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Antioxidant Activity Cultivated of Soursop (*Annona muricata* L.) Leaves Extract in Jayasari Village, Pangandaran District, West Java

Indah Zahara[1], Effionora Anwar[2*], Dian Ratih Laksmitawati[3], Deni Rahmat[2]

[1]*Doctoral Program, Faculty of Pharmacy, Universitas Pancasila, Jakarta, Indonesia.*
[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Pancasila, Jakarta, Indonesia.*
[3]*Department of Biomedical and Clinical Pharmacy, Faculty of Pharmacy, Universitas Pancasila, Jakarta, Indonesia.*
*Corresponding Author. E-mail: effionora.anwar@univpancasila.ac.id

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ABSTRACT: Indonesia is a source of raw materials for tropical medicines that treat various diseases. One of the places to plant soursop leaves in West Java is Jayasari Village, Pangandaran District. *Annona muricata* Linn (Soursop) leaves is a medicinal plant widely used as an anti-diabetic, anti-inflammatory, insecticide, antimalarial, anticancer, antibacterial, and antioxidant. Soursop leaves have many benefits because they contain phytochemical compounds. This study aims to determine the phytochemical content, total flavonoid content, and antioxidant activity of *A. muricata* leaves. This research was carried out in several steps, including extraction, evaporation, phytochemical testing, total flavonoid content, and antioxidant activity. Phytochemicals were extracted with 96% ethanol by maceration. Measurement of flavonoids in extracts was determined using standard quercetin and UV-Vis spectrophotometry method. Antioxidant activity was carried out through a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical removal test. The results showed that the ethanol extract of soursop leaves contained alkaloids, flavonoids, phenolics, saponins, and tannins. The results of measurements of flavonoids from the ethanol extract of soursop leaves showed that 1 g of the extract contained 8.32 mg of quercetin equivalent. The ethanol extract of soursop leaves has antioxidant activity, as indicated by the scavenging of DPPH radicals with an IC_{50} of 56.73 ppm.

KEYWORDS: phytochemistry; muricata; soursop; flavonoid; antioxidant.

1. INTRODUCTION

Indonesia, a nation endowed with abundant biodiversity, has been widely acknowledged as a repository of varied plant life, a significant portion of which exhibits invaluable medicinal attributes. Jayasari Village, located in Pangandaran District, West Java, is recognized for its significant reservoir of raw materials for tropical medicine. The village is of notable importance due to its cultivation of *Annona muricata* Linn., commonly called soursop. This plant has attracted considerable interest for its wide-ranging therapeutic properties. The leaves of *A. muricata* have historically been employed in folk medicine and traditional healing practices due to their diverse effects, encompassing antidiabetic, anti-inflammatory, insecticidal, antimalarial, anticancer, antibacterial, and antioxidant activities. The presence of a wide range of phytochemical compounds inherent in the leaves is responsible for these qualities [1–3].

The evaluation of *A. muricata*'s antioxidant properties has been a focal point in numerous scientific investigations due to its capacity to mitigate free radical-induced damage [4]–[6]. Free radicals are molecules characterized by their high reactivity, which renders them capable of inducing cellular damage and contributing to the development of diverse degenerative ailments, including cancer, diabetes, heart disease, and premature aging. An effective strategy for mitigating the deleterious impacts of free radicals involves the utilization of antioxidant compounds, which possess the capability to safeguard cellular structures against oxidative harm [4–6].

Numerous studies have been conducted to investigate the antioxidant properties of *A. muricata*. However, the exploration of A. *muricata*'s antioxidant activity continues to be a compelling subject, as the geographical

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origin of the plant influences it. The geographical location in which a plant thrives, encompassing various environmental elements such as climate, soil composition, and other growth-related factors, can exert a substantial influence on the plant's chemical composition, including its antioxidant compounds. Hence, it is imperative to comprehend how these environmental factors impact the antioxidant activity of plants [7–11].

This study aims to conduct a comprehensive analysis of the antioxidant activity of soursop leaf extract obtained from Pangandaran. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, known for its efficacy in assessing free radical scavenging capacity, will be employed for this purpose. Furthermore, this study will thoroughly examine the composition of antioxidant compounds, including flavonoids, phenolics, alkaloids, saponins, and tannins, found in soursop leaves.

2. MATERIALS AND METHODS

2.1. Material

Aluminium foil (Bestfresh), $AlCl_3$ (Brataco), Ascorbic acid (Merck), Blender HR2068/20 (Philips), DMSO (Merck), DPPH (sigma aldrich), 96% ethanol (OneMed), 1% $FeCl_3$ (Merck), filter paper (Whatman), freeze drying (LZ-MFD-A110 Brand Labozon USA), glassware (pyrex), 1% glacial acetic acid (Merck), H_2SO_4 (Merck), HCl (Merck), $HgCl_2$ (Merck), magnetic stirrer (DAIHAN Scientific Indonesia), Methanol pro analysis (Merck), NaOH (Brataco), Quercetin (Merck), Soursop leaves (Pangandaran, West Java), UV-Vis Spectrophotometry (Shimadzu 1800), Analytical balances (ABS 220-4 Analytical Balance).

2.2. Preparation of soursop leaf extract

Plant determination was carried out at the Bogoriense Herbarium, Botany Division, Biology Research and Development Center - Indonesian Institute of Sciences (LIPI) Cibinong, Jl. Raya Jakarta - Bogor KM. 46 Cibinong Bogor, 169110 West Java. The results of this determination are represented by letter number B-361/V/DI.05.07/1/2022, which states that the plant used in the study is indeed a soursop leaf plant (*Annona muricata* L.). The soursop leaves are cleaned with water, cut into small pieces, and dried for three days. After drying, they are blended and weighed. Then, 150 grams of the dried soursop leaves are soaked in 96% ethanol for 30 minutes with continuous stirring. The mixture is covered and left undisturbed for 24 hours with occasional stirring. After filtration, the filtrate is transferred to another container and left for one day to allow the alcohol to evaporate completely. To create soursop leaf extract with different concentrations, a 10% dimethyl sulfoxide (DMSO) solution is prepared by combining 10 ml of DMSO with 90 ml of distilled water [12,13].

2.3. Phytochemical Screening [12,13]

1. Alkaloid test
   A total of 1 mg of extract plus 2 mL of HCl was then stirred and filtered. Filtrate is added two drops of $HgCl_2$. If a yellow, orange, or white precipitate forms, it indicates that the sample contains alkaloids.
2. Flavonoids test
   A total of 1 mg of extract was added, and methanol 4 mL then heated. The filtrate was added H_2SO_4. The formation of red color indicates the presence of flavonoids.
3. Phenolic test
   A total of 1 mg of extract is added two drops of 1% FeCl3. Positive extracts contain phenols when they produce solid green, red, purple, blue, or black colors.
4. Saponin test
   A total of 1 mg sample was put into a test tube, then 5 mL of water and one drop of HCl were added and shaken for 20 seconds, and changes occurred. If foam forms (does not disappear for 20 minutes), it indicates the presence of saponins.
5. Tannin test
   Extract (1 mg) was added of 10 mL of water and boil for 5-10 minutes. The mixture was filtered and added 1% FeCl3. The dark blue or greenish-black color indicates the presence of tannins.

2.4. Determination of total flavonoid levels

Total flavonoid content was measured using the colorimetric method, with the total content expressed as quercetin equivalent (mg/g extract). Measurements were made by constructing a calibration curve using a standard quercetin solution and measuring the absorbance at a wavelength of 510 nm with a UV-Vis spectrophotometer. Flavonoid content was calculated based on the regression equation of the calibration curve.

2.5. Antioxidant activity

The present study involved the evaluation of the antioxidant activity of soursop leaf extract at various concentrations (10, 20, 30, 40, and 50 ppm) through the utilization of the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The DPPH radical is employed as a scavenging agent in this experiment, capable of sequestering unpaired electrons from the antioxidants under investigation. Introduction of soursop leaf extract into the DPPH radical solution initiates a redox reaction, wherein the antioxidant transfers electrons and subsequently reduces the DPPH radical to yield a stable, yellow molecule. The measurement of color change was conducted at specific wavelengths utilizing a UV-Vis spectrophotometer. Subsequently, the percentage of DPPH radical capture was determined by evaluating light absorption intensity. There is a positive correlation between the capture percentage and the antioxidant activity of soursop leaf extract, indicating that higher concentrations of the extract result in increased antioxidant activity. The present methodology elucidates soursop leaf extract's capacity to effectively neutralize free radicals, thereby highlighting its potential as a valuable source of antioxidants capable of safeguarding cellular integrity against oxidative stress-induced harm [14–16].

3. RESULTS

3.1. Extraction

The thick extract of soursop leaves is dark green (Figure 1), yielding 15.42%. Compared to other studies that took soursop leaves from various places in South Sulawesi, soursop leaf extract from Pangandaran produced higher yields. The results showed that soursop leaf extract from Makassar had a yield of 12.23%, from Jeneponto of 11.72%, while from Mamuju only had a yield of 10.72% [17].

Figure 1. soursop leaf extract

3.2. Phytochemical screening

Phytochemical screening is a scientific technique utilized to identify and evaluate the chemical constituents present in plant simplicia. The present study employs a qualitative approach to conduct a phytochemical analysis to identify the specific class of chemical compounds found within the sample. These chemical compounds can typically be categorized as alkaloids, phenolics, flavonoids, saponins, and tannins. The phytochemical screening procedure involved the visual examination of colour alterations and the formation of precipitates resulting from the reaction between the sample and different chemical reagents. The observed reaction is an initial indicator for determining the presence or absence of a specific group of chemical compounds in the sample. An illustration of this phenomenon involves the alteration in colour that transpires upon the introduction of a distinct reagent, thereby serving as an indication of the existence of alkaloid, phenolic, flavonoid, saponin, or tannin compounds within the simplicia[18–20]. The results of the phytochemical screening showed that these compounds were qualitatively suspected to be contained in the soursop leaf extract (Table 1).

Table 1. Phytochemical screening of soursop leaf extract

Compound	Parameter	Result	
Alkaloids	Yellow-orange precipitate	Positive	
Phenols	Deep Black	Positive	
Flavonoids	Red	Positive	
Saponin	The foam lasts ± 20 minutes	Positive	
Tannin	Greenish Black	Positive	

3.3. Total flavonoid content

The analysis results indicate a linear relationship between flavonoid content and soursop leaf extract. The linear regression equation is y = 0.018x + 0.0882, where "y" represents the flavonoid content and "x" denotes the soursop leaf extract. The coefficient of determination (r^2) for the linear regression model is found to be 0.9719, which signifies a high level of fit between the data points and the regression line (Figure 2).

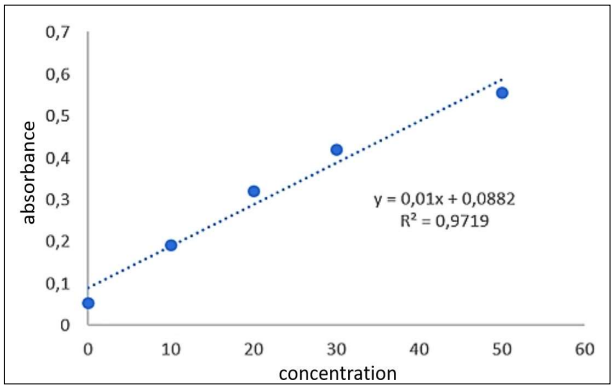

Figure 2. Regression Equation, the result of measuring the absorbance value (Total flavonoid content) of soursop leaf extract

Based on the linear regression equation, the total flavonoid content in soursop leaf extract from Pangandaran is 8.32 ± 0.39 mg. This value is significantly lower than the average value of above 20 mg/g extract reported in various other studies for soursop leaf extracts from different regions.

3.4. Antioxidant activity

The IC_{50} value, representing the concentration required to inhibit 50% of antioxidant activity, was determined for the soursop leaf extract and ascorbic acid to assess their respective antioxidant capacities. A lower IC_{50} value indicates a higher level of antioxidant activity. The IC_{50} value of soursop leaf extract was determined using a linear regression equation from the curve of the relationship of sample concentration to percent inhibition with the equation y = ax + b, sample concentration (ppm) as axis (x) and percentage value

of inhibition as axis (y) (Figure 3).

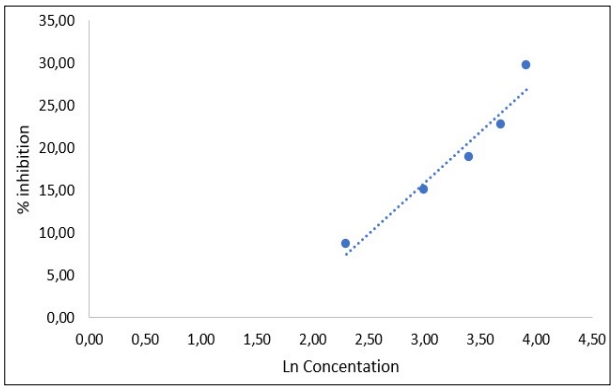

Figure 3. Regression equation, the result of measuring the absorbance value (antioxidant) of soursop leaf extract with the DPPH method

Based on the linear regression equation between the concentration of extract results and the percentage of inhibition, IC_{50} values were obtained at 56.84 ± 0.55 ppm. In comparison, ascorbic acid exhibited a very strong IC_{50} value of 11.73 ± 0.06 ppm, indicating its potent antioxidant activity compared to the soursop leaf extract.

4. DISCUSSION

The findings indicated that soursop leaves sourced from Pangandaran showed higher concentrations of secondary metabolites than those obtained from other locations. The results of previous investigations showed that soursop leaf extract obtained from Selangor, Malaysia, did not show the presence of saponins and tannins following a comprehensive phytochemical screening procedure. The Selangor soursop leaf extract analysis revealed no phenolic content, including flavonoids. Moreover, no significant differences were observed between the extraction processes carried out under high and low temperature. Both types of extracts obtained from Selangor showed negative regarding flavonoid content [21].

Flavonoids represent a class of naturally occurring polyphenolic compounds well known for their strong antioxidant characteristics. Flavonoids function as antioxidants, protecting cellular structures against oxidative stress caused by free radicals [22–24]. Research conducted in Selangor, Malaysia, revealed no flavonoid content in *A. muricata* or commonly known as soursop. These findings reveal that soursop leaf extract lacks secondary metabolites of the phenol type, resulting in the loss of the potential of specific secondary metabolites with antioxidant properties. Based on this, it can be suspected that soursop leaf extract from Pangandaran has the potential to be superior to soursop leaf extract from various other regions.

Flavonoids detected in soursop leaf extract are thought to be one of the factors that make soursop leaf extract have high antioxidant potential. Although it is not known what type of flavonoids contain exactly. Compared with various existing studies, the antioxidant activity of soursop leaf extract derived from food has a stronger potential. Another study states that soursop leaf extract extracted with the same solvent (ethanol) only has an antioxidant potential of 141.127 ppm, where the value only shows antioxidant activity with moderate category [25]. While soursop leaf extract derived from Pangandaran has an antioxidant activity value of 56.84 ppm.

5. CONCLUSION

This study shows that the yield extract of soursop leaf from Pangandaran is 15.42%. Although the extract from Pangandaran had a lower concentration of flavonoids (8.32 ± 0.39 mg) compared to extracts from other places, which had an average value above 20 mg/g. The results of the study show antioxidant activity in the strong category, where the IC_{50} value was known to be 56.84 ± 0.55 ppm.

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