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Characterization and Identification of Phytochemical Compounds Soursop Leaf Extract (*Annona muricata* L.)

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ABSTRACT. *Soursop leaves (Annona muricata L.) have long been used in traditional medicine in Indonesia to treat various diseases. This study aimed to characterize and identify phytochemical compounds present in soursop leaf extract. The analysis was carried out using UV-Visible spectrophotometric, FT-IR spectroscopy, and phytochemical screening to identify the presence of bioactive compounds such as alkaloids, flavonoids, and tannins. UV-Visible spectrophotometric analysis showed a characteristic of the isoflavone spectrum with two absorption peaks of 310-330 nm. FT-IR revealed strong absorption at 3380 cm^{-1} (O-H group), 1737 cm-¹ (C=O group), and 718 cm^{-1} (C-H alkene group), along with moderate absorption at 2922 cm^{-1} and 2852 cm^{-2} (C-H alkane group) and 1650 cm^{-1} (C=C alkene group). The total flavonoid content of the extract was 89.201 mg/g at 1000 ppm and 87.220 mg/g at 500 ppm. These findings indicate that soursop leaves are rich in flavonoids and other bioactive compounds with potential medicinal value.*

Keywords: *Soursop leaves, UV-Visible, FT-IR, phytochemical screening, total flavonoids*

INTRODUCTION

Soursop (*Annona muricata* L.) is a tropical plant widely used in traditional medicine across Indonesia. Soursop leaves have been used for centuries to treat headaches, fever, toothaches, coughs, and asthma. The soursop leaves are also reported to possess properties that may benefit individuals with heart disease, diabetes, gout, rheumatism, hypertension, high cholesterol, mouth ulcers, anemia, insomnia, and anti-inflammatory and anti-cancer effects [1] [2]. These pharmacological activities are attributed to bioactive compounds such as alkaloids, tannins, flavonoids, saponins, and terpenoids.

Environmental conditions, including climate, temperature, and soil characteristics, strongly influence the phytochemical profile of medicinal plants. Such variations may lead to differences in the type and concentration of secondary metabolites [1]. Therefore, comprehensive phytochemical characterization is essential to validate traditional uses and to provide scientific evidence of their therapeutic potential.

Phytochemical screening is a widely used approach to identify classes of active compounds, namely regarding their chemical structure, biosynthesis, natural distribution, biological functions, isolation, and comparison of the composition of chemical compounds from various plants. Phytochemical screening involves isolating and comparing chemical compounds from different plant species and researching their chemical structure, biosynthesis, natural distribution, and biological function. Phytochemical tests can be performed on a wide variety of plant parts, including those with therapeutic properties, such as leaves, stems, fruits, flowers, and roots [2].

Based on the results of research conducted by [3] with the title Phytochemical Screening of Soursop Leaf Extract (*Annona muricata* L) and its Effect on the Corrosion Rate of Carbon Steel ST 37, phytochemical analysis of soursop leaves revealed the presence of saponins, triterpenoids, tannins, alkaloids, and flavonoids in the extract. Likewise, research conducted by [4] with the title Phytochemical Analysis of soursop leaf extract

(*Annona muricata* L) from the city of Langsa, positively contains alkaloids, terpenoids, steroids, saponins, flavonoids, phenols, and tannins. However, variations in metabolite profiles across different geographical origins suggest the need for further investigation.

Based on these considerations, the present study aimed to characterize the secondary metabolites of *A. muricata* leaf extract through phytochemical screening, UV-Visible phytochemical screening, and FT-IR spectroscopy, focusing on determining the total flavonoid content. Therefore, this current research is expected to provide scientific insights into the phytochemical composition of soursop leaves (*Annona muricata* L) and support their potential application in medicinal and pharmaceutical fields

RESEARCH METHODS

Sampling

Fresh soursop leaves (*Annona muricata* L.) were collected from Rusunawa 2, Ibnu Sina, Universitas Islam Malang. Purposive sampling was employed to select intact, healthy leaves that were green in color, free from yellowing or browning, and of varying sizes [5].

Preparation of Simplicia

The collected leaves were manually sorted to remove contaminants and then washed thoroughly. The clean leaves were oven-dried at 60–65 °C for 48 hours, ground into powder using a blender, and sieved to obtain a uniform particle size of 100 mesh [6].

Determination of Moisture Content

The simplicia powder was weighed in amounts of up to 1 gram and then inserted into the Moisture Analyzer. The sample was then left to wait for 10 minutes to obtain the results of the water content of the simplicia. The maximum allowable limit was 10% for simplicia [7].

Determination of Ash Content

Approximately 1 gram of simplicia (W1) was placed in a silicate crust previously ignited and weighed (W0). The simplicia was heated using a furnace at 400-600 °C for 4-6 hours, until a white ash was formed or the sample had reached a constant weight. The ash formed inside was cooled in a desiccator and then weighed [8]. Ash content was calculated using the following equation:

$$\%\ \text{Total Ash} = \frac{W2 - W0}{W1} \times 100\%$$

Extract Preparation

A total of 50 grams of simplicia powder was macerated with 400 mL of 96% ethanol solvent until the simplicia was completely submerged in a 1:10 ratio for 3 × 24 hours in a state protected from light, with stirring for 1 hour every 24 hours. The mixture was filtered using cotton to get the macerate. Then the second maceration was carried out by adding 100 mL of 96% ethanol solvent and stirring for 1 hour. The macerate was concentrated with a rotary evaporator at a temperature of 70-80°C to obtain a thick ethanol extract from soursop leaves [9].

Extract Characterization

UV-Vis Spectrophotometer

UV-Visible Spectrophotometer characterization was carried out to determine the absorbance spectrum and wavelength of the sample. Measurements were made using a Thermo Scientific GENESYS brand UV-Vis spectrophotometer with a 200-800 nm wavelength.

FT-IR

The soursop leaf extract was characterized for its functional groups by IR spectrophotometer. Fourier Transform Infrared (FT-IR) spectra were analyzed using a spectrometer in the range 4000-500 cm^{-1}. Measurements were made using pressed, transparent KBr pellets, after which the pellets were placed on the IR spectrometer, and the wavelength was determined. A mixture of KBr and soursop leaf extract was also prepared into a pressed, transparent pellet, which was then placed in an FT-IR device for measurement [9].

Identification of Compounds Group

Alkaloid Screening

Mayer's Test

Test tubes are prepared and labeled (write according to the sample name and test name). Then, drop 20 drops of liquid extract from the plant sample into a test tube. Then add Mayer's reagent to the liquid extract of the plant sample, using as many as 10 drops through the test tube wall, and shake gently. The presence of alkaloids is indicated by the formation of a white or yellow Page 3 of 7 precipitate after adding Mayer's reagent [2].

Wagner's Test

Test tubes were prepared and labeled (write according to the sample and test names). Then, 20 drops of liquid extract from the plant sample were dropped into a test tube. Then, Wagner's reagent was added to the liquid extract of the plant sample, and as many as 10 drops were placed through the test tube wall; the mixture was then shaken gently. If a brown precipitate appears after the addition of Wagner's reagent, then the sample is positive for alkaloids [2].

Dragendroff's Test

Dragendorff reagent is prepared by mixing bismuth nitrate with potassium iodide, producing a black precipitate of bismuth (III) iodide which then dissolves in excess potassium iodide to form potassium tetraiodobismutat [10]. When the Dragendorff reagent was added to the sample containing alkaloids, an orange or red precipitate was formed. The intensity of the color and the amount of precipitate formed were used as qualitative indications of the presence and concentration of alkaloids in the sample [11].

Bouchardat Test

The Bouchardat test reacted with the basic nitrogen atoms in the alkaloid structure. However, the reagents used and the mechanism of complex formation differed slightly. Bouchardat's reagent contained a solution of iodine in potassium iodide. When this reagent was added to alkaloids, a brown-black precipitate was formed. The formation of this precipitate indicated the interaction between the iodine atom in the reagent and the nitrogen atom in the alkaloid molecule. The intensity of the color and the amount of precipitate formed served as qualitative clues to the presence of alkaloids in the sample [12].

Flavonoid Screening

Shinoda Test

The test solution was evaporated to dryness, then 2-3 drops of ethanol were added to the Mg powder, and a few drops of 5 M hydrochloric acid. The appearance of a red to purplish-red coloration indicates the presence of flavonone, flavonol, flavanonol, and dihydroflavonol compounds [12].

10% Sodium Hydroxide Test

The test was performed using 10% NaOH by placing the extract in a test tube, adding 2-4 drops of the 10% NaOH solution, and observing the color change, which shifted from yellow to a brownish yellow [13].

Saponin Screening

Foam Test

The sample extract was placed in a test tube, and 10 mL of hot water was added. The mixture was then shaken for approximately 1 minute. The presence of saponins was considered positive when a stable foam measuring 1-10 cm in height persisted for at least 10 minutes [14].

Tannin Screening (Polifenol)

Braymer's Test

A total of 2 mL of the sample was placed in a test tube. The sample was treated with two drops of 10% $FeCl_3$. A positive result was indicated by a color change from green to blue-black [15].

Base Solution Test

Test tubes were prepared and labeled (write according to the name of the sample and test). A total of 20 drops of liquid extract from plants (both leaves and twigs) were put into the test tube. Then, 20 drops of a 10% ammonium hydroxide (NH_4OH) solution were added to the test tube containing the liquid extract through the test tube wall while gently shaking. Observe what happens. Record the observation. Positive results of tannins (polyphenols) formed a fluorescent yellow color [16].

Steroid Screening

Salkowski Test

The extract was added to 1-2 mL of concentrated H_2SO_4 through the test tube wall. The presence of unsaturated steroids was indicated by the appearance of a red ring [17].

Liberman Bouchard Test

A total of 1 mg of ethanol solid sample was placed on a drip plate, and six drops of anhydrous acetic acid were added. The mixture was stirred with a spatula until it dissolved. Furthermore, one drop of concentrated sulfuric acid (H_2SO_4) was added. If a purple to orange color is formed in the solution, it indicates a triterpenoid compound, while blue or green indicates a steroid compound [18].

Total Flavonoid Test

Searching for The Highest Wavelength

0.5 mL of quercetin solution at a concentration of 100 ppm was taken, then 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M CH_3COONa, and 2.5 mL of distilled water were added. The solution was homogenized using a vortex and incubated for 40 minutes in low light, and the highest wavelength was tested on a spectrophotometer with an absorbance range of 415-440 nm.

Preparation of Quercetin Standard Solution

A total of 10 mg of quercetin was dissolved in 96% ethanol (p.a) with a volume of 10 mL. Take 1 mL of 1000 ppm parent concentration solution then added 96% ethanol (p.a) up to 10 mL, so as to obtain a test concentration of 100 ppm. Then Pipette as much as 2, 4, 6, 8 and 10 mL and dissolved with 96% ethanol (p.a) up to 10 mL, and obtained the final concentration of 20, 40, 60, 80 and 100 ppm.

At each concentration, 0.5 mL of quercetin solution was taken, followed by 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.5 mL of distilled water. The solution was homogenized using a vortex and incubated for 40 minutes in minimal light. And tested using a spectrophotometer with a maximum wavelength of 434 nm.

Determination of Total Flavonoid

A total of 10 mg of test sample was dissolved in 96% ethanol (p.a) with a volume of 10 mL. Taken 1mL of 1000 ppm parent concentration solution and then added 96% ethanol (p.a) up to 10 mL, so that the test concentration of 100 ppm was obtained. Pipette up to 5 mL, then dissolve in 96% ethanol to a final volume of up to 10 mL, to achieve a final concentration of 50 ppm.

At each concentration, 0.5 mL of the test sample solution was taken, and then 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.5 mL of distilled water were added. The solution was homogenized using a vortex, and incubated for 40 minutes in minimal light. And tested using a spectrophotometer with a maximum wavelength of 434 nm. The total flavonoid content contained in the extract was expressed as mg quercetin equivalent (QE)/g fresh sample.

RESULT AND DISCUSSION

Water Content and Ash Content Test

The water content test aims to avoid the rapid growth of mold in the extract. The moisture content of *Annona muricata* L. simplicia was 1.012%. This indicates that the soursop leaf simplicia has met the water content criteria according to [7], which is below 10%. A low water content is desirable because it reduces the risk of microbial contamination and enzymatic degradation, thereby ensuring better stability during storage. The total ash content of the simplicia was 1.14%, which falls within the recommended limit of not more than 8% [19]. Ash content reflects the total amount of inorganic material in plant samples, primarily mineral elements. The relatively low ash value obtained indicates good purity of the simplicia and supports its potential for further phytochemical and pharmacological studies.

UV-Vis Spectrophotometer

UV-Vis spectrophotometric analysis was carried out in the 200-800 nm wavelength range according to the following graphic (Figure 1).

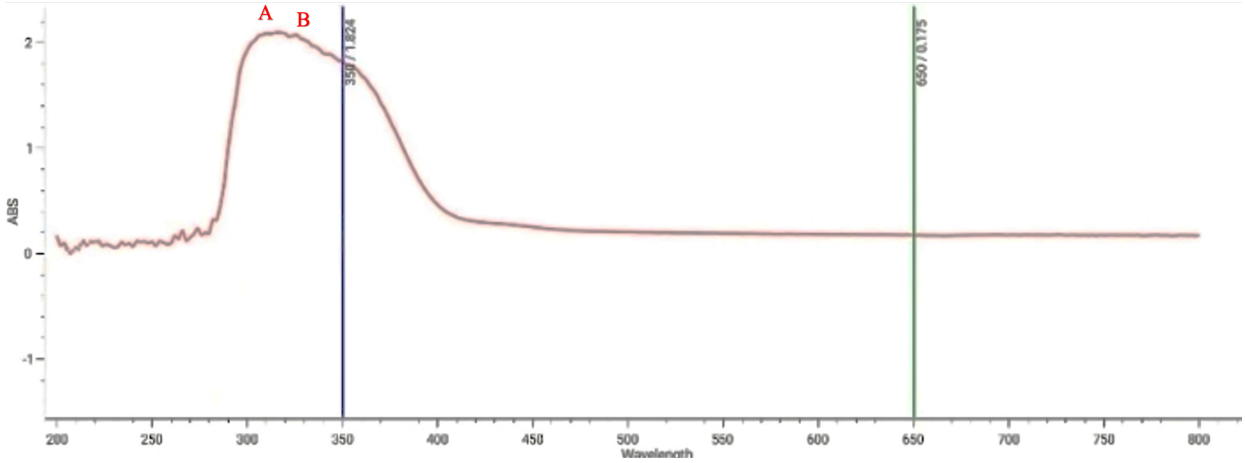

Figure 1. Results UV-Vis Spectroscopy from soursop leaf extract.

Based on Figure 1, the UV-Vis spectrophotometry results of soursop leaf extract show two distinct peaks. The wavelength at peak A is 310 nm. In contrast, peak B has a wavelength of 330 nm. These results show a typical spectrum of isoflavone-type flavonoids, characterized by two peaks in the wavelength range of 310-330 nm [20]. The detection of such peaks confirms the presence of chromophoric groups associated with polyphenolic structures, supporting the role of flavonoids as antioxidant agents.

FT-IR

An analytical technique used in chemistry and biochemistry to identify and study the molecular structure of various compounds is Infrared Spectroscopy (FT-IR). This technique utilizes the interaction between infrared radiation and molecules. In the context of the study, FT-IR was employed to identify the various functional groups present in the extract. By analyzing the absorption at specific wavelengths, it is possible to determine the presence of compounds such as alcohols, phenols, alkenes, and other compounds that potentially have biological activity [21].

The results of the analysis using FT-IR show that the soursop leaf extract exhibits absorption of several functional groups. The FT-IR spectrum of the soursop leaf extract is shown in Figure 2, with the peaks in the spectrum labeled separately. The functional groups obtained from FT-IR analysis can be used to estimate the constituent compounds in soursop leaf extract [9]. The results of the observations using FT-IR are presented in Figure 2.

Based on Figure 2, it can be seen that the soursop leaf extract exhibits a strong absorption for the stretching vibration of the O-H group of alcohol or phenol compounds, as shown in region A, with a wavenumber of 3380 cm^{-1}. Regions F, G, H, and I show the second strong absorption of C-O group stretching vibrations from alcohol compounds, carboxylic acids, esters, and ethers.

The third strong absorption for the bending vibrations of the C-H group in alkene compounds is observed in region J, with a wavenumber of 718 cm^{-1}. The fourth strong absorption for C=O group stretching vibrations from alcohol, carboxylic acid, ester, and ether compounds is observed in region D at a wavenumber of 1737 cm^{-1}.

Regions B and C showed moderate absorption for the C-H group of alkane compounds with wave numbers 2922 cm^{-1} and 2852 cm^{-1}. The highest peak is shown in region B. The next moderate absorption for the -C=C group of alkenes is observed in region E, with a wavenumber of 1650 cm^{-1}.

Phytochemical screening

Phytochemical screening aims to analyze the content of bioactive compounds in soursop leaf extract [9]. The results of the phytochemical screening test are presented in Table 1.

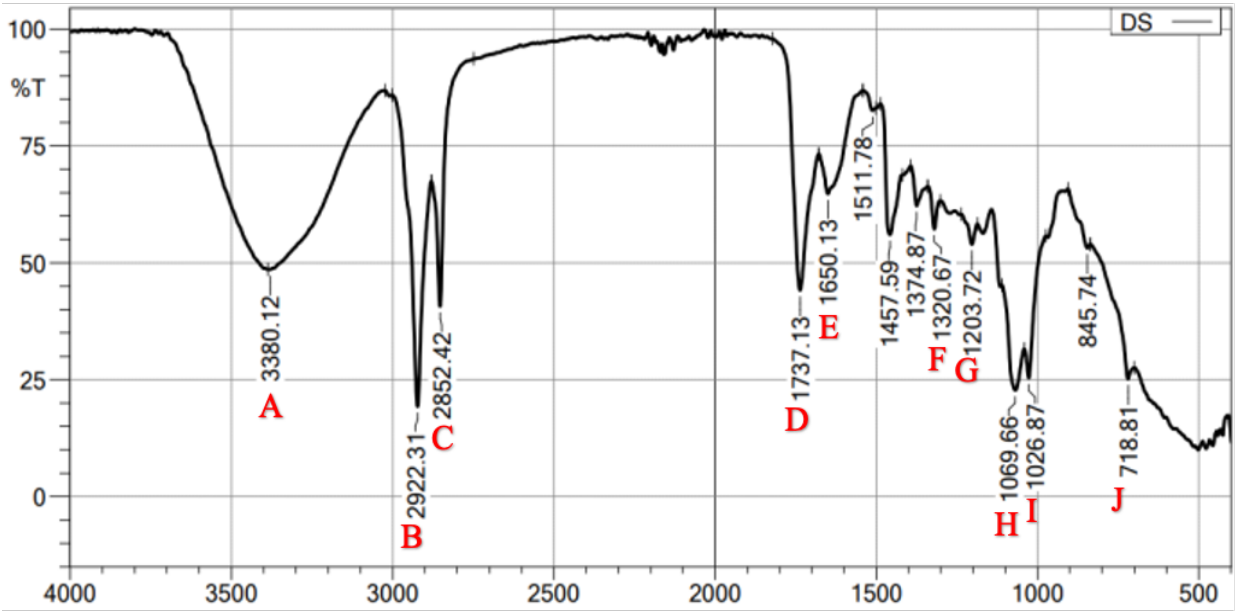

Figure 2. FT-IR analysis results of soursop leaf extracts.

Table 1. Phytochemical screening test results of extracts after maceration.

Test	Reagent	Description	Result
Alkaloid	Mayers	White precipitate formed	+
	Wagners	Brown precipitate formed	+
	Dragendroff's	No precipitate formed	-
	Bouchardat	Brown precipitate formed	+
Flavonoid	Mg+HCl	Orange in color	+
	Natrium Hydroxide 10%	Reddish yellow in color	+
Saponin	Aquadest	No foam layer formed	-
Tannin	Braymer's	Greenish black in color	+
	Base Solution	Does not change color	-
Steroid	Salkowski	No ring formed	-
	Liberman Bouchard	Does not change color	-

Based on Table 1, alkaloid compound test results are strong because the four reagent parameters show three positive results. Strong results were obtained in the flavonoid compound test because the two reagent parameters showed positive results. The saponin test does not show the presence of saponin compounds because the reagent parameters yield negative results. The tannin test obtained moderate compound results because the two reagent parameters only showed one positive result. No steroid compounds were detected in the steroid test, as both parameters yielded negative results.

The difference in research results compared to [4] is notable, as the content of secondary metabolite compounds, as determined by the phytochemical screening method, showed positive results for alkaloids, steroids, flavonoids, and tannins. But in this study, steroids showed negative results. The difference is because two samples were tested in the research [4], namely dried soursop leaves and fresh soursop leaves, whereas in this study, only fresh soursop leaves were tested.

Total Flavonoid

This test is conducted to measure the quantity of flavonoid compounds present in soursop leaf extract (*Annona muricata* L.). Sampling time, ambient temperature, light exposure, humidity, and other factors affect the total flavonoid content in plants. According to research by Helmidanora and Sukawaty [22], determining flavonoid content by the UV-Vis spectrophotometric method shows absorption in the visible light spectrum due to conjugated aromatic structures [23]. Quercetin measured its maximum wavelength in the 415-440 nm wavelength range.

The quercetin extract was prepared at a concentration of 100 ppm and then tested for absorbance at a maximum wavelength of 434 nm, yielding an absorbance value of 1,200. The total flavonoid content of the extract was then calculated based on the linear regression equation of the standard curve (y=ax+b).

The absorbance of the quercetin standard solution yielded a linear regression equation of y = 0.0124x + 0.0339, with a correlation coefficient of R^2 = 0.998. These results indicate that the higher the concentration used, the higher the absorbance. The linear regression equation obtained was then used to calculate the value of total flavonoid content of soursop leaf extract (*Annona muricata* L.).

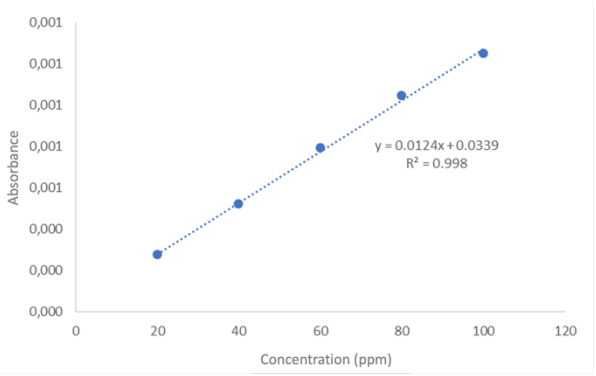

Figure 3. Quercetin standard curve.

Based on the quercetin standard curve results, the equation y = 0.0124x + 0.0339, with a regression coefficient (R^2) of 0.998, was obtained. This equation was used to determine the total flavonoid content in the sample. The absorption results were measured using the maximum wavelength to obtain the absorption. Then the absorption of the sample was entered into the equation y = 0.0124x + 0.0339, and the value of x (concentration in ppm) was obtained. The x value was then used to calculate the total flavonoid content.

Then, each extract is prepared at concentrations of 1000 and 500 ppm, tested for absorption at a maximum wavelength of 434 nm,

and an absorbance value of 1.200. The total flavonoid content of the extract is then determined. The total flavonoid content in the sample is presented in Table 2.

Table 2. Total Flavonoid test results of extracts after maceration.

Sample Concentration (ppm)	Concentration (µg/mL)	Total Flavonoid Content (mg/g)
1000	89.20161	89.201
500	43.61022	87.220

In this study, the total flavonoid content at a sample concentration of 1000 ppm showed a value of 89.201 mg QE per gram of extract. At the same time, the total flavonoid content at a sample concentration of 500 ppm showed a value of 87.220 mg QE per gram of extract. This study's results align with research [24], which indicates that the total flavonoid content of extracts determined using spectrophotometric methods offers advantages in sensitivity, selectivity, and specificity to the sample.

Flavonoid compounds act as antioxidants, preventing oxidative stress and cell damage, and thus play a crucial role in the development of degenerative diseases [18]. The total flavonoid content in a sample is represented as QE (Quercetin Equivalent), which indicates milligrams of quercetin equivalent per gram of extract.

CONCLUSION

Based on the research results, it can be concluded that soursop leaves (*Annona muricata* L.) contain flavonoid, alkaloid, and tannin chemical compounds. FT-IR analysis showed the absorption of several O-H, C-O, C-H, C=O, and -C=C functional groups. UV-Vis Spectrophotometric analysis showed a typical spectrum of isoflavone type flavonoids, the results of the analysis of total flavonoid content at a sample concentration of 1000 ppm showed a value of 89.201 mg/g while the total flavonoid content at a concentration of 500 ppm showed a value of 87.220 mg/g, this indicates that soursop leaf extract (*Annona muricata* L.) has antioxidant potential to fight free radicals.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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