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Antioxidant Activity Analysis Using DPPH and Total Phenol Methods of Soursop Leaf Extract (*Annona muricata* L.)

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Abstract

Soursop leaves (*Annona muricata* L.) are known to contain various bioactive compounds with potential antioxidant properties. This study aimed to evaluate the antioxidant activity of soursop leaf extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and to determine its total phenolic content using the Folin–Ciocalteu method. Antioxidant activity was assessed at extract concentrations of 500 and 1000 ppm, with quercetin used as a positive control. The results showed that the soursop leaf extract exhibited very strong antioxidant activity, with an IC_{50} value of 9,486 ppm. The total phenolic content of the extract was relatively high, ranging from 102,929 to 195,702 mg GAE/g extract. The strong antioxidant activity observed is likely associated with the high content of phenolic compounds, which play a crucial role in scavenging free radicals. These findings suggest that soursop leaf extract has significant potential as a natural antioxidant source for applications in the functional food and pharmaceutical industries.

Keywords: Soursop leaves; antioxidant activity; DPPH method; total phenolic content; *Annona muricata* L.

Abbreviations: DPPH (2,2-diphenyl-1-picrylhydrazyl); GAE (Gallic Acid Equivalent); IC_{50} (Half Maximal Inhibitory Concentration); TPC (Total Phenolic Content); UV-Vis (Ultraviolet-Visible); ppm (Parts per million); SD (Standard Deviation); UNISMA (Universitas Islam Malang); GAE/g (Gallic Acid Equivalent per gram)

INTRODUCTION

Soursop (*Annona muricata* L.) is a tropical plant widely used in traditional medicine in Indonesia (Rohman & Riyanto, 2020). Soursop leaves have been empirically used to treat various health complaints, such as headaches, fever, toothaches, coughs, and asthma. Furthermore, soursop leaves are also reported to have pharmacological potential as antihypertensive, antidiabetic, anti-gout, anti-inflammatory, anti-cholesterol, and anticancer agents. These biological activities are closely related to their bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and terpenoids, which act as natural antioxidants (Putra et al., 2020; Sari et al., 2021).

Phenolic and flavonoid compounds are groups of secondary metabolites that contribute significantly to the antioxidant activity of plants (Putri & Nugroho, 2021). Antioxidants play a crucial role in counteracting free radicals that can cause oxidative stress and contribute to various degenerative diseases. One commonly used method to evaluate antioxidant activity is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method due to its simplicity, rapidity, and high sensitivity. Meanwhile, determining total phenol content is used to estimate the amount of phenolic compounds that potentially contribute to antioxidant activity (Rahmawati et al., 2022; Nugroho & Lestari, 2023).

The phytochemical profile of medicinal plants is significantly influenced by environmental factors such as climate, temperature, altitude, and soil characteristics. Differences in these factors can lead to variations in the types and levels of secondary metabolites produced by plants, thus affecting their biological activity. Therefore, phytochemical analysis and antioxidant activity testing are essential to validate traditional medicinal properties and provide a scientific basis for developing natural ingredients as antioxidant sources (Widyaningrum et al., 2020).

Several previous studies reported that soursop leaf extract contains alkaloids, flavonoids, tannins, saponins, and terpenoids, which have antioxidant potential (Harborne, 2019). Research by Handayani et al. (2021) showed that soursop leaf extract has strong antioxidant activity based on the DPPH test. Similar results were also reported by Prasetyo et al. (2023), who stated that the

high total phenol content in soursop leaf extract is positively correlated with its antioxidant activity. However, differences in growing location and extraction method can cause variations in antioxidant activity and total phenol levels, so further research is needed.

Based on this description, this study aims to analyze the antioxidant activity of soursop (*Annona muricata* L.) leaf extract using the DPPH method and determine its total phenol content. The results are expected to provide scientific information on the potential of soursop leaves as a natural antioxidant source and support the development of their use in the health and pharmaceutical sectors.

MATERIALS AND METHODS

Study area

Soursop leaf samples were taken at the location of Rusunawa 2 UNISMA (now Grand Unisma Stay) located on the UNISMA Malang campus, which is located at Jalan Mayjen Haryono No. 193, dinoyo, Lowokwaru, Malang City, precisely at the location of Rusunawa 3, 1st floor, with estimated coordinates around: -7.9621, 112.6288 (Latitude -7.9621, Longitude 112.6288)

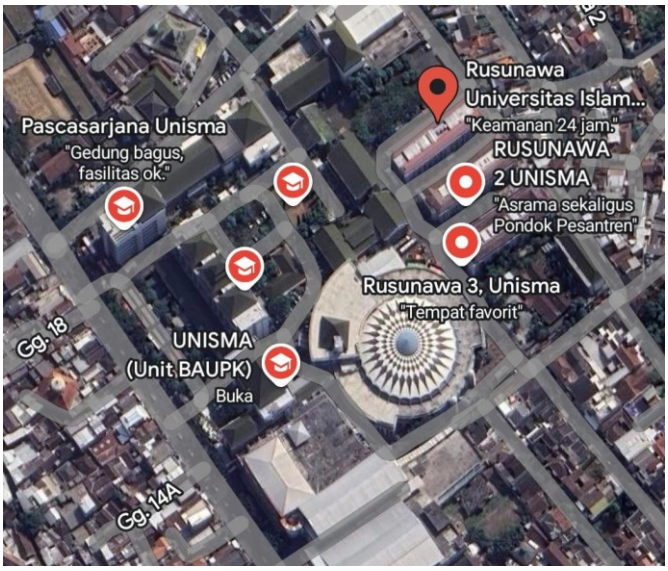

Figure 1. 7°57'43.6"S 112°37'43.7"E (Rusunawa 2, Universitas Islam Malang)."

Procedures

Tools and materials

The tools used in this study included analytical scales, measuring cups, plastic wrap, aluminum foil, spatulas, micropipettes, pipettes, blue tips, UV-Vis spectrophotometry, measuring flasks, vortex, test tubes, and a beaker. The materials used are quercetin, gallic acid, folin reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH), 96% ethanol, distilled water, and soursop leaf extract (*Annona muricata* L.).

Preparation of Quercetin Comparison Solution

Take 10 mg of quercetin, weigh it, and mix it with 10 mL of ethanol. Then made five concentrations: 10, 20, 30, 40, 50 ppm. Taken mL of each quercetin solution and 3 mL of DPPH were added. Incubated for 30 minutes and measured using UV-Vis spectrophotometry (Husain et al., 2024).

Preparation of Weighed DPPH Solution

2 mg DPPH was then mixed with 50 mL of ethanol. Then, aluminum foil was used to coat and cover the volumetric flask to protect it from light and prevent oxidation (Husain et al., 2024).

Wavelength Determination

3 mL of DPPH was taken and incubated for 30 minutes. Then, the wavelength was measured in the range of 400–600 nm (Husain et al., 2024).

Analysis of Antioxidant Activity of Soursop Leaf Extract

A stock solution of 1000 ppm was made, 50 mg of extract was added with 50 mL of ethanol. Then it was made with a concentration of respectively. 1 mL was taken and 3 mL of DPPH was added. Then incubated for 30 minutes and the absorbance was measured using a UV-Vis spectrophotometer (Husain et al., 2024).

Total Phenol Analysis of Soursop Leaf Extract

Preparation of Gallic Acid Stock Solution

Preparation of standard solution by weighing 10 mg of gallic acid then dissolving it in 10 ml of ethanol and concentrating it to 100 ppm, 80 ppm, 60 ppm, 40 ppm, 20 ppm (Baragain et al., 2021).

Determination of Gallic Acid Calibration Curve

Gallic acid solution with various concentrations was taken 100µL in a vial bottle and 0.75 ml of 7% Na was added. The CO3 was then incubated for 5 minutes at room temperature. After incubation, 0.75 ml of Folin-Ciocalteu reagent was added and incubated for 15 minutes. UV-Vis spectrophotometry at a wavelength of 735 nm was

Data analysis

Data analysis was performed using a graph depicting the relationship between sample concentration and percentage inhibition (% inhibition) to illustrate antioxidant activity. In this test, soursop leaf extract was tested at concentrations of 500 and 1000 ppm. As a comparison or positive control, quercetin solutions were used with varying concentrations of 10, 20, 30, 40, and 50 ppm to compare the effectiveness of the extract's antioxidant activity against standard antioxidant compounds

RESULTS AND DISCUSSION

Antioxidant Analysis of Soursop Leaf Extract

The antioxidant activity test of soursop (*Annona muricata* L.) leaf extract was conducted using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, a spectrophotometric method commonly used to determine the antioxidant capacity of a compound. The principle of this method is based on the ability of antioxidant compounds to reduce stable DPPH free radicals through the mechanism of electron or hydrogen atom donation (Widowati et al., 2020). This reaction causes a color change in the DPPH solution from purple to paler, which is indicated by a decrease in absorbance (Husain et al., 2024).

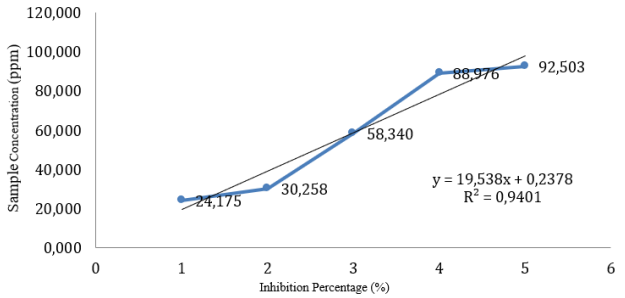

Figure 2. Linear Regression Result of Antioxidant Activity with Quercetin.

Figure 2 presents the results of the linear regression analysis between quercetin concertation and the percentage of DPPH free radical inhibition. Based oh the graph, the regression equation is y = 19,538x + 0,2378 with a coefficient of determination (R^2) value, which is close to 1, indicates a very strong positive correlation between the increase in sample concentration and the resulting inhibitor power. This confirms that the antioxidant activity of quercetin is directly proportional it its concentration; the higher the concentration of quercetin, the higher the percentage of free radical inhibition (Husain et al., 2024).

Table 1. Results of Quercetin Antioxidant Activity Test.

Sample	IC50 (ppm)	Category Antioxidants
Quercetin	2,547	Very strong

The antioxidant activity of quercetin, at 2.547 ppm, indicates very strong antioxidant activity and is comparable to that of soursop (*Annona muricata* L.) leaf extract. The high antioxidant activity of standard quercetin indicates that flavonoid compounds play a crucial role as free radical scavengers. According to domestic research, flavonoids can stabilize free radicals through proton donation and inhibition of oxidation chain reactions (Harborne, 2019; Rohman & Riyanto, 2020). Quercetin is a flavonoid compound found abundantly in plants. Quercetin is used as an antioxidant because it has a catechol group on the B ring and 3-OH groups on the A and C rings, which trap free radicals. Quercetin is an excellent source of antioxidants and can be used as an anti-inflammatory agent (Melanie et al., 2023).

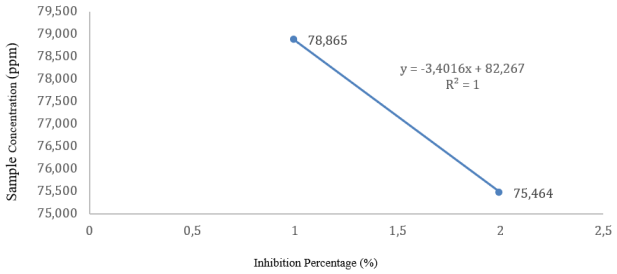

Figure 3. Linear Regression Results of Antioxidant Activity with Soursop Leaf Extract.

Figure 3 display the linear regression analysis of the antioxidant activity of soursop leaf extract. The graph yields a regression equation of y= -3,4016x + 82, 267 with a coefficient of determination (R^2) of 1. This perfect R^2 value indicates a robust linear correlation between the concentration and the inhibition percentage. This data confirms that the soursop leaf extract possesses antioxidant properties, where an increase in concentration correlates with the measured antioxidant activity (Melanie et al., 2023)

Table 2. Results of Antioxidant Activity Test of Soursop Leaf Extract.

Sample	IC50 (ppm)	Category Antioxidants
Soursop Leaf Extract	9,486	Very strong

The results of the antioxidant activity test of soursop leaf extract yielded an IC50 value of 9.486. This IC50 value indicates that soursop leaves have very strong antioxidant activity. This IC50 value indicates the concentration of extract required to inhibit the activity of DPPH free radicals. This value indicates very strong antioxidant activity.

A compound is said to have strong antioxidant activity if the IC50 value is less than 50 µg/mL, moderate if the value is between 50 and 100 µg/mL, and weak if the value ranges from 100 to 250 µg/mL (Husain et al., 2024). Soursop leaf extract is classified as a very strong antioxidant because the antioxidant compound has an IC50 value < 50.

The potent antioxidant activity of soursop leaf extract is thought to originate from its phenolic, flavonoid, and acetogenin compounds. Several studies in Indonesia have reported that soursop leaves have a high total phenol content, which correlates positively with antioxidant activity using the DPPH method (Putri & Nugroho, 2021; Sari et al., 2022).

Total Phenol Analysis of Soursop Leaf Extract

The total phenol test is an analytical method used to determine the content of phenolic compounds in an extract sample, which play an important role as bioactive compounds with antioxidant activity. The principle of this test is based on the reaction between phenolic

compounds and the Folin-Ciocalteu reagent under alkaline conditions, which produces a blue complex due to the reduction of phosphomolybdate-phosphotungstate ions. The intensity of the color formed is proportional to the amount of phenolic compounds in the sample and is measured spectrophotometrically at a specific wavelength, so that the total phenol content can be calculated and expressed as gallic acid equivalents (Kesuma et al., 2022).

Determination of total phenol content of soursop (*Annona muricata* L.) leaf extract was carried out using the Folin–Ciocalteu method with gallic acid as a reference standard. In this test, a standard gallic acid solution was used at various concentrations of 20, 40, 50, 80, and 100 ppm to form a calibration curve, while the soursop leaf extract was analyzed at concentrations of 500 and 1000 ppm. The absorbance measurement results were then used to calculate the total phenol content of the extract, expressed as gallic acid equivalents (mg GAE/g extract).

Determination of Gallic Acid Calibration Curve

The standard curve of gallic acid was made from standard gallic acid solutions with varying concentrations of 20; 40; 50; 60; 80 and 100 ppm and then reacted with Folin–Ciocalteu reagent. The color of the resulting solution varied from light blue to deep blue, indicating that the solution contained phenol. Next, 7% Na2CO3 solution was added, resulting in a color variation from bluish white to deep blue. The absorbance of the solution was then measured using Visible Spectrophotometry at a wavelength of 744 nm. The results obtained were in the form of absorbance values of gallic acid solutions at each concentration as shown in the following table.

Table 3. Gallic Acid Standart Measurement Results.

Sample	ppm concentration	Absorbance
Blank	0	0,079
Standart 1	20	0,167
Standart 2	40	0,782
Standart 3	60	0,133
Standart 4	80	1,856
Standart 5	100	2,313

The absorbance value obtained was connected with each concentration of the standard gallic acid solution to obtain a calibration curve of the standard gallic acid solution in the form of a concentration versus absorbance curve graph as shown in Figure 1. A linear regression equation of y = 0.0268x - 0.439 was obtained which was used to determine the total phenol content in the soursop leaf ethanol extract sample. The gallic acid stock solution was made at several concentrations measured using UV-Vis spectrophotometry to obtain the absorbance value and then a gallic acid calibration curve was made as in (Figure 1).

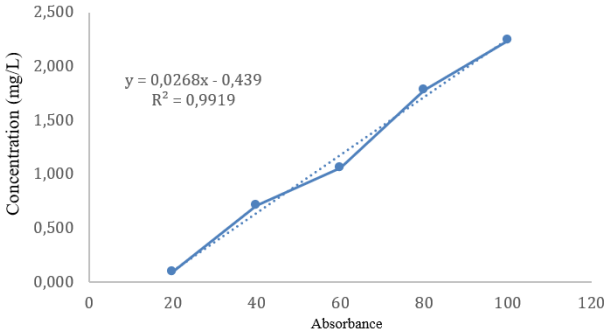

Figure 4. Linear Regression Results of Gallic Acid a wavelength of 744 nm

Figure 4 presents the results of the linear regression equation obtained from the standard curve of gallic acid is y = 0.0268x - 0.439, where y is the absorbance and x is the concentration. The gallic acid correlation coefficient R2 = 0.9919. A gallic acid correlation coefficient close to 1 indicates a linear relationship between concentration and the resulting absorption, in other words, an increase in the analyte absorption value is directly proportional to the increase in accordance with the acceptance criteria of 0.99 (Kusmiyati et al., 2022).

Determination of Total Phenol Content in Samples

The total phenol value of the sample was obtained from the absorbance value of each sample measured and then entered into the standard linear regression equation of gallic acid y = 0.0268x - 0.439 to obtain the concentration (ppm) of each sample. The total phenol content value was then calculated against the weight of the weighing and dilution of the sample solution that had been carried out so that the total phenol content of the sample was calculated as Gallic Acid Equivalent (GAE) as can be seen in Table 4.

Table 4. Results of Total Flavonoid Content of Soursop leaf Extract.

Sample Concentration (ppm)	Concentration (µg/mL)	Total Phenol (mg/g)
1000	102,929	102,929
500	97,851	195,702

Based on the results of the calculation of total phenol content using the spectrophotometric method with the standard curve equation (y = ax + b), the total phenol content of soursop leaf extract was obtained at two concentrations, namely 1000 and 500. At a concentration of 1000, the total phenol content of soursop leaf extract from three consecutive replications was 102.929 mg/L, 82.259 mg/L, and 115.246 mg/L with an average value of 100.145 mg GAE/g and a standard deviation (SD) of 3.610. Meanwhile, at a concentration of 500, the total phenol content obtained from three replications was 195.702 mg/L, 218.835 mg/L, and 178.837 mg/L, with an average value of 197.791 mg GAE/g and a standard deviation (SD) of 2.396. These results show that soursop

leaf extract has a fairly high phenolic compound content, which has the potential to act as a natural antioxidant.

Phenolic compounds are a group of secondary metabolites that play an important role in antioxidant activity due to their ability to donate hydrogen atoms or electrons to neutralize free radicals (Rashid et al., 2021). The high total phenol content in soursop leaf extract indicates its great potential as a source of natural antioxidants. Variations in total phenol levels obtained at different concentrations can be influenced by dilution factors, extraction efficiency, and the interaction of phenolic compounds with the Folin–Ciocalteu reagent during the analysis process (Rahman et al., 2022). In addition, solvent conditions and sample concentration also affect the amount of phenolic compounds detected. Soursop leaves (*Annona muricata* L.) are known to contain phenolic compounds such as flavonoids, tannins, and gallic acid, which contribute directly to antioxidant activity (Adewole et al., 2023). Therefore, the results of this study strengthen the evidence that soursop leaf extract has the potential to be developed as a natural antioxidant ingredient in the fields of functional foods and pharmaceuticals.

The results of this study indicate that the soursop leaf extract (*Annona muricata* L.) collected from the Rusunawa 2 area of the Islamic University of Malang possesses very strong antioxidant activity and high total phenolic content. The IC_{50} value obtained (9.486 ppm) categorizes this extract as a very active antioxidant, as it is well below the 50 ppm threshold typically used to define "very strong" antioxidant capacity. This potency is comparable to the quercetin used as a positive control in this experiment.

The high antioxidant activity observed is closely related to the total phenolic content, which ranged from 102.929 to 195.702 mg GAE/g. Phenolic compounds, including flavonoids which are abundant in soursop leaves, act as primary antioxidants by donating hydrogen atoms to free radicals, thereby neutralizing them. The DPPH assay confirmed this mechanism, where the purple color of the DPPH radical faded to yellow upon reduction by the antioxidant compounds present in the extract.

The environmental conditions of the sampling site at Rusunawa 2, Universitas Islam Malang, may have influenced the secondary metabolite profile of the leaves. Factors such as soil nutrients, sunlight exposure in the Malang highland area, and the age of the trees at the time of collection contribute to the synthesis of bioactive compounds. Previous studies have suggested that Annona muricata grown in different geographical locations can exhibit variations in their chemical constituents. However, the findings in this study align with the general characteristic of soursop leaves as a rich source of natural antioxidants.

The correlation between high phenolic content and low IC_{50} values in this research reinforces the potential of soursop leaves from the UNISMA campus as a raw material for herbal medicine or functional food supplements. Further isolation of specific phenolic compounds is recommended to identify which sub-groups are most responsible for the radical scavenging activity observed in this local sample.

CONCLUSIONS

Soursop (*Annona muricata* L.) leaf extract has been shown to possess very strong antioxidant activity based on the DPPH method with an IC_{50} value of 9.486 ppm. This activity is supported by its high total phenol content, which is around 102.929–195.702 mg/g of extract. These results indicate that the phenolic compounds in soursop leaves play a significant role in antioxidant activity, thus offering potential for development as a natural antioxidant source.

Competing Interests: The authors declare that there are no competing interests or potential conflicts that could have influenced the outcomes of this study.

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