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ANTIOXIDANT ACTIVITY TEST ON ETHANOL EXTRACT OF SOURSOP LEAVES (*Annona muricata* L.) USING DPPH METHOD (1,1-DIPHENYL-2-PICRYLHIDRAZYL)

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Abstract: Soursop plant parts that are often used by the community, namely the fruit and leaves. Ripe soursop fruit is usually developed to produce juice and lunkhead products. Ripe soursop fruit contains vitamin C which is known to have a wrong function, namely to increase immunity, so that it can protect the body from pathogens. Soursop leaves are often used by the community in the form of a decoction of the leaves for traditional medicine. The research about antioxidant test on ethanol extract of soursop leaves (*Annona muricata* Linn) had been conducted using DPPH method (1,1-diphenyl-2-picrylhdirazyl). This research aims to indicate the antioxidant activity of soursop leaves. Samples were collected from Lambaro Skep, a region of Banda Aceh. Then, the sample was dried by air before macerated using ethanol 96%. After that, the filtrate is evaporated using rotary evaporator and concentrated again using a hairdryer. The concentrated extract is then tested for antioxidant activity using DPPH method at maximum wavelength of 517 nm. The result shows young soursop leaves extract has 14.462 ppm of IC_{50} value, while old soursop leaves extract has IC_{50} value of 9.626 ppm.

Keywords: *Annona muricata* L., antioxidant, DPPH, and IC_{50} value

Abstrak: Bagian tanaman sirsak yang sering dimanfaatkan oleh masyarakat, yaitu buah dan daunnya. Buah sirsak masak biasanya dikembangkan menghasilkan produk juice dan dodol. Buah sirsak masak mengandung vitamin C yang sudah diketahui salah fungsinya yaitu untuk meningkatkan kekebalan tubuh, sehingga mampu melindungi tubuh dari patogen. Daun sirsak sering dimanfaatkan oleh masyarakat berupa ekstrak rebusan daunnya untuk obat tradisional. Telah dilakukan penelitian tentang uji aktivitas antioksidan pada ekstrak etanol daun sirsak *(Annona muricata* Linn*)* dengan menggunakan metode DPPH *(1,1-difenil-2-pikrilhidrazil)*. Tujuan dari penelitian ini untuk mengetahui aktivitas antioksidan yang terkandung dalam daun sirsak. Sampel diperoleh dari Lambaro Skep, Banda Aceh. Sampel dikering anginkan dan kemudian diekstraksi dengan cara maserasi menggunakan pelarut etanol 96%, filtratnya diuapkan dengan *rotary evaporator* dan dipekatkan lagi menggunakan *hairdryer*. Ekstrak yang diperoleh kemudian dilakukan uji aktivitas antioksidan menggunakan metode DPPH pada panjang gelombang 517 nm. Hasil pengujian aktivitas antioksidan menggunakan metode DPPH diperoleh nilai IC_{50} sebesar 14,462 ppm untuk daun sirsak muda, dan 9,626 ppm untuk daun sirsak tua.

Kata kunci: *Annona muricata* L., Antioksidan, DPPH, dan IC_{50}

INTRODUCTION

Soursop is one of plants which lives in tropical area. It has many benefits, including its leaves up to the stem. Fabrice, *et al*. (2017) explained that soursop leaves were used for natural treatment in the tropics, because it has many advantages for health, and also as antioxidant sources in order to suspend the formation of peroxide and palm olein. According to Afroz, *et al*. (2020), the soursop leaves can be utilized as anticancer, antidiarrhea, antitumor, and anti-diabetes.

The chemicals contained in soursop leaves are flavonoids, tannin, phytosterols, calcium oxalate, and alkaloids (Puspitasisari, *et al*., 2016). According to Justino, *et al*. (2018), others chemical such as acetogenin, flavonoids, tannin, alkaloids, coumarins and terpenoids also found in that leaves. The plants which built by that chemicals are frequently used as antioxidants.

Antioxidants defer and postpone the oxidation reaction which driven by free radicals or neutralize and destroy the free radicals which cause cell and biomolecule damage like DNA, proteins, and lipoproteins (Sie, 2013). Besides that, antioxidants are also found in noni leaves (Irianti, *et al*., 2015), purple sweet potato (Pratiwi, *et al*., 2019), mixed katuk leaves (Fathoni, *et al.*, 2020), fern stalk (Whadaningsih, *et al*., 2011), mangosteen skin (Puspitasari, *et al*., 2016), avocado seeds (Liberty, *et al*., 2012), stem Bouea macrophylla Griff (Rudiana, *et al*., 2018), and tomato (Andayani, *et al*., 2008). Data for antioxidant test is analyzed using DPPH method (1,1-diphenyl-2-picrylhidrazyl).

DPPH is used because this method is simple and proper for the small number of samples and short time for operation (Andayani, *et al.*, 2008). Then, Tonellia, *et al*., (2019) explained that DPPH test is selected because it have been very popular in chemistry and can be used to evaluate both of the chemicals performance as free radicals or hydrogen donor, and antioxidant capacity. DPPH is the simple method and only need UV-vis spectrophotometer. The presence of hydrogen or electron donor (radical scavenger antioxidants) decreases the absorption intensity. It is indicated by radical solution color loses that align with electrons gain (Irianti, *et al*., 2015).

Based on the background above, the researchers conducted study on antioxidants test on ethanol extract of soursop leaves (*Annona muricata* L.) using DPPH method (1,1-diphenyl-2-picrylhidrazyl).

METHOD

The sample used in this research is 500 grams of young light green soursop leaves, and 500 grams of old dark green soursop leaves. The leaves are obtained from Lambaro Skep, Banda Aceh. The research tools are jars, evaporator, analytical balance, spatula, mixer, beaker glasses, suction ball, measuring pipette, drop pipette, blender, test tubes, Erlenmeyer flasks, volumetric flasks, funnel, cuvette, watch glass, and UV-vis spectrophoto-meter. Then, the chemicals are young and old soursop leaves, ethanol 96% (C_2H_5OH), distilled water (H_2O), crystal of ascorbic acid ($C_6H_8O_6$), filter paper, aluminum foil, and 1-1-diphenyl-2-picrylhidrazyl (DPPH).

The research used young and old soursop leaves, then separated from its stem and weighed as much as 500 grams each of wet weight, after that dried by air for 7 days. So, the dried soursop leaves are then cut into small pieces and mashed using blender, then sieved to collect a finer powder (Aminah, *et al*., 2005).

Both of young and old soursop leaves powder are then macerated in 2 liters of ethanol 96% for 24 hours. After that, it is poured into a tightly closed container and protected from the sunlight. The mixture of soursop leaves and ethanol is separated form its filtrate and residue. The macerated filtrate of is then evaporated using vacuum evaporator, and then thickened using hair dryer. Finally, extract is stored until it used for test of antioxidants activity.

The control solution was prepared by dissolving 0,001 grams of DPPH into ethanol up to 25 mL, in order to obtain a 0,1 mM DPPH solution. Then, it measured the absorbance at 400 – 600 nm.

Before measured the absorbances, first step is a must to prepare blank solution of 100 ppm by weighing 1 mg ethanol extract of young soursop leaves and dissolving into ethanol up to 10 mL. After that, prepared test solutions with various concentrations, specifically 2, 4, 6, 8, 10 ppm, and all is incubated for 30 minutes at room temperature. Then, measured the absorbance at maximum wavelength. The percentage of inhibition was determined using vitamin C as control absorbance with the same various concentrations as samples. To determine the absorbance and inhibition percentage of old extract soursop leaves, it used the same procedures. Furthermore, data of inhibition percentage of young soursop leaves is compared with percentage of inhibition of old soursop leaves.

After absorbance is obtained, the DPPH radical scavenger activity

percentage can be calculated as inhibition percentage using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Ak} - \text{As}}{\text{Ak}} \text{x } 100\%$$

where,

Ak = Absorbance of control solution

As = Absorbance of samples

RESULT AND DISCUSSION

Maceration

This research uses maceration for extraction because of its simplicity. The sample is only soaked in suitable solvent, and this method can decrease the chemicals damage, because it is operated without heating. It uses ethanol 96% as solvent, because it has polar properties, universal, and easy to obtain.

Maceration method is carried out for 3 x 24 hours with few strirring process. It aims to mix soursop leaves powder and solvent evenly. The maceration process is conducted in a place that is protected from direct sunlight. The macerated soursop leaves is then filtered and the residue is macerated again using the same solvent and repeated 3 times until the extract is colorless. After that, filtering process is carried out to separate filtrate and its residue. Then, the filtrate is evaporated using rotary evaporator to remove the solvent, so that the extract can be stored for a longer time. The concentrating of extract is stopped when the extract is free from the solvent, and it is again concentrated using a hairdryer to obtain a denser extract.

Antioxidant Activity Test using DPPH Method

This text is carried out using DPPH method, because it is one of quantitative methods to indicate the antioxidant activity (Bahriul, *et al*., 2014). The method is based on DPPH color change caused by the reaction between DPPH free radical with one electron or hydrogen atom released by the compounds contained in materials to form a yellow 1,1-diphenyl-2-picrylhidrazyl compound (Irnawati, *et al*., 2017).

Test of antioxidant activity using DPPH method is measured using UV-Vis spectrophotometer at a maximum wavelength in region of 400 – 600 nm. The control solution used to determine the maximum wavelength, namely DPPH and ethanol with a ratio of 1:3. The measurement of maximum wavelength is carried out to find put the maximum absorption which is read by UV-vis spectrophotometer. The result of control solution measurement for maximum wavelength at 400 – 600 nm can be seen in Figure 1.

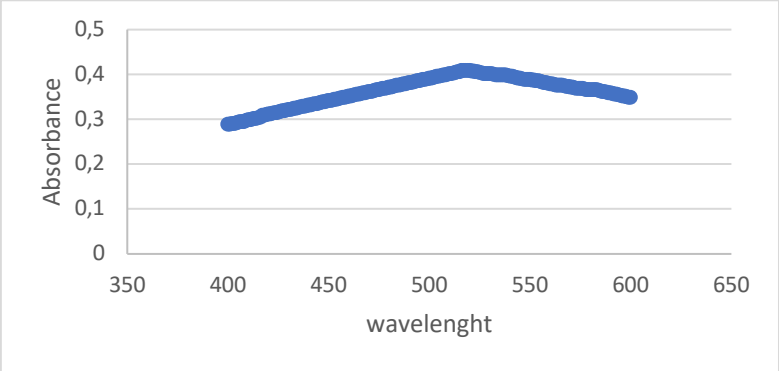

Figure 1. The maximum wavelength of DPPH solution

According to figure 1, the maximum wavelength of DPPH solution is 517 nm with an absorbance value of 0.410. The samples to be tested for DPPH were ethanol extract of either young soursop leaves or old soursop leaves, while the comparison used was vitamin C. According to Parwati, *et al*., (2014), vitamin C can be used as comparison in this research because it has strong natural antioxidant.

Measurement of antioxidant activity using the DPPH method using spectrophotometry in the sample solution and the comparison solution was measured at a wavelength of 517 nm, which is the maximum wavelength of DPPH. Bahriul, *etl al*., (2014) explained that the measurement of antioxidant activity by spectrophotometry was carried out at a wavelength of 517 nm, which is the maximum wavelength of DPPH.

Table 1 DPPH test result for ethanol extract of young soursop leaves, old soursop leaves, and vitamin C

Control absorbance	Test solution	Concentration	Absorbance	% Inhibition
0.410	Young soursop leaves	2	0.401	2.195
		4	0.384	6.341
		6	0.350	14.634
		8	0.315	23.17
		10	0.271	33.902
0.410	Old soursop leaves	2	0.376	8.292
		4	0.343	16.341
		6	0.284	30.731
		8	0.243	40.731
		10	0.196	52.195
0.410	Vitamin C	2	0.294	28.292
		4	0.236	42.439
		6	0.178	56.585
		8	0.130	68.292
		10	0.069	83.170

The difference in absorbance values obtained indicated that the ability to reduce a DPPH radical between young and old soursop leaf test solutions was not similar. Based on the results of the absorbance in table 1, it can be concluded that the older the leaves are, the smaller the absorbance value. The absorbance decrease is caused by difference of antioxidant compounds, where the older the leaves are, the more antioxidant compounds composed the leaves (Bahriul, *et al*., 2014).

The higher the antioxidant content in a compound, the faster the purple color on DPPH will fade to yellow. This color fading causes a decrease of absorbance value. Talapessy, *et al*., (2013) explained that the higher extract concentration, the stronger fading indicated by yellow formation, because at higher concentration, the more contained compounds and antioxidant activity, so the absorbance will be decreased.

The concentration of the sample of the test solution is directly proportional to the percentage of inhibition, where the higher the concentration of a test solution, the greater the value of % inhibition obtained. It can be seen in table 1 that the 2 ppm of ethanol extract test solution of young soursop leaves has a % inhibition value of 2.195, while at a concentration of 10 ppm, it has a % inhibition value of 33.902. Likewise, the test solution for the ethanol extract of old soursop leaves at a concentration of 2 ppm has a % inhibition value of 8.292, and at 10 ppm of 52.195.

The decrease in absorbance value in different leaves could be caused by differences in the content of antioxidant compounds, where the more secondary metabolites contained in the leaves, the stronger the antioxidant activity. Likewise with the comparison, namely vitamin C, Vitamin C has a smaller absorbance value compared to the absorbance value of young soursop leaves and old soursop leaves, this is because vitamin C is a strong antioxidant compound, so the absorbance value obtained will be smaller. along with the increasing concentration of vitamin C.

IC_{50} determination

The antioxidant activity test in this study used the IC_{50} paramete is a value that can indicate the ability to inhibit 50% free radicals by a sample concentration. IC_{50} can be calculated using regression obtained from the concentration relationship samples with a percentage of inhibiting free radical activity (percentage inhibition). The IC_{50} value obtained from a linear regression equation which states the relationship between the concentration

of the test extract and the percent of radical scavenging. The smaller the IC_{50} value of, the more active the extract / fraction (test compound) is as an antioxidant (Rohman, *et al*., 2007).

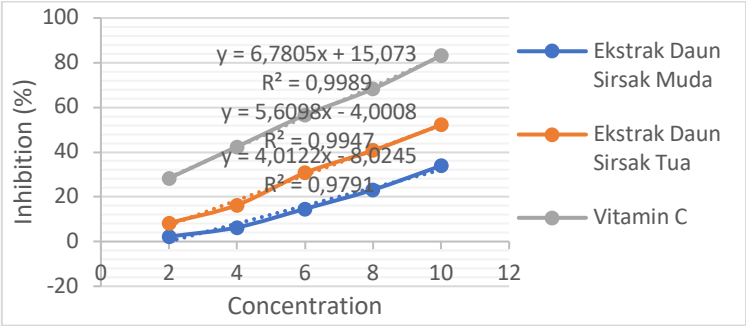

Figure 2. The relation between concentration and inhibition percentage

Based on figure above, it obtains regression equation of Y = 4.0112X – 8.0245 for ethanol extract of young soursop leaves, and Y = 5.6098X – 4.0008 for ethanol extract of old soursop leaves, and Y = 6.7805X + 15.073 for vitamin C. Whereas, the result of antioxidant activity test based on IC_{50} is presented in table 2.

Table 2 IC_{50} values for ethanol extract of both young and old soursop leaves, and vitamin C

Test solution	Regression equation	R^2	Y	IC_{50} (ppm)
Old soursop leaves	5.6098x – 4.0008	0.9947	50	9.626
Young soursop leaves	4.0122x – 8.0245	0.9791	50	14.462
Vitamin C	6.7805x + 15.073	0.9989	50	5.151

The IC_{50} value of each soursop leaves and vitamin C sample was determined based on the regression equation. The grouping of the antioxidant power of a compound based on the IC_{50} value by Molyneux (2004) can be seen in Table 3.

Table 3 Level of Antioxidant Power

Antioxidant Intensity	IC_{50} values (ppm)
Very strong	< 50
Strong	50 – 100
Moderate	100 -150
Weak	150 – 200
Very weak	> 200

The results of the final calculation show that the relationship between the IC_{50} value and the antioxidant ability of a compound is inverse, where the smaller the IC_{50} value obtained, the stronger the compound's ability to act as an antioxidant. Based on table 2, the IC_{50} value obtained for young soursop leaves extract is 14.462 ppm, for old soursop leaves extract is 9.626 ppm, while the IC_{50} value generated from vitamin C is 5.151 ppm.

Based on the IC_{50} values, it can be explained that vitamin C as a comparison or positive control is a stronger

antioxidant when compared to the soursop leaves extract used as the sample of this research. This shows that vitamin C is stronger in reducing free radical compounds in DPPH, compared to ethanol extract of young soursop leaves and ethanol extract of old soursop leaves.

When the samples were compared to vitamin C with IC_{50} under 50 ppm, the antioxidant activity of the ethanol extract of young soursop leaves and old soursop leaves is not too different from vitamin C. Minarni, *et al*., (2017) stated that soursop leaves have high antioxidant activity, their antioxidant activity is almost comparable to the standard vitamin C. Therefore, the ethanol extract of young soursop leaves and old soursop leaves are very good to be used as natural antioxidants. The levels of antioxidant activity in order from large to small were ascorbic acid, ethanol extract of old soursop leaves, and ethanol extract of young soursop leaves.

The difference in antioxidant activity at different leaves ages according to Arianti *et al*., (2007) is due to differences in the concentration of secondary metabolites component. The more secondary metabolites contained, the stronger the antioxidant activity. This shows that the growth phase (plant age) affects secondary metabolites with compounds which have antioxidant activity. Based on the results of these studies indicate that the use of soursop leaves to treat a disease is appropriate, because it contains antioxidant compounds.

CONCLUSION

The results of the antioxidant activity test using the DPPH method, obtained IC50 of 14,462 ppm for young soursop leaves, 9,626 ppm for old soursop leaves, and vitamin C of 5.151 ppm. Based on these results, it can be concluded that young and old soursop leaves are very good to use as natural antioxidants because they are very strong antioxidants with an IC50 value of less than 50 ppm. Thus soursop leaves can be used to increase immunity, because it contains antioxidant activity.

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