Jurnal Ners Volume 8 Nomor 1 Tahun 2024 Halaman 59 - 68
JURNAL NERS
Research & Learning in Nursing Science
http://journal.universitaspahlawan.ac.id/index.php/ners

ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT AND ETHYL ACETATE FRACTION OF SOURSOP (Annona muricata) LEAVES IN VITRO

Jeli Marlita[1], Tanti Azizah Sujono[2]
[1]Master of Pharmacy, Postgraduate School, University of Muhammadiyah Surakarta
[2]Department of Pharmacology and Clinical Pharmacy, University of Muhammadiyah Surakarta
jelimarlita@gmail.com, tanti_azizah@ums.ac.id

Abstract

Antioxidants play an active role and are responsible for counteracting free radicals in the body through the process of inhibiting oxidation reactions. Secondary metabolite compounds of phenolic and flavonoid groups are compounds that are widely found in Annona muricata plants that have potential as antioxidants. The purpose of this study was to determine the antioxidant activity of soursop (Annona muricata) leaves using two methods, namely Ferric Reduction Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The extraction method used is maceration method with 70% ethanol solvent, then fractionation using liquid-liquid extraction method, antioxidant test using DPPH and FRAP methods with measurement of levels using UV-Vis spectrophotometry. From the results of maceration, the yield of ethanol extract was 21% from 500 g dry simplisia of soursop leaves and the yield of ethyl acetate fraction was 32.78% from 50 g thick ethanol extract. The results of antioxidant activity test by FRAP method on ethanol extract and ethyl acetate fraction samples were 7.790 and 10.026 mgAAE/g, respectively. Both samples showed very strong antioxidant activity. In DPPH method, ethanol extract showed strong antioxidant activity with IC50 value of 63.947 µg/mL and ethyl acetate fraction showed very strong antioxidant activity with IC50 value of 6.702 µg/mL. From the results, it can be concluded that the ethyl acetate fraction showed very strong antioxidant activity in the DPPH and FRAP test methods, while the ethanol extract showed very strong antioxidant activity in the FRAP test method and strong antioxidant activity in the DPPH test method.

Keywords: *Antioxidant, Annona muricata, ethanol extract, ethyl acetate fraction, DPPH, FRAP*

✉ Corresponding author :
Address : University of Muhammadiyah Surakarta
Email : jelimarlita@gmail.com

INTRODUCTION

Various types of free radicals are generated from both inside and outside the cell. Free radicals can cause cell damage at the macromolecular level and can damage the structure of the cell itself (Badmus et al., 2022). In counteracting and preventing cell damage due to free radicals, the body can produce antioxidant compounds in the form of enzymes such as SOD (superoxide dismutase), glutathione, and catalase. Antioxidants are molecules that can interact with and inhibit the spread of free radicals**.** chain oxidation reaction caused by a reactive free radical (Irivibulkovit et al., 2018; Tamahiwu et al., 2023). Some of the mechanisms of action of antioxidant inhibition include hydrogen donation, radical removal, and singlet oxygen quenching (Irivibulkovit et al., 2018).

Antioxidant-rich plants have a very important role to counteract the type of free radicals and prevent oxidative stress at the cellular and tissue levels (Badmus et al., 2022). One plant that has strong antioxidant potential is Annona muricata. This plant comes from the hot tropics of South America and is now widely found in various countries in Asia, one of which is Indonesia (Adedapo et al., 2022). It has been described in previous research, secondary metabolites in Annona muricata that show therapeutic activity in biological systems include flavonoids, alkaloids, phenolics and tannins (Olasehinde et al., 2022). The content of antioxidant compounds in soursop leaves (*Annonan muicata*) can be useful in preventing chain reactions in free radicals, where these reactions become the pathogenesis of cell damage that can cause various types of diseases (Justino et al., 2018).

Non-enzymatic antioxidant activity testing on plant samples can be done through various methods such as, 2,2-dienyl-1-picrylhydrazyl (DPPH), Ferric Reduction Antioxidant Power (FRAP), Ferrous Ion Chealaing (FIC). Based on its mechanism of action, the FRAP method can determine the antioxidant activity of a sample based on the compound's ability to reduce Fe^{3+} ions to stable Fe^{2+} ions. This ion change is anologized as the ability of antioxidant compounds in a sample (Halvorsen et al., 2002). The DPPH method occurs through the process of radical reduction by an antioxidant by transferring hydrogen atom electrons which is characterized by a change in color on DPPH from violet to a stable pale yellow color (Irivibulkovit et al., 2018).

The use of different methods in antioxidant activity tests can sometimes give different results (Maesaroh et al., 2018). This is because in the DPPH test the free radical suppression mechanism is not only by electron transfer, but can also occur with the hydrogen atom transfer mechanism, while in the FRAP test the free radical suppression mechanism is only based on electron transfer, not a combination of single electron transfer (SET) and hydrogen atom transfer (HAT) (Safitri et al., 2020). The novelty of this study is to determine the antioxidant activity of the same sample with different test methods that have not previously been carried out on soursop leaves (*Annona muricata*). Based on this, researchers are interested in comparing the antioxidant activity of the DPPH and FRAP methods on the same sample, namely ethanol extract and ethyl acetate fraction of soursop leaves (*Annoan muricata).*

RESEARCH METHODS

Equipment and Materials

Various equipment used in this study include glassware (Iwaki and Pyrex), blender, analytical balance (Ohaus), separatory funnel, rotary evavorator (Heidolph), water bath, micropipette, cuvette, UV-Vis spectrophotometry.

Various materials used in this study are soursop leaves (Annoan muricata) obtained from the Tawomangu area, Karanganyar district, Central Java which was taken in May 2023. 70% ethanol (technical), ethyl acetate (technical), n-hexan (technical), Na_2CO_3 (Brand), $AlCl_3$ (Brand), 2,2-diphenyl-1-picrylhydrazyl (DPPH) p.a (Brand), oxalic acid, NaOH, KH_2PO_4, potassium ferricyanide, $FeCl_3$, trichloroacetic acid, ascorbic acid p.a (Brand), and quersetin p.a (Brand).

Research Procedure

1. Extraction

A total of 500 g of simplisia powder was extracted using a solvent of 5 liters of 70% ethanol for 3 days in a closed container at room temperature while occasionally stirring so that the simplisia was well dissolved. The macerate obtained was evaporated using a rotary evaporator at 60^o C, then the results obtained were concentrated on a waterbath at 60^o C until a thick extract was obtained.

2. Fractionation

A total of 25 g of thick ethanol extract of soursop leaves dissolved in ethanol:water (9:1 v/v) was put into a separatory funnel and 40 mL of n-hexane was added. Then shaken until there are two phases that separate. The ethanol fraction obtained was fractionated again with ethyl acetate, after which it was shaken and allowed to stand until perfect separation occurred. The ethyl acetate fraction obtained was concentrated on a waterbath at 60^{o} C.

3. Total phenolic content

Measurement of total phenolics from ethanol extracts and ethyl acetate fractions was determined using the Folin-Ciocalteu method, according to research described by Ningsih, et al (2016). A total of 0.1 mL of sample (0.08 % b/v) was added to 1.0 mL of Folin-Ciocalteau reagent previously diluted in a ratio of 1:10 v/v and Na_2CO_3 (7.5 % b/v). The mixture was then vortexed for ± 20 seconds and stored at 25^{o} C for 30 minutes. The blank used is the solvent from the sample. The standard solution used was 1000 ppm gallic acid (0.1 b/v) which was then made with five concentrations of 25, 50, 75, 100, and 125 ppm. The absorbance of the sample was read at a wavelength of 765 nm using UV-Vis spectrophotometry (Ningsih et al., 2016).

4. Total flavonoid content

Total flavonoids from ethanol extract and ethyl acetate fraction of sisak leaves were determined using the method described by Ningsih, et al (2016). Each sample was taken as much as 0.5 mL (0.05% b/v) added with 0.5 $AlCl_3$ (2% v/v) and kept at 25^{o}C for 30 minutes. The color change to yellow indicates the presence of flavoniods in the sample. The absorbance of the sample was then measured using UV-Vis spectrophotometry at a wavelength of 420 nm. Quersetin solution was used as standard solution (20-100 µg/mL). The calibration curve was plotted to calculate the total flavonoid content and the result was expressed as quercetin equivalent (QE) (Ningsih et al., 2016; Fatma et al., 2023).

5. Antioxidant activity test DPPH method

Antioxidant activity test with DPPH method refers to the research of Suhendi, et al (2013) with slight modifications. A total of 0.394 g of DPPH was dissolved with 10 mL ethanol, thus obtaining 0.1 M DPPH solution. From the solution, 100 µL was pipetted and put into a 100 mL volumetric flask and added ethanol to the limit mark, thus obtaining a concentration of 0.1 mM DPPH solution.

The sample mother solution was made by weighing 10 mg of each sample dissolved with ethanol in a 10 mL volumetric flask, so that a concentration of 1000 ppm was obtained, then 5 concentration series were made. Measurement of IC_{50} by adding 1.5 mL of DPPH (0.1 mM) with 1.5 mL of sample from each concentration variation. After that, it was allowed to stand for 30 minutes and measured the absorbance at a wavelength of 517 nm (Suhendi et al., 2013).

6. Antioxidant activity assay FRAF method

1) Phosphate buffer solution 0.2 M pH 6.6

The solution is made by weighing 2 g NaOH and dissolved with CO_2-free distilled water to exactly 250 mL in a volumetric flask. Then as much as 6.8 g KH_2PO_4 was dissolved with 250 mL of CO_2-free distilled water in a volumetric flask. After that, 16.4 mL of NaOH was pipetted into a volumetric flask and mixed with 50 mL of KH_2PO, then measured to pH 6.6 and sufficed with CO_2-free distilled water up to 200 mL.

2) Oxalate acid 1%

The solution was made by dissolving 1 g of oxalic acid in CO_2-free distilled water in a 100 mL volumetric flask.

3) Potassium Ferrisianide 1%

The solution is made by dissolving 1 g of potassium ferricyanide in distilled water and diluted in a 100 mL volumetric flask.

4) $FeCl_3$ 0,1 %

The solution was prepared by dissolving 0.1 g $FeCl_3$ in distilled water and diluted in a 100 mL volumetric flask.

5) Trichloroacetic acid (TCA) 10%

The solution was prepared by dissolving 10 g of TCA in distilled water and diluted in 100 mL volumetric flask.

6) Preparation of sample solution

Ethanol extract and ethyl acetate fraction of soursop leaves were weighed with 3 replications of 10 mg each. Each ethanol extract and ethyl acetate fraction

was dissolved with 96% ethanol as much as 10 mL and then homogenized.

7) Standard curve solution

Stock solution was made by weighing 25 mg of ascorbic acid dissolved with 1% oxalic acid in a 25 mL volumetric flask. Furthermore, from the 1000 ppm stock solution, 0.6; 0.7; 0.8; 0.9; and 1.0 mL were taken respectively and placed in different 10 mL volumetric flasks and diluted with 1% oxalic acid to 10 mL and homogenized to obtain a concentration series of 60, 70, 80, 90, 100 ppm ascorbic acid.

8) Antioxidant test with FRAP method

Antioxidant measurements on samples were carried out according to the method used by Raharjo and Haryoto (2019). Samples taken 1 mL were added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of K_3Fe (CN) 6% then incubated for 20 minutes at 50°C. After incubation, 1 mL of TCA was added and centrifuged at 3000 rpm for 10 minutes. Next, 1 mL of supernatant was pipetted and put into a test tube, then 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added. The solution was left for 10 minutes and the absorbance was measured at a wavelength of 720 nm. The blank used was oxalic acid solution. Calibration curve was made using ascorbic acid solution with various concentrations. The results of the antioxidant activity test with the FRAP method are expressed in mg ascorbic acid equivalent/g extract (Raharjo & Haryoto, 2019; Hanipah et al., 2021).

RESULTS AND DISCUSSION

The extraction results of the maceration method used showed that the soursop leaf ethanol extract produced was blackish green with a thick consistency and a slightly pungent aroma of soursop leaves with a percent yield obtained of 21% from 500 g of dried soursop leaf simplisia. Furthermore, the partition process with the liquid-liquid extraction method obtained a green ethyl acetate fraction, a concentrated consistency with a percent yield of 32.78% from 50 g of soursop leaf ethanol extract. The purpose of calculating the yield is to determine the level of secondary metabolites carried by the solvent but not to determine the specific type of compound (Aminah et al., 2017). The results of the yield calculation are presented in **Table 1**.

Table 1. Yield Values of Ethanol Extract And Ethyl Acetate Fraction of Soursop Leaves

Sample	Weight (Kg)	Yield (%)
Soursop leaf	6 Kg	
Soursop leaf dry powder	0.5 kg	(0.5 Kg/6 Kg) x 100% = 8.33%
Thick ethanol extract	0.105 Kg	(0.105 Kg/0.5 Kg) x 100% = 21%
Ethyl acetate fraction	0.01639 Kg	(0.01639 Kg/0.5 Kg) x 100 = 32.78%

Soursop (*Annonan muricata*) is one of the plants that has many pharmacological activities from the secondary metabolites produced. From several studies, parts of the plant such as leaves, seeds and fruits of soursop have good effectiveness as antidiabetics and anticancer (Justino et al., 2018; Sanni et al., 2020). In addition, the metabolite content of soursop leaf extract is known for its strong antioxidant ability (Florence et al., 2014; Tamahiwu et al., 2023). Compounds that have an important role as antioxidants are phenolics and flavonoids (Nur et al., 2019).

Measurement of phenolic and flavonoid levels is an important measurement to determine the antioxidant power in a sample, where phenolics and flavonoids play a very important role in antioxidant activity (Nur et al., 2019). In addition, phenolic compounds in the form of flavonoids have a very important role as free radical neutralizers based on the number and location of their -OH groups. So with this, the higher the total phenolics and flavonoids, the greater the antioxidant ability to suppress the activity of free radicals (Nur et al., 2019).

In this study, the measurement of total phenolics and flavonoids used gallic acid and quersetin standard solutions, respectively. Gallic acid standard solution made with five concentrations obtained a linear regression equation $y = 0.0025x + 0.1394$, $R^2 = 0.9962$ which will be used as a formula for calculating total phenolics. Gallic acid is a phenolic compound and is a natural antioxidant compound and is stable and relatively cheap (Rahayu & Inanda, 2015), while the standard solution used in the total flavonoid test is quersetin. This compound is a flavonoid compound of the flavonol group that has a keto

group at C-4 and has a hydroxyl group that has activity in counteracting free radicals (Aminah et al., 2017). Measurement of the standard curve of quersetin solution obtained regression equation y = 0.4009x + 0.0374, R^2 = 0.9852. The standard curve of gallic acid and quersetin solution is presented in **Figure 1.**

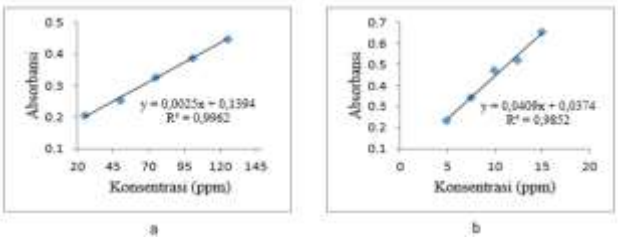

Figure 1. (a) Garlic acid standar curve (b) Quercetin standar curve

The equation obtained in **Figure 1** is used as a formula in the determination of phenolic and flavonoid levels in the sample. The phenolic and flavonoid levels of ethanol extract and ethyl cetat fraction of soursop leaves are presented in **Table II** and **Table III** below.

Table 2. Total Phenolic Content of Ethanol Extract and Ethyl Acetate Fraction of Soursop Leaves

Sample	Absorbance	Mean ± SD	Total phenolic content (mg GAE/g extract)
Ethanol extract	0.257	0.243 ± 0.020	41.573
	0.221		
	0.252		
Ethyl acetate fraction	0.317	0.334 ± 0.018	77.840
	0.332		
	0.353		

Note : GAE (*Gallic acid equivalent*)

Table 3. Total Flavonoid Content of Ethanol Extract and Ethyl Acetate Fraction of Soursop Leaves

Sample	Absorbance	Mean ± SD	Flavonoid content (mg QE/g extract)
Ethanol extract	0.357	0.350 ± 0.017	7.643
	0.331		
	0.362		
Ethyl acetate fraction	0.529	0.537 ± 0.009	12.207
	0.546		
	0.535		

Note: QE *(Quercetin equivalent*)

From the measurement of total phenolic and flavonoid levels, the phenolic and flavonoid levels of the ethyl acetate fraction were higher than those of the ethanol extract. This can be caused because, samples from ethanol extracts still contain many compounds with different levels of polarity. According to (Mazzutti et al., 2018), the nonpolar compound characteristics of carbon dioxide can interfere with the solubility of phenolic compounds of medium to high polarity. This can keep compounds with antioxidant potential dissolved in small amounts.

Antioxidant activity test DPPH method

The antioxidant activity test with the DPPH method can be used on solid and liquid sample types and works by measuring the overall antioxidant capacity of the sample. The reaction mechanism in the method is based on oxidation-reduction reactions, where the DPPH compound is a synthesized free radical that is soluble in polar solvents (Hairunisa et al., 2021). Samples containing antioxidant compounds will react with DPPH by reducing hydrogen atoms to obtain electron pairs (Theafelicia & Wulan, 2023). Qualitative analysis in this method can be seen by observing the color that occurs. The DPPH radical compound from the purple DPPH solution will fade to yellow (**Figure 2**) when reduced by antioxidants in the sample solution and will produce stable DPPH-H (DPPH hydrazine) (Baliyan et al., 2022; Hairunisa et al., 2021).

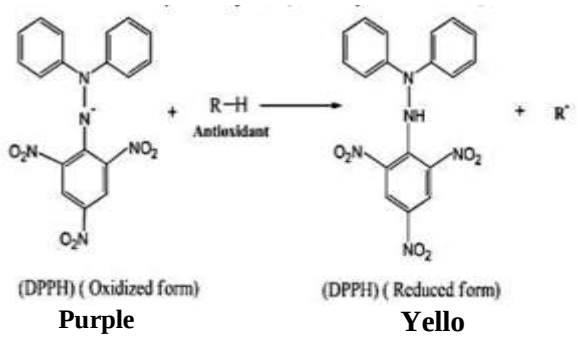

Figure 1. Reaction mechanism of DPPH with antioxidant compounds (Hairunisa et al., 2021)

As a comparison solution, ascorbic acid (vitamin C) was used because vitamin C has strong antioxidant activity (Hairunisa et al., 2021). The

purpose of this comparison is to see whether the tested sample has the potential as an antioxidant like vitamin C (Hairunisa et al., 2021). The results of the antioxidant activity test in this study showed that the ethanol extract and ethyl acetate fraction had strong and very strong antioxidant activity as indicated by the percent inhibition and IC_{50} value obtained. Quoted from Novatam & Supartono (2014), about the classification of antioxidants, that IC_{50} values <50 µg/mL are categorized as very strong and IC_{50} values of 50-100 µg/mL are categorized as strong (Novatam & Supartono, 2014). The relationship between the concentration of vitamin C and each sample against the percent inhibition on DPPH inhibition is presented in **Figures 3 to 5** below:

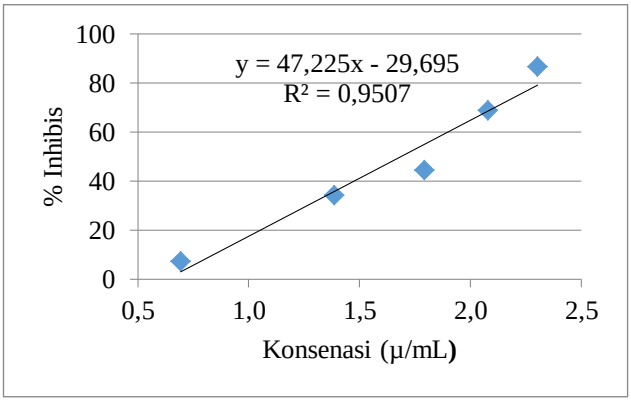

Figure 3. Correlation between ascorbic acid concentration and % inhibition against DPPH

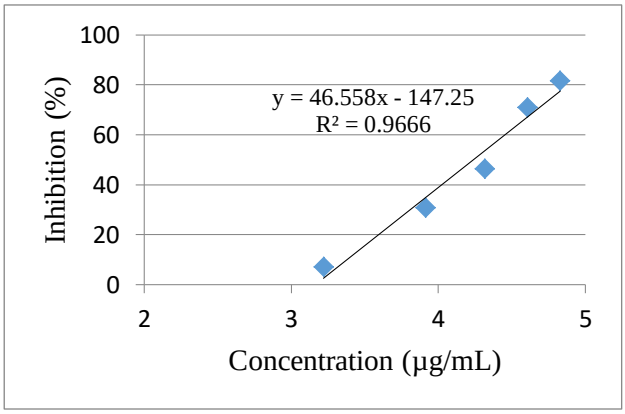

Figure 4. Correlation between soursop leaf ethanol extract concentration and % inhibition against DPPH (n=3)

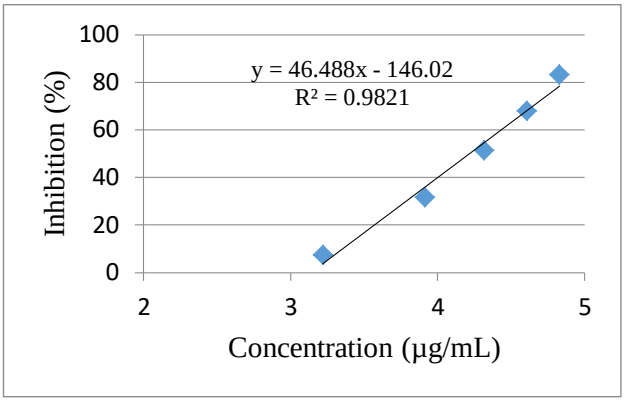

Figure 5. Correlation between concentration of soursop leaf ethyl acetate fraction and % inhibition against DPPH (n=3)

Antioxidant activity with DPPH method is indicated by IC_{50} value. The smaller the IC_{50} value, the greater the antioxidant activity. The IC_{50} value indicates the required concentration of a sample to reduce or counteract free radicals. The antioxidant activity of ethanol extract and ethyl acetate fraction as well as vitamin C (ascorbic acid) is presented in **Figure 6**.

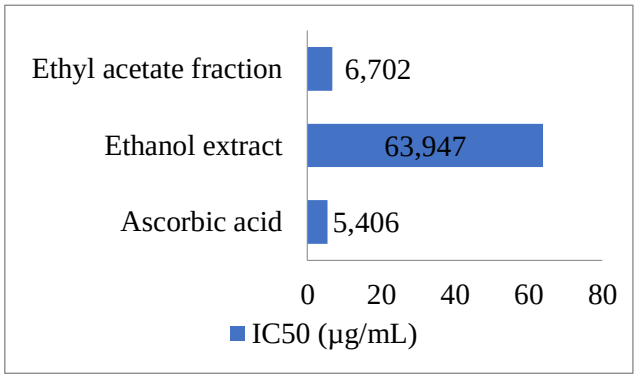

Figure 6. IC_{50} values of ascorbic acid, ethanol extract, and ethyl acetate fraction of soursop leaves

The results of the calculation of the IC_{50} value of each sample showed that the ethyl acetate fraction provided strong inhibition characterized by an IC_{50} value close to the IC_{50} value of vitamin C as a standard. When compared with ethanol extract, ethyl acetate fraction has greater inhibitory activity. The IC_{50} value of the ethyl acetate fraction is 6.702 µg/mL, which means that the sample concentration of ± 6.702 µg/mL is able to counteract free radicals from DPPH, while the ethanol extract requires a sample concentration of

± 63.947 µg/mL to be able to reduce the activity of free radicals in DPPH.

FRAP method antioxidant activity test

In principle, the FRAP method is based on the ability of a compound to reduce iron ions (Fe^{3+}) to complex iron ions (Fe^{2+}) which is characterized by the presence of a concentrated blue color in an acidic environment (Munteanu & Apetrei, 2021). The blue color is formed due to the presence of antioxidant compounds that reduce ferricyanide to ferrocyanide which binds Fe^{3+} in solution and forms a blue complex (**Figure 7**) (Shi et al., 2022). The color produced can be quantified by UV-Vis spectrophotometric method at a wavelength of 720 nm, the results of which will show the reducing power of antioxidants in a test sample (Munteanu & Apetrei, 2021).

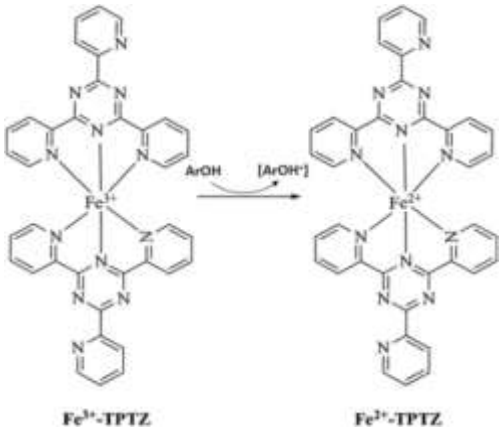

Figure 7. Mechanism of Ferric Reduction Antioxidant Power (FRAP) reaction (Shi et al., 2022)

The use of ascorbic acid as a standard in this study is because ascorbic acid is an antioxidant that has and is able to react with free radicals contained in the body (Wibawa et al., 2020). The mechanism of ascorbic acid as an antioxidant works by stabilizing free radicals by completing the electron deficiency of free radicals so that a chain reaction does not occur that can cause oxidative stress in the body (Kane et al., 2017).

Ascorbic acid as a standard solution was made at five concentrations and the absorbance measurement results are presented in **Table 4**. The absorbance values obtained were then entered into the regression equation of the standard curve of ascorbic acid (Figure 8), the regression value obtained was y = 0.0086x - 0.4249 with a value of R^2 = 0.9993 where (x) is the concentration and y is the absorbance.

Table 4. Absorbance Value of Ascorbic Acid

Concentration (ppm)	Absorbance
60	0.094
70	0.173
80	0.266
90	0.346
100	0.437

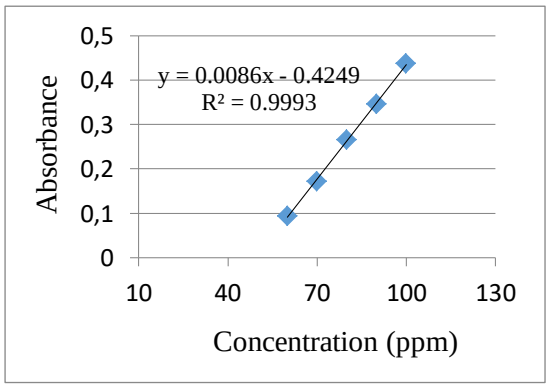

Figure 8. Ascorbic acid calibration curve (n=3)

The equation obtained is used to calculate the antioxidant activity value of the test sample by inserting the absorbance value of the sample into the equation obtained from the measurement of the ascorbic acid standard curve. The FRAP value is expressed in mg ascorbic acid equivalent/g extract (AAE) (Raharjo & Haryoto, 2019). Ascorbic acid equivalent (AAE) is a standard used to measure the amount of ascorbic acid in a material. The results of antioxidant activity calculation by FRAP method are presented in **Table 5**.

Table 5. Antioxidant Activity of Ethanol Extract and Ethyl Acetate Fraction of Soursop Leaves by FRAP Method

Sample	Replicate	Absorbance	Antioxidant activity (mg AAE/g extract)	Mean ± SD
Ethanol extract	1	0.240	7.731	7.790 ± 0.051
	2	0.247	7.813	
	3	0.248	7.824	
Ethyl acetate fraction	1	0.429	9.929	10.026 ± 0.139
	2	0.432	9.964	
	3	0.451	10.185	

Note : AAE (*Ascorbic acid equivalent*)

The results obtained are presented in **Table 5** showing the antioxidant activity of the ethanol extract and ethyl acetate fraction were 7.790 and 10.026 mg AAE/g extract, respectively. Based on these results, the higher the antioxidant activity of the sample equivalent to ascorbic acid equivalence, the greater the $Fe2^{+}$ complex formed(Yulianti, 2021). This shows that the ethanol extract has lower antioxidant activity than the ethyl acetate fraction, because the content of phenolic compounds in the ethyl acetate fraction is more dissolved due to the loss of nonpolar compounds in the fractionation process (Mazzutti et al., 2018), so that the total phenolic and flavonoid content of the ethyl acetate fraction is greater than the ethanol extract. The higher the total phenolic and flavonoid values, the greater the antioxidant ability to suppress the activity of free radicals (Nur et al., 2019). However, both samples showed antioxidant activity characterized by their ability to reduce $Fe3^{+}$ ions to complex Fe^{2+} ions. This proves the presence of antioxidant compounds in the sample that are able to donate electrons to react with free radicals to produce stable products (Wibawa et al., 2020).

Both methods showed strong antioxidant activity in the ethanol extract and very strong in the ethyl acetate fraction of soursop leaves. However, in the DPPH test, the antioxidant capacity of the ethyl acetate fraction sample was greater than the FRAP test results. This is because in the DPPH test, free radical suppression is not only by electron transfer mechanism, but can also occur with hydrogen atom transfer mechanism, while in the FRAP test, the free radical suppression mechanism is only based on electron transfer, not a combination of SET (Single Electron Transfer) and HAT (Hydrogen Atom Transfer). Based on this explanation, it can allow for different antioxidant potential or capacity in the same sample, because not all methods have the ability to scavenging at the same time (Safitri et al., 2020).

CONCLUSION

The results of the antioxidant activity test of soursop leaf ethanol extract in the FRAP test were 7.790 mg AAE/g extract (very strong category) and 63.947 µg/mL DPPH test (strong category), while the ethyl acetate fraction had a very strong antioxidant activity category from both methods with an antioxidant activity value of 10.026 mg AAE/g in the FRAP test and 6.702 µg/mL in the DPPH test. This is consistent with the higher total phenolic and flavonoid content of the ethyl acetate fraction compared to the ethanol extract. However, both samples showed antioxidant activity.

REFERENCES

Adedapo, A. A., Oni, O. A., Falayi, O. O., Ogunmiluyi, I. O., Omobawale, T. O., Oyagbemi, A. A., & Yakubu, M. A. (2022). Annona muricata mitigates glycerol-induced nephrotoxicities in male albino rats through signaling pathways of angiotensin conversion enzyme, kidney injury molecule-1, and antioxidant properties. *Scientific African*, *16*, 225. https://doi.org/10.1016/j.sciaf.2022.e01225

Aminah, A., Tomayahu, N., & Abidin, Z. (2017). Penetapan Kadar Flavonoid Total Ekstrak Etanol Kulit Buah Alpukat (Persea americana Mill.) Dengan Metode Spektrofotometri UV-Vis. *Jurnal Fitofarmaka Indonesia*, *4*(2), 226–230. https://doi.org/10.33096/jffi.v4i2.265

Badmus, Rafiu, & Fatoki. (2022). The protective effect of ethanol leaf extract of Annona muricata against doxorubicin toxicity via modulations of hematological, serum biochemical, antioxidant enzymes, and lipid peroxidation. *Phytomedicine Plus*, *2*(3), 100328. https://doi.org/10.1016/j.phyplu.2022.100328

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules*, *27*(4). https://doi.org/10.3390/molecules27041326.

Fatma, H., & Rahman, S. (2023). PENGARUH PEMBERIAN EKSTRAK DAUN SALAM (EUGENIA POLYANTHA) TERHADAP KADAR GLUKOSA DARAH PUASA PADA PESERTA PROLANIS DIABETES MELLITUS TIPE 2 DI KLINIK IMAN. Jurnal Kesehatan Tambusai, 4(3), 3277-3284.

Florence, N. T., Benoit, M. Z., Jonas, K., Alexandra, T., Désiré, D. D. P., Pierre, K., & Théophile, D. (2014). Antidiabetic and antioxidant effects of Annona muricata (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. *Journal*

of Ethnopharmacology, *151*(2), 784–790. https://doi.org/10.1016/j.jep.2013.09.021

Hairunisa, I., Mentari, I. A., Julianti, T., Wikantyasning, E. R., Cholisoh, Z., Ningsih, S. C., & Muslim, M. R. F. (2021). Antioxidant Activities in Different Parts of Pulasan (Nephelium mutabile Blume) from East Borneo. *IOP Conference Series: Earth and Environmental Science*, *736*(1), 0–9. https://doi.org/10.1088/1755-1315/736/1/012018

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., Wold, A. B., Haffner, K., Baugerød, H., Andersen, L. F., Moskaug, O., Jacobs, D. R., & Blomhoff, R. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition*, *132*(3), 461–471. https://doi.org/10.1093/jn/132.3.461.

Hanipah, H., Nurjanah, A., Saebah, N., Farizki, R., & Faradilla, H. F. (2021). Peran Perawat Sebagai Edukator Kepada Pasien Rumah Sakit X. In Jurnal Forum Kesehatan: Media Publikasi Kesehatan Ilmiah (Vol. 11, No. 1, pp. 15-19).

Irivibulkovit, K. S., Ouanthavong, S. N., & Ameenoi, Y. S. (2018). Paper-Base DPPH Assay Antioxidant Activity Analysis. *Analitical Siences*, *34*(July), 795–800.

Justino, A. B., Miranda, N. C., Franco, R. R., Martins, M. M., Silva, N. M. da, & Espindola, F. S. (2018). Annona muricata Linn. leaf as a source of antioxidant compounds with in vitro antidiabetic and inhibitory potential against α-amylase, α-glucosidase, lipase, non-enzymatic glycation and lipid peroxidation. *Biomedicine and Pharmacotherapy*, *100*(January), 83–92. https://doi.org/10.1016/j.biopha.2018.01.172

Kane, S. N., Mishra, A., & Dutta, A. K. (2017). The Effect of Astaxanthin and Regular Training on Dynamic Pattern of Oxidative Stress on Male under Strenuous Exercise. *Journal of Physics: Conference Series*, *755*(1). https://doi.org/10.1088/1742-6596/755/1/011001

Maesaroh, K., Kurnia, D., & Al Anshori, J. (2018). Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP dan FIC Terhadap Asam Askorbat, Asam Galat dan Kuersetin. *Chimica et Natura Acta*, *6*(2), 93. https://doi.org/10.24198/cna.v6.n2.19049

Mazzutti, S., Rodrigues, L. G. G., Mezzomo, N., Venturi, V., & Ferreira, S. R. S. (2018). Integrated green-based processes using supercritical CO2 and pressurized ethanol applied to recover antioxidant compouds from cocoa (Theobroma cacao) bean hulls. *Journal of Supercritical Fluids*, *135*, 52–59. https://doi.org/10.1016/j.supflu.2017.12.039

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7). https://doi.org/10.3390/ijms22073380

Ningsih, I. Y., Zulaikhah, S., Hidayat, M. A., & Kuswandi, B. (2016). Antioxidant Activity of Various Kenitu (Chrysophyllum Cainito L.) Leaves Extracts from Jember, Indonesia. *Agriculture and Agricultural Science Procedia*, *9*, 378–385. https://doi.org/10.1016/j.aaspro.2016.02.153

Novatam, S. M., & Supartono, E. K. (2014). Identifikasi Betasianin Dan Uji Antioksidan Ekstrak Buah Bit Merah (Beta vulgaris L). *Indonesian Journal of Chemical Science*, *5*(3), 3–6. https://doi.org/10.5962/bhl.title.81730

Nur, S., Sami, F. J., Awaluddin, A., & Afsari, M. I. A. (2019). Korelasi Antara Kadar Total Flavonoid dan Fenolik dari Ekstrak dan Fraksi Daun Jati Putih (Gmelina Arborea Roxb.) Terhadap Aktivitas Antioksidan. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *5*(1), 33–42. https://doi.org/10.22487/j24428744.2019.v5.i1.12034

Olasehinde, O. R., Ph, D., Afolabi, O. B., Ph, D., Owolabi, O. V., Ph, D., Akawa, A. B., & Omiyale, O. B. (2022). GC – MS analysis of phytochemical constituents of methanolic fraction of Annona muricata leaf and its inhibition against two key enzymes linked to type II diabetes. *Scientific African*, *16*, e01178. https://doi.org/10.1016/j.sciaf.2022.e01178

Raharjo, D., & Haryoto. (2019). Antioxidant Activity of Mangrove Sonneratia caseolaris L using the FRAP Method. *International Summit on Science Technology and Humanity*, 623–629.

Rahayu, M. P., & Inanda, L. V. (2015). Determination of Total Acetate Extracts Phenol Content and Dichloromethane-Ethyl

Acetate Fraction of Mundu (Garcinia dulcis. Kurz) Stem Bark. *Biomedika*, *8*(2), 37–44. www.biomedika.ac.id

Safitri, F. W., Abdul, A., & Qonitah, F. (2020). Uji Aktivitas Antioksidan Ekstrak Etanol Daun Adas (Foeniculum Vulgare Mill) Dengan Metode DPPH Dan FRAP. *Pharmed: Journal of Pharmaceutical Science and Medical Research*, *3*(2), 43. https://doi.org/10.25273/pharmed.v3i2.7456

Sanni, O., Erukainure, O. L., Oyebode, O. A., & Islam, M. S. (2020). Fractions from Annona muricata attenuate oxidative stress in pancreatic tissues, inhibits key carbohydrate digesting enzymes and intestinal glucose absorption but enhances muscle glucose uptake. *Journal of Food Biochemistry*, *44*(6), 1–10. https://doi.org/10.1111/jfbc.13211

Shi, L., Zhao, W., Yang, Z., Subbiah, V., & Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environmental Science and Pollution Research*, *29*(54), 81112–81129. https://doi.org/10.1007/s11356-022-23337-6

Suhendi, A., Haryoto, Indrayudha, P., Muhtadi, & Azizah, T. (2013). Determination of antioxidant activity of extract, polar, and semipolar fraction of Cynometra ramnifolia Linn leaf using DPPH assay. *Acta Pharmaciae Indonesia*, *1*(1), 22–25.

Tamahiwu, N. E. R., Bodhi, W., Datu, O. S., & Fatimawali, F. (2023). UJI AKTIVITAS ANTIDIABETES EKSTRAK ETANOL DAUN LABU KUNING (CUCURBITA MOSCHATA) PADA TIKUS PUTIH JANTAN (RATTUS NORVEGICUS). Jurnal Kesehatan Tambusai, 4(3), 2416-2429.

Theafelicia, Z., & Wulan, S. N. (2023). Comparison of Various Methods for Testing Antioxidant Activity (DPPH, ABTS, and FRAP) on Black Tea (Camellia sinensis) Zerlinda. *Jurnal Teknologi Pertanian*, *24*(1), 35–44.

Wibawa, J. C., Wati, L. H., & Arifin, M. Z. (2020). Mekanisme Vitamin C Menurunkan Stres Oksidatif Setelah Aktivitas Fisik. *JOSSAE : Journal of Sport Science and Education*, *5*(1), 57. https://doi.org/10.26740/jossae.v5n1.p57-63

Yulianti, N. (2021). Uji Aktivitas Antioksidan Ekstrak Kulit Pisang Kapas (Musa paradisiaca L.) dengan Metode FRAP dan DPPH pada Sediaan Hand and Body Lotion. *E-Journal Polkestama*, *17*(2), 86–92. https://doi.org/10.37160/bmi.v17i1.743