, 150, 200, dan 250 ppm. Each extract solution was added with 0.6 mL DPPH and the absorbance was measured by UV-Vis spectrometer at a wavelength of 515 nm (Artanti *et al.,* 2006)

Addition of natural antioxidant

The crude extract of *D. petandra* prepared in a series concentration of 200; 400; 600; 800 and 1000 ppm, and for each concentration of 50 mL of crude extract was added with a CPO as much as 50 mL. (comparison of CPO and antioxidants 1:1 (v/v)). The mixture of CPO and antioxidant additives was homogenized for 5 minutes and allowed for 24 hours. The mixture of solution forms 2 layers between antioxidants and CPO. Then CPO was tested for quality based on the specific parameters. (Shahid *et al.,* 2018)

CPO quality parameter test

1. Determination of free fatty acid levels

   The CPO sample was heated at a temperature of 60 – 70 °C. Weighed 3 g was added 50 mL of 96% ethanol. After that, the sample was heated on a water bath at 40°C until the oil was completely dissolved. Added 3-5 drops of PP indicator to the sample solution, then titrated with 0.1 N NaOH until the colour turned to pink (Deisberanda *et al.,* 2019). Free fatty acid levels were calculated based on Equation 1:

$$\text{FFA} = \frac{\text{V NaOH x N NaoH x Mw Fatty acid}}{\text{Weigh of Sample (g)}} \quad \ldots(1)$$

   The CPO test without the addition of antioxidants was carried out as a standard comparison

2. Determination of acid values

   The CPO sample was heated at a temperature of 60 - 70 °C. Weighed 3 g of liquid CPO sample was added 50 mL of 96% ethanol. After that, the sample was heated on a water bath at 40°C until the oil was completely dissolved. Added 3-5 drops of PP indicator to the sample solution, then titrated with 0.1 N KOH until the colour turned to pink (Deisberanda *et al.,* 2019). Acid values were calculated based on Equation 2:

$$\text{Acid Number} = \text{FFA} \times \frac{\text{Mr KOH}}{\text{Mw Fatty acid/10}} \quad \ldots(2)$$

   The CPO test without the addition of antioxidants was carried out as a standard comparison

3. Deterioration of bleachability index

   As much as 0.1 g of sample was dissolved with n-hexane. The absorbance of the sample was measured at a 319 nm for the UV range and at 446 nm for the Visible range (Hasibuan, 2020). DOBI numbers were calculated based on Equation 3:

$$\text{DOBI} = \frac{\text{absorbance at } \lambda \text{ 446 nm}}{\text{absorbance at } \lambda \text{ 319 nm}} \quad \ldots..(3)$$

   The CPO test without the addition of antioxidants was carried out as a standard comparison

RESULT AND DISCUSSION

Antioxidant Activity

The method used in the antioxidant activity test was the DPPH (1,1-diphenyl-2-

picryhydrazyl) method (Shah & Modi, 2015). If a compound has activity as an antioxidant, there will be a decrease in the absorbance value of DPPH solution at a wavelength of 515 nm. The decreasing in absorbance of DPPH solution was indicated by the degradation of the DPPH color from purple to yellow. The process of degradation color that occurs is directly proportional to the concentration of the extract addition. The percentage of inhibition of *D. petandra* antioxidant activity using the DPPH method is presented in Table 1.

Table 1. The result of antioxidant activity of *D. petandra* ethanolic extract test using DPPH

Concentration	% inhibition 1	% inhibition 2	IC_{50} 1 (ppm)	IC_{50} 2 (ppm)
0	0	0		
50	87.155	86.658		
100	87.500	86.891	6.472	6.266
150	87.729	87.239		
200	88.073	87.470		
250	88.303	87.819		
	$\bar{x}$		6.369	

The antioxidant activity of ethanol fraction of 6.369 ppm, which meant that the ethanol extract of the mango parasite had high antioxidant activity. The antioxidant activity of *D. petandra* ethanol extract is caused by the presence of phenolic. The coumpunds have hydroxyl active group, that can be anti free radicals. The smaller the IC_{50} value obtained, the higher the antioxidant activity

The Effectiveness of Natural Antioxidant Extract Addition on The Quality of CPO

Before adding antioxidant extract

CPO quality testing was carried out in two steps, before and after addition of antioxidants from the *D. petandra* ethanol extract. The Tests were conducted on the parameters of free fatty acids, acid value, and DOBI. The results of testing the quality of CPO before adding antioxidants are shown in Table 2. From the results of free fatty acid test, it showed that CPO oil has not met the standards of Indonesian standart (SNI 01 – 2901 – 2006) which is 0.5%. CPO sample exceeds FFA as much as 6.8% from the standard. The acid value is used to measure the amount of free fatty acids contained in one gram of oil or fat. The acid value that obtained was 9.1 mg KOH/g. greater than the standard, which is 6.9 mg KOH/g. The increasing levels of FFA and acid values were caused by the increased activity of the lipase enzyme in CPO. In addition, in impure (non-refining) state, FFA undergoes an autoxidation process to produce primary oxidation products (peroxides and dienes) and secondary derivatives (carbonyls, aldehydes, trienes) (Goudoum *et al.,* 2017).

Table 2. Quality of CPO before addition *D. petandra* ethanolic extract

Parameters	Quality	SNI Standard
FFA (%)	7.30	0.5 max
Acid value (mg KOH/g)	16.00	6.90 max
DOBI	1.25	1.68 min

DOBI is an index which indicates the rate of oxidation of the samples of processing the palm oil due to carotenoids breakdowns. DOBI can be used as an indicator of CPO quality, and shows the oxidative stability of CPO. DOBI is obtained from the ratio of absorbance in the visible range and absorbance in the UV range. The result indicate DOBI analysis value for CPO are 1.25. This shows that the quality CPO sample has out of space from the standard of CPO oil based on Palm Oil Refiners Association of Malaysia (PORAM) The DOBI analysis result is bad if the analysis result is < 1.68. Pro-oxidant metals such as copper and iron in CPO increase thermal autoxidation. Iron and copper act as catalysts during the oxidation process where the double bonds of fatty acids react with oxygen. Both of these metals have been caming from the engine or tank due to mechanical wear or corrosion during the storage process (Chong, 2012). Therefore, this reaction causes the decreased in DOBI and an increased in acidity.

After adding antioxidant extract

The addition of *D. petandra* extract, can increase the quality of the CPO, as presented on Table 3.

Table 3. Quality of CPO after addition *D. petandra* ethanolic extract

Concentration of Antioxidant (ppm)	CPO sample (50 mL)		
	Free fatty acid (%)	Acid value (mg KOH/g)	DOBI
200	6.1	11.1	1.258
400	5.8	10.5	1.263
600	5.0	10.2	1.272
800	4.6	9.7	1.285
1000	4.2	8.6	1.300

The addition of natural antioxidant was found that there was a decreasing value of free fatty acids. The best decreasing value in FFA levels after adding antioxidants with a concentration of 1000 ppm was 3.1%, from the initial level of 7.3% to 4.2%. Results of effectiveness of *D. petandra* ethanolic extract on the descrease of FFA presented in Figure 1.

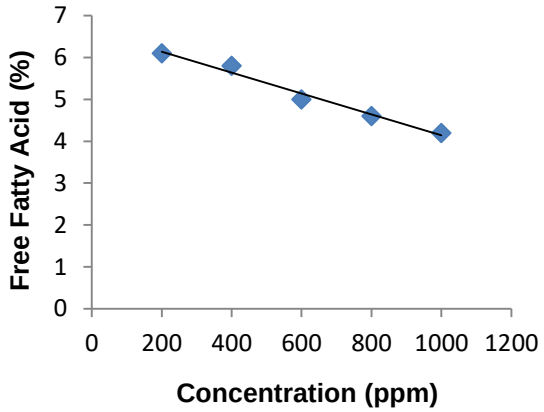

Figure 1. Effectiveness of *D. petandra* ethanolic extract on the descrease of free fatty acid

Similar results were also obtained in the measurement of acid values. The best decreasing value in acid value after the addition of antioxidants with a concentration of 1000 ppm was 8.6 mg KOH/g from 16 mg KOH/g to 7.4 mg KOH/g. Results of effectiveness of *D. petandra* ethanolic extract on the descrease of acid value presented in Figure 2.

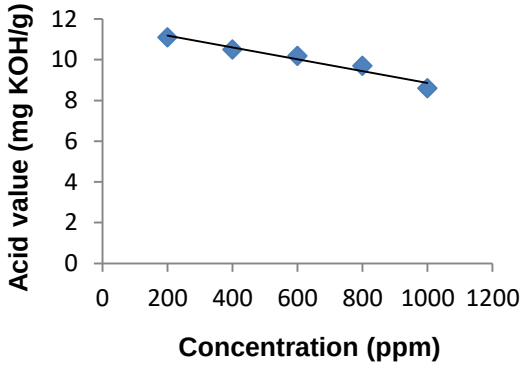

Figure 2. Effectiveness of *D. petandra* ethanolic extract on the descrease of acid value

The greater of antioxidant concentration addition, the value of free fatty acids and acids in CPO will be smaller. Figure 3 showed an increasing value in DOBI numbers after adding *D. petandra* ethanolic extract. The 1000 ppm range had the most significant increasing value in DOBI rate of 0.047 from 1.253 (before the addition of antioxidants) to 1.3. The phenolic compounds contained in natural antioxidants act as free radical scavenger and reduce oxidative degradation of lipids.

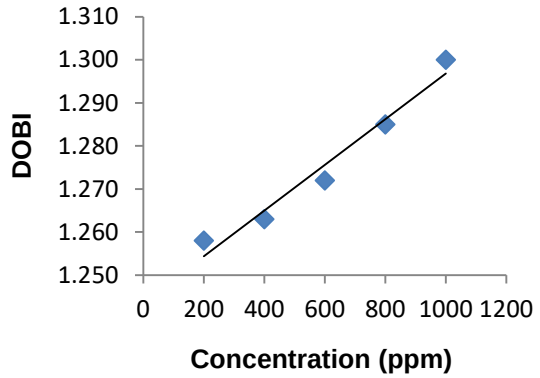

Figure 3. Effectiveness of antioxidant extract on the increase of DOBI

CONCLUSION

D. petandra ethanol extract has antioxidant activity that maintains the quality of CPO from oxidation during storage. Although the decrease in FFA levels and acid values, as well as the increase in DOBI numbers did not meet the Indonesian standards of SNI 01 – 2901 – 2006, but the addition of natural antioxidants can reduce the CPO oxidation process.

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