



## Antioxidant Activity of Fresh Decinte, Fresh Sari, and Fresh Ethanol Extract of Soursop (*Annona muricata* L.) Leaves Using DPPH Method

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### Abstract

Antioxidants are compounds that are able to ward off or reduce free radicals in the body so that they can prevent several diseases. Soursop leaves (*Annona muricata* L.) are used as a traditional herbal medicine to prevent or treat cancer, as well as a natural antioxidant, which is usually used by people by boiling it. The purpose of this study was to look at the phytochemical screening by comparing fresh boiled water, fresh juice, and a fresh extract of soursop leaves, as well as test the antioxidant activity with the addition of DPPH (1,1-diphenyl-2-picrylhydrazyl) and measured using a UV-Vis spectrophotometer at 516 nm. The results of the soursop leaf phytochemical screening showed that there were groups of chemical compounds such as flavonoids, glycosides, tannins, and saponins. The results of testing the antioxidant activity by looking at the maximum wavelength of DPPH, using a UV-Vis spectrophotometer, obtained IC<sub>50</sub> values for fresh juice of 770.067 µg/mL, fresh decoction of 1,022.8422 µg/mL, and ethanol extract of 299.733 µg/mL. Based on the results of the study, it was shown that the ethanol extract had stronger antioxidant activity than a fresh decoction and the fresh juice of soursop leaves.

**Keywords:** Antioxidant, DPPH, Phytochemical Screening, UV-Vis Spectrophotometry, Soursop Leaf

### Abstrak

Antioksidan adalah senyawa yang mampu menangkal atau meredam radikal bebas yang terdapat di dalam tubuh sehingga dapat menghambat beberapa penyakit. Daun sirsak (*Annona muricata* L.) digunakan sebagai obat herbal tradisional untuk mencegah atau mengobati penyakit kanker, serta sebagai antioksidan alami yang biasanya masyarakat menggunakan dengan cara merebusnya. Tujuan penelitian ini adalah untuk melihat skrining fitokimia dengan membandingkan dari air rebusan segar, sari segar dan ekstrak segar daun sirsak serta uji aktivitas antioksidan dengan penambahan DPPH (1,1-diphenyl-2-picrylhydrazyl) dan diukur menggunakan spektrofotometer UV-Vis pada panjang gelombang 516 nm. Hasil skrining fitokimia daun sirsak bahwa terdapat kandungan golongan senyawa kimia seperti golongan flavonoid, glikosida, tanin dan saponin. Hasil pengujian aktivitas antioksidan dengan melihat panjang gelombang maksimum DPPH, menggunakan alat spektrofotometer UV-Vis, diperoleh nilai IC<sub>50</sub> pada sari segar sebesar 770,067 µg/mL, rebusan segar sebesar 1.022,8422 µg/mL, dan ekstrak etanol sebesar 299,733 µg/mL. Berdasarkan hasil penelitian menunjukkan bahwa ekstrak etanol memiliki aktivitas antioksidan lebih kuat dibanding rebusan segar dan sari segar daun sirsak.

**Kata kunci:** Antioksidan, DPPH, Skrining Fitokimia, Spektrofotometri UV-Vis, Daun Sirsak

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## INTRODUCTION

The human body continuously produces free radicals as a by-product of the body's normal metabolic processes. Under normal circumstances, the formation of free radicals will be followed by the formation of antioxidants. However, pollution, ultra-violet radiation, smoking, unhealthy diets, high-fat foods, food additives, and other factors enter the body unknowingly, causing the rate of increase in free radical production to accelerate<sup>1</sup>.

Excessive production of free radicals and a lack of antioxidants have an impact on the pathogenesis of several diseases in humans due to oxidative stress in cells<sup>2</sup>. The most appropriate step to reduce oxidative stress is to reduce exposure to free radicals and optimize the body's defenses by increasing antioxidant intake<sup>1</sup>.

Antioxidants are a substance that can neutralize free radicals by preventing and inhibiting the formation of new free radicals and repairing damage by radicals by giving hydrogen or electrons into radical molecules<sup>3</sup>. Some plants that contain antioxidants include Barangan bananas, which have an IC value of 50,180.8 µg/mL, Porang tubers with an IC value of 93.04 µg/mL and so forth<sup>3,4</sup>.

The plant that is often used by the community is soursop leaf, which is used as a traditional herbal medicine to prevent and treat diseases, one of which is cancer, and which people usually use by boiling. One of the

chemical constituents of soursop leaves that has potential as an antioxidant so that it can treat several diseases, such as cancer, includes flavonoids, alkaloids, and tannins<sup>5</sup>. Although boiled water from fresh soursop leaves has long been used as medicine, However, no one has conducted research on the antioxidant activity of fresh boiled water, fresh juice, or a fresh extract of soursop leaves (*Annona muricata* L.) using the DPPH method. Therefore, researchers are interested in conducting phytochemical screening studies and antioxidant activity tests by comparing fresh boiled water, fresh juice, and a fresh extract of soursop leaves (*Annona muricata* L.).

## METHODOLOGY

This study used a cold extraction method (maceration). The research phase began with data collection, plant identification, the preparation of fresh boiled water, fresh extract, and ethanol extract, phytochemical screening tests, and antioxidant activity tests using the DPPH method using a UV-Vis spectrophotometer.

### *Sample Processing*

The plants used in this study were fresh soursop (*Annona muricata* L.) leaves taken at Dolok Merawan, Serdang Bedagai Regency, North Sumatra Province. The method of taking soursop leaves (*Annona muricata* L.) was carried out purposefully, i.e., without comparing them with similar plants from other regions.



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***Preparation of fresh boiled water (dekok) of soursop leaves (*Annona muricata* L.)***

Preparation of fresh boiled water from soursop leaves (*Annona muricata* L.) is carried out by collecting fresh soursop leaves, wet sorting, namely separating soursop leaves from other plant parts, dirt, or other foreign materials, and then washing the collected soursop leaves to remove adhering dirt. Washing is done with running tap water and drained.

As much as 30 grams of fresh soursop leaves were put into a beaker and added with distilled water up to 300 mL. Then heat it in a water bath for 30 minutes. The 30 minute time is calculated after the temperature in the beaker has reached 90°C<sup>6</sup>.

***Making Fresh Soursop Leaf Extract***

The preparation of fresh soursop leaf extract (*Annona muricata* L.) is carried out by collecting fresh soursop leaves, wet sorting, namely separating the soursop leaves from other plant parts, dirt, or other foreign material, and then washing the collected soursop leaves to remove impurities. attached. Washing is done with running tap water and drained. Soursop leaves were weighed up to 30 g and then blanched for 5 minutes. Soursop leaves are drained and then crushed using a blender simultaneously added water up to 300 mL. Soursop leaf extract that has been mashed, then filtered, and then boiled for 5 minutes before being stored in a sterile glass bottle<sup>7</sup>.

***Preparation of Fresh Soursop Leaf Ethanol Extract***

The preparation of soursop leaf ethanol extract (*Annona muricata* L.) is carried out by collecting fresh soursop leaves, wet sorting, namely separating the soursop leaves from other plant parts, impurities, or other foreign materials, and then washing the collected soursop leaves to remove impurities. attached. Washing is done with running tap water and drained<sup>8</sup>.

The preparation of fresh soursop leaf extract was carried out by maceration using 96% ethanol solvent in a way, 10 parts (30 grams) of fresh soursop leaves were put into a vessel, then poured 75 parts (225 mL) of liquid extract and then closed and left for 5 days and protected from sunlight while stirring occasionally. After 5 days, the mixture is filtered, and the dregs are squeezed out. Soak the dregs again with enough solvent (75 mL) to obtain 100 parts of macerate. Then transferred into a closed vessel, left in a cool place and protected from light for 2 days then filtered again. Collect the second maserat with the first<sup>9</sup>.

***Phytochemical Screening***

Phytochemical screening tests were carried out to determine the content of secondary metabolites in plants such as alkaloids, flavonoids, tannins/polyphenols, saponins, and steroids/terpenoids<sup>10</sup>. Generally, this class of secondary metabolites has antioxidant activity<sup>11</sup>.



### ***Antioxidant Testing***

#### ***Determination of Maximum Absorption Wavelength***

Pipette 1 mL of a 0.5 mM DPPH solution (200 ppm concentration), then put it into a 5 mL volumetric flask and make up the volume with methanol up to the mark line (40 ppm concentration), then observe the absorbance at a wavelength of 400–800 nm<sup>12</sup>.

#### ***Preparation of Sample Mother Solution (Fresh Boiled Water, Fresh Extract and Fresh Extract of Soursop Leaves***

5 mL of fresh soursop leaf extract was pipetted, then put into a 50 mL volumetric flask dissolved with methanol, and the volume was made up with methanol up to the mark line (concentration 100,000 ppm) in LIB I. Then pipette 2.5 mL of solution (LIB I) into a volumetric flask; 25 mL were dissolved in methanol, and the volume was made up to the mark (concentration 10,000 ppm) in LIB II.

5 mL of fresh decoction of soursop leaves was pipetted, then put into a 50 mL volumetric flask dissolved with methanol, and the volume was made up with methanol up to the mark line (concentration 100,000 ppm) in LIB I. Then 1.25 mL of LIB I was taken and put into a 25 mL volumetric flask. dissolved with methanol, then the volume was made up with methanol to the mark line (concentration 5000 ppm) in LIB II.

Pipetted fresh ethanol extract of soursop leaves as much as 5 mL, then

put into a 50 mL volumetric flask dissolved with methanol then the volume was made up with methanol up to the mark line (Concentration 100,000 ppm) LIB I.

#### ***Preparation of Sample Test Solution (Fresh Boiled Water, Fresh Extract and Fresh Extract of Soursop Leaves***

0.25 mL of LIB II main solution; 0.5; 0.2; 0.75; and 0.1 mL were then each put into a 5 mL volumetric flask (to obtain a test solution concentration of 500, 1000, 1500, 2000, and 2500 ppm. Into each volumetric flask was added 1 mL of 0.5 mM DPPH solution (200 ppm concentration) then the volume was made up with methanol up to the mark line, then allowed to stand in a dark place. Measurements were made three times after standing for 30 minutes at operating time.

LIB II mother liquor pipette as much as 0.5; 0.8; 1; 1.2; and 2 mL were then each put into a 5 mL volumetric flask (to obtain a test solution concentration of 500, 800, 1000, 1200, and 2000 ppm). Into each volumetric flask was added 1 mL of 0.5 mM DPPH solution (200 ppm concentration) then the volume was made up with methanol up to the mark line, then allowed to stand in a dark place. Measurements were made three times after standing for 30 minutes at operating time.

0.05 mL of LIB I main solution was pipetted; 0.1; 0.15; 0.2 and 0.25 mL, then each was put into a 5 mL volumetric flask (to obtain a test solution concentration of 1000, 2000, 3000, 4000,



and 5000 ppm). Into each volumetric flask was added 1 mL of 0.5 mM DPPH solution (200 ppm concentration) then the volume was made up with methanol up to the mark line, then allowed to stand in a dark place. Measurements were made three times after standing for 30 minutes at operating time.

#### **Determination of Percent Attenuation**

The antioxidant capacity was measured as the decrease in the absorption of the DPPH solution (depletion of the purple DPPH color) due to the addition of the test solution. The absorption value of the DPPH solution before and after the addition of the test solution is calculated as a percent dissipation.

$$\%Immersion = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \%$$

#### **Value Determination IC<sub>50</sub>**

The IC<sub>50</sub> value is a number that indicates the concentration of the test sample (ppm), which provides a DPPH reduction of 50% (able to inhibit or dampen the oxidation process by 50%). A value of 0% means that it has no antioxidant activity, while a value of

100% means total absorption, and the test needs to be continued with dilution of the test solution to see the limit of its activity concentration. The results of the calculations are entered into the regression equation with the extract concentration (ppm) as the abscissa (X axis) and the % attenuation value (antioxidant) as the coordinate (Y axis).

## **RESULT AND DISCUSSION**

### **Phytochemical Screening**

A phytochemical screening was carried out to obtain information on the class of secondary metabolites contained in soursop leaf samples (fresh boiled water, fresh juice, and fresh extract of soursop leaves). The phytochemical screening tests carried out included tannins, alkaloids, flavonoids, steroid/triterpenoid saponins, and glycosides. The results of the phytochemical screening of fresh boiled water, fresh juice, and fresh extract of soursop leaves showed a class of chemical compounds, which can be seen in Table 1.

**Table 1.** Phytochemical Screening

No	Test	Fresh extract	Fresh cider	Fresh Boiled Water
1	Flavonoid	+	+	+
2	Alkaloid	+	+	+
3	Tannin	+	+	+
4	Saponin	+	+	+
5	Steroid/triterpenoid	-	-	-
6	Glikoside	-	-	-



### Antioxidant Activity Test

The ability of antioxidants in plants to neutralize DPPH radicals by releasing DPPH electrons results in a color change from purple to yellow or a decrease in the intensity of the purple color of the DPPH solution, where this

color change causes a decrease in the absorbance of DPPH<sup>13</sup>. The results of the percent DPPH damping activity test in fresh boiled water, fresh juice, and fresh extract solutions can be seen in Tables 2, 3, 4, and 5.

**Table 2.** Percent Activity Test of DPPH Preservation on Soursop Leaf Fresh Extract

Test Solution Concentration (ppm)	Measurement (A)			Average	% Damping
	1	2	3		
DPPH	0.996	0.996	0.996	0.996	-
500	0.924	0.923	0.924	0.923	7.32
1000	0.824	0.824	0.824	0.824	17.26
1500	0.670	0.670	0.670	0.670	32.73
2000	0.456	0.456	0.456	0.456	54.21
2500	0.151	0.150	0.150	0.150	84.93

**Table 3.** DPPH Percent Activity Test on Soursop Fresh Boiled Water

Test Solution Concentration (ppm)	Measurement (A)			Average	% Damping
	1	2	3		
DPPH	0.996	0.996	0.996	0.996	-
500	0.831	0.827	0.823	0.827	16.96
800	0.592	0.593	0.593	0.592	40.56
1000	0.442	0.442	0.441	0.441	55.72
1200	0.304	0.305	0.307	0.305	69.37
2000	0.125	0.124	0.124	0.124	87.55

**Table 4.** Percent Activity Test of DPPH Damping on Fresh Extract of Soursop Leaves

Test Solution Concentration (ppm)	Measurement (A)			Average	% Damping
	1	2	3		
DPPH	0.996	0.996	0.996	0.996	-
1000	0.358	0.358	0.356	0.357	64.156
2000	0.308	0.308	0.309	0.308	69.076
3000	0.180	0.180	0.180	0.180	81.927
4000	0.124	0.124	0.123	0.123	87.650
5000	0.085	0.085	0.085	0.085	91.465

Based on the table above, it can be seen that there was a decrease in % attenuation with increasing concentration of the solution from fresh boiled water, fresh extracts and fresh extracts as well as Vitamin C as a positive control. Decreasing the %

damping value indicates an increase in antioxidants. The decrease in % damping occurs because fresh boiled water, fresh juice, and fresh extract are able to neutralize DPPH (1,1 diphenyl-2-picrylhydrazyl) by giving electrons to DPPH so that atoms with unpaired



electrons get an electron pair and no longer become radicals. This is indicated by a solution that changes from dark purple to bright yellow, and the absorbance at the maximum wavelength decreases<sup>13</sup>.

### Value Analysis IC<sub>50</sub> (Inhibitory Concentration)

Determination of the results of the DPPH capture method is to calculate IC<sub>50</sub>. The results of calculating the IC<sub>50</sub> value from a solution of fresh boiled water, fresh juice, and fresh extract can be seen in Table 6.

Table 5. Percent Activity Assay of DPPH Reducing on Vitamin C

Test Solution Concentration (ppm)	Measurement (A)			Average	% Damping
	1	2	3		
DPPH	0.933	0.933	0.933	0.933	-
50	0.211	0.215	0.216	0.214	77.06
100	0.162	0.519	0.156	0.156	82.95
200	0.109	0.107	0.105	0.107	88.53
400	0.074	0.075	0.077	0.075	91.96

Table 6. The Value of Fresh Boiled Water, Fresh Essence and Fresh Extracts

Sample	Regression Equation	50 (ppm)
Fresh Sari	$Y = 0.033188X + 24.443$	770.067
Fresh Boiled Water	$Y = 0.0469 X + 2.0287$	1,022.8422
Fresh Extract	$Y = 0.05259X + 65.763$	299.733
Vitamin C Solution	$Y = 0.1556X + 44.76$	33.6760

Based on the table above, soursop leaf extract has an IC<sub>50</sub> value of 770.067 ppm, fresh boiled water of soursop leaves has an IC<sub>50</sub> of 1,022.8422 ppm, and fresh soursop leaf extract has an IC<sub>50</sub> of 299.733 ppm. From the three samples tested, fresh soursop leaf extract has the category "ppm, fresh boiled water of soursop leaves has an IC<sub>50</sub> of 1,022.8422 ppm, and fresh soursop leaf extract has an IC<sub>50</sub> of 299.733 ppm. From the three samples tested, fresh soursop leaf extract has the category "weak," while fresh soursop leaf extract and soursop leaf boiled water do not enter the predetermined category because there is almost no

antioxidant activity contained therein. Meanwhile, vitamin C, with an IC<sub>50</sub> value of 33.6760 ppm, has antioxidant activity in the very strong category. This is because vitamin C is a pure compound<sup>14</sup>. So from the three samples, fresh ethanol extract of soursop leaves had better antioxidant activity than fresh decoction and fresh juice of soursop leaves.

A substance has antioxidant properties if the IC<sub>50</sub> value is less than 200 ppm. If the IC<sub>50</sub> value obtained is between 200-1000 ppm, then the substance is less active but still has potential as an antioxidant<sup>15</sup>.



The difference in the IC<sub>50</sub> value obtained is caused by the amount of antioxidants contained therein and the ability of each compound to donate electrons to DPPH; the more electrons given to DPPH, the lower the absorbance value, which means an increase in the inhibition value and a decrease in the IC<sub>50</sub> value<sup>16</sup>.

## CONCLUSION

The results of screening for chemical compounds found in fresh boiled water, fresh juice, and a fresh ethanol extract of soursop leaves (*Annona muricata* L.) are chemical compounds belonging to the tannins, flavonoids, alkaloids, and saponins groups. Antioxidant activity testing using the DPPH method was obtained in a fresh ethanol extract of soursop leaves, which was 299.733 ppm; a soursop leaf extract was 770.067 ppm; and a fresh soursop leaf decoction was 1.022.8422 ppm. Meanwhile, vitamin C is 33.6760 ppm, so vitamin C has the strongest antioxidant activity compared to fresh boiled water, fresh juice, and fresh soursop leaf extract.

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