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Antioxidant Activity of Methanol and n-Hexane Fractions of the Barf of Kersen (*Muntingia Calabura*) Extracts

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ABSTRACTS

Antioxidants are substances that can slow or prevent the oxidation process or compounds that protect cells from the harmful effects of reactive oxygen free radicals. Plants containing antioxidants are chemotactonomically characterized by chemical compounds derived from phenolics such as flavonoids, coumarin, xanthones, benzophenone, tannin, lignin, and anthraquinone. Muntingia calabura is one of the plants that is well known in Indonesian forests. This plant has several benefits, including as a trasditional medicine. The community uses this plant, where as a shade plant for shade and the fruit is consumed directly. However, until now the community has not used this plant as a medicinal plant and there has also been no research on Kersen (Muntingia calabura) from this area. The purpose of this study was to determine the antioxidant activity of the bark Muntingia calabura (Kersen) methanol and n-hexane fraction. The method used is the extraction and the DPPH method. The results obtained showed that the antioxidant activity of the bark of Muntingia calabura methanol fraction (IC50 124.58 ppm) was higher than the n-hexane fraction (IC50 244.95 ppm). From the IC50 values, of the both fractions showed that the methanol fraction was very active against antioxidants because it had an IC50 value close to the IC50 value of the positive control 112.872 ppm, while the hexane fraction was less active against antioxidants but still had the potential as an antioxidant.

Keywords: antioxidants, extraction, DPPH, Muntingia calabura (Kersen)

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1. Introduction

Antioxidants are substances that can slow or prevent the oxidation process or compounds that protect cells from the harmful effects of reactive oxygen free radicals. The human body actually has an antioxidant system in the form of an enzyme. These enzymes include superoxide dismutase, catalase, and glutathione, but the amount is often not enough to neutralize free radicals that enter the body in excessive amounts, so that foods that contain antioxidants are needed, such as flavonoids, vitamin A, vitamin C, vitamin E (Prakash, 2001). The balance between antioxidants and free radicals is the main key to preventing chronic diseases caused by free radicals.

To find out which plants provide antioxidants are able to be detected by utilizing phytochemical knowledge. Phytochemistry is one of the branches of organic chemistry that examines chemical compounds contained in plants, which are related to active substances, chemical structures, biosynthesis, metabolism, physiological, and pharmacological effects. Plants that contain antioxidants, chemotactonomically are characterized by chemical compounds derived from phenolics such as flavonoids, coumarin, xanthones, benzophenone, tannin, lignin and anthraquinone (Ersam, 1999).

Kersen (Muntingia calabura) is one of the plants that is well known in Indonesian forests. Based on previous research, it was reported that the plant has anti-nociceptive (Yusof, M, et al.,2013), anti-microbial (Arum, YP et al., 2012), and antioxidants (Balan et al., 2014). One of the area that this plant grow well is Alor island - Nusa Tenggara Timur. The community use this plant, where as a shade plant for shade and the fruit is consumed directly. However, the people until now have not been use as a medical plant and no researches for this plant in a medicinal area.

2. Materials and Methods

2.1. Material and instrument

The materials used consisted of the bark of Muntingia calabura , organic solvents such as n-hexane, methanol, aquades, filter paper ascorbic acid and DPPH. The tools used in this study are glassware (beakers, measuring cups, small vial glasses, large vial glasses, Erlenmeyer, and glass funnels), maceration vessels, drip pipettes, rotary vacuum evaporators, digital scales, and UV spectrophotometers.

2.2. Methods

2.2.1. Extraction

Dry powder of Muntingia calabura bark, taken from Alor Island of NTT-Indonesia, was macerated in methanol. The extract from methanol was then partitioned with n-hexane repeatedly to the maximum. The partition produced two

fractions, namely n-hexane fraction and methanol fraction. The fractions were then evaporated so that the concentrated fractions of n-hexane and concentrated fractions of methanol were obtained.

2.2.2. Antioxidant Activity Assay

The method used to measure antioxidant activity is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The first step, making a DPPH radical solution of 6 × 10−5 M was carried out by dissolving 1.182 mg DPPH into 50 mL of methanol. Making the test solution was carried out by dissolving 10 mg of the sample into 1 mL of methanol. The test solution is piped 33.33 µL and inserted into a tube protected from light, then 1 mL DPPH is added. The mixture of the solution is the vortexed mixer for 10 seconds to homogenize it. The next, the solution was incubated at 37 °C for 30 minutes. During the process of reduction by antioxidants, the DPPH radical solution will change color from purple to pale yellow. This decrease in absorbance was measured in a UV spectrophotometer at a wavelength of 515 nm (As). The blank solution used consisted of 33.33 µL methanol in 1 mL DPPH which was measured at the same wavelength (Ab). Ascorbic acid is used as a positive control. The treatment in the DPPH test was carried out with three repetitions (triple). Radical inhibition activities can be calculated using the formula below:

$$\%\ Inibition\ = \left[\frac{Ab - As}{Ab}\right] \times 100$$

Ab: blank absorbance, As: test compounds absorbance

After obtaining the inhibition percentage of each concentration, the equation y = ax + b is determined by linear regression calculation where "x" is the concentration (µg / mL) and "y" is the percentage of inhibition. Antioxidant activity is expressed by inhibition Concentration of 50% is the concentration of a test solution that can reduce DPPH radicals by 50% (Liu, et al., 2010)

3. Results and Discussion

The sample antioxidant activity was measured by measuring absorption intensity from each sample after DPPH was added using a UV-Vis spectrophotometer at a certain wavelength. Based on this, before the measurement of antioxidant activity, the determination of the maximum wavelength of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was carried out. The experimental results show a maximum uptake of DPPH 515 nm. This shows that the measurement of uptake of the reduction of all test solutions against DPPH free radicals is carried out at that wavelength.

Quantitatively, the ability of a compound to reduce radical DPPH is indicated by a decrease in the absorbance of the test solution calculated against a blank solution. The smaller the absorbance value, the stronger the compound is in reducing DPPH radicals. The absorbance data of methanol fraction against DPPH reduction with concentration variants obtained then calculated the inhibition percentage using the formula and obtained the results, as shown in Table 1.

Table 1. Data from the Absorbance of Methanol Fraction with DPPH Reduction

Sample	Concentration (ppm)	Absorbance			Average absorbance	% Inhibition
		I	II	III		
Methanol fraction	500	0.1516	0.1521	0.1526	0.1521	77.2679
	250	0.1840	0.1846	0.185	0.1845	72.4256
	125	0.2096	0.2094	0.2093	0.2094	68.7042
	75	0.325	0.3245	0.3239	0.3244	51.5169
	50	0.4169	0.4167	0.4167	0.4167	37.7223
	25	0.5398	0.5396	0.5404	0.5399	19.3095
Blank		0.6696	0.6692	0.6685	0.6691	

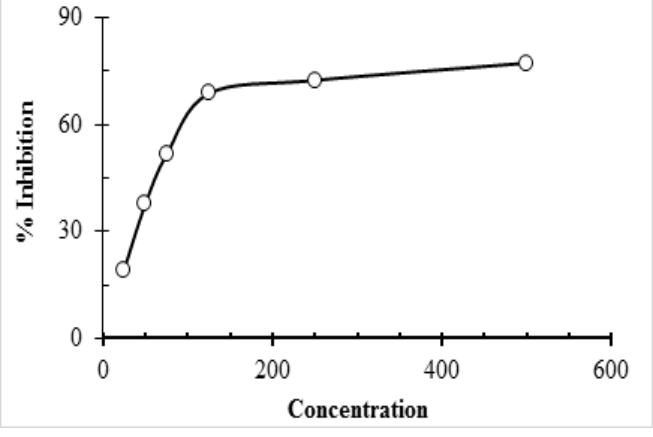

Fig.1. Regression equation curve for IC_{50} methanol fraction

After the inhibition percentage obtained from each concentration, y equation = ax + b is determined by linear regression calculation where x is the concentration (ppm) and y is the percentage of inhibition as shown in Figure 1. The IC_{50} obtained by integrating the y = 50 value on each replication and averaging IC_{50} 124.58 ppm on the methanol fraction.

The absorbance data of n-hexane fraction against DPPH reduction with concentration variants obtained then also calculated the inhibitory percentage using the same formula and obtained the results in the Table 2 and linear regression curve shown in Figure 2. From the calculation obtained the value of IC_{50} 244.95 ppm.

The positive control used in this study is ascorbic acid (vitamin C). The absorbance data of Vitamin C against DPPH reduction with concentration variants obtained and percent inhibition shown Table 3 and Figure 3. From the inhibition percentage of the ascorbic acid standard solution and its linear regression curve as, the value of IC_{50} it is 112,872 ppm. Vitamin C is an antioxidant that dissolves in water. The use of a positive control in this antioxidant activity test was to determine how strong the potential of antioxidants present in the methanol fraction and the fraction of n-hexane of the bark of *Muntingia calabura* when compared with vitamin C.

Table 2. Data from the Absorbance of n-hexane Fraction with DPPH Reduction

Sample	Concentration (ppm)	Absorbance			Average absorbance	% Inhibition
		I	II	III		
n-hexane fraction	500	0.1274	0.1279	0.1268	0.1273	91.3176
	250	0.5845	0.5846	0.5842	0.5844	60.1418
	125	1.007	1.001	1.006	1.0004	31.7691
	75	1.1774	1.1772	1.1774	1.1773	19.7039
	50	1.3278	1.3277	1.3277	1.3277	9.4461
	25	1.3613	1.3613	1.3605	1.3610	7.175
Blank		0.6696	0.6692	0.6685	0.6691	

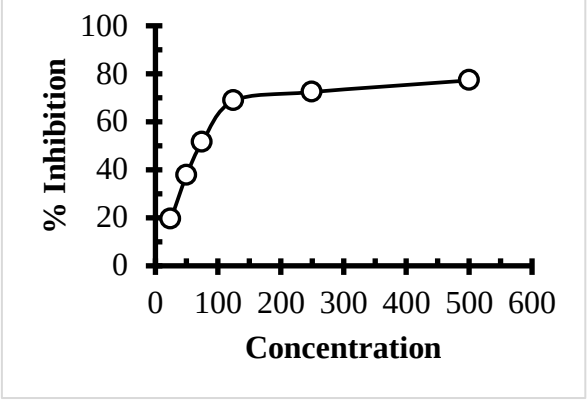

Fig. 2. Regression equation curve for IC_{50} hexan fraction

Table 3. Data from the Absorbance of Vitamin C with DPPH Reduction

Sample	Concentration (ppm)	Absorbance			Average absorbance	% Inhibition
		I	II	III		
Vitamin C	500	0.205	0.205	0.205	0.2050	60.7805
	250	0.235	0.235	0.234	0.2346	55.1176
	125	0.263	0.265	0.263	0.2636	49.5695
Blank		0.522	0.5228	0.5233	0.5227	

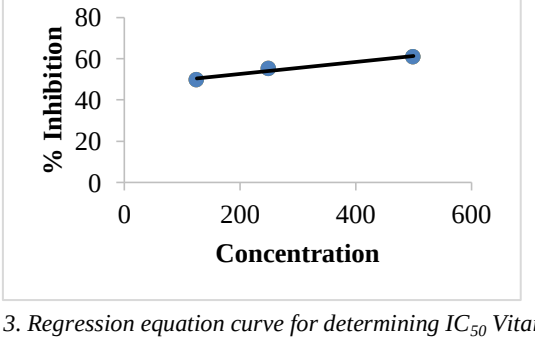

Fig. 3. Regression equation curve for determining IC_{50} Vitamin C

If the value of the IC_{50} samples at or near the value of IC_{50} controls positive it can be said that it has the potential as one of the most powerful antioxidant alternatives. According to Hanani et al (2005), a test material is said to have strong antioxidant activity if it has an IC50 of fewer than 200 µg / mL, whereas for pure compounds it has an IC50 of fewer than 100 µg / mL. In addition, Molyneux (2004) states that if the IC50 obtained the range from 200-1000 ppm, then the substance is less active, but still has the potential as an antioxidant.

4. Conclusions

Based on the results of the study, it can be concluded that the antioxidant activity of the bark of Muntingia calabura methanol fraction (IC_{50} 124.58 ppm) is higher than the n-hexane fraction (IC_{50} 244.95 ppm). From the IC_{50} both fractions showed that the methanol fraction was very active against antioxidants because it had an IC_{50} close to the IC_{50} of the positive control 112.872 ppm, while the hexane fraction was less active against antioxidants but still had the potential as an antioxidant.

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Conflict of interest: Non declare