

# Artículo Original

# Phytochemical compounds, vitamin C levels, and antioxidant activity of soursop leaves (*Annona muricata Linn*) tea powder under various drying durations

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## ABSTRACT

**Introduction:** Soursop leaves (*Annona muricata Linn*) possess numerous properties that play a significant role in preventing various diseases, such as antibacterial, antioxidant, anticancer, anti-inflammatory activities, and their ability to act as immunomodulators. Degenerative diseases are primarily caused by the harmful effects of free radicals. Oxidative stress induced by free radicals leads to various degenerative diseases that can damage the body. Antioxidants mitigate this by donating electrons to free radicals, thereby playing a crucial role in the body's defense mechanisms.

**Aim:** This study aims to analyze the phytochemical compounds, vitamin C levels, and antioxidant activity of soursop leaf tea powder under varying drying durations.

**Methods:** This quantitative research employed a descriptive design based on laboratory testing. Phytochemical compounds were analyzed qualitatively, vitamin C levels were measured using the titration method, and antioxidant activity was determined using Ultra Violet-Visible (UV-Vis) Spectrophotometry with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A oneway Analysis of Variance (ANOVA) was used for statistical analysis, as the data obtained followed a normal distribution.

**Results:** The results revealed that all samples tested positive for phytochemical compounds, including flavonoids, saponins, tannins, alkaloids, and terpenoids. A significant effect of drying duration variations on vitamin C levels and antioxidant activity was observed, with a p-value < 0.05.

**Correspondencia:** Siti Ika Fitrasyah ikafitrasyah@gmail.com **Conclusion:** In conclusion, soursop leaves are a promising source of natural bioactive compounds, with drying at 50°C for 3 to 6 hours effectively preserving vitamin C content and enhancing antioxidant activity. However, extending the drying time to 12 hours may lead to a slight reduction in these beneficial properties. Statistical analyses confirm that drying time significantly affects both vitamin C levels and antioxidant activity in soursop leaf tea powder.

## **KEYWORDS**

Antioxidant Activity, Drying Durations, Soursop Leaves, Tea Powder, Vitamin C Levels.

## **INTRODUCTION**

On a global scale, the percentage of deaths attributed to noncommunicable diseases (NCDs) steadily increased to 73.9% in 2019. Consequently, the percentage of deaths from NCDs declined to 70.0% in 2020 and 65.3% in 2021. Advancements in the prevention, diagnosis, and treatment of NCDs have contributed to a consistent reduction in premature mortality<sup>1</sup>. Consistent with global trends, Indonesia has experienced a steady decline in the prevalence of degenerative diseases over the past ten years. The prevalence of degenerative diseases diagnosed by doctors in Indonesia (all ages) decreased between 2013 and the present, with cancer prevalence dropping from 1.4% to 1.2%, heart disease from 1.5% to 0.85%, stroke from 12.1% to 8.3%, and diabetes mellitus from 2.1% to  $1.7\%^{2,3}$ .

Understanding the role of oxidative stress is crucial in addressing degenerative diseases. Oxidative stress, driven by free radicals, disrupts the balance between reactive oxygen species (ROS) production and antioxidant defenses, leading to cellular damage and the progression of conditions like cardiovascular disease and cancer. Antioxidants from diet or endogenous systems help neutralize ROS and protect biomolecules such as nucleic acids, proteins, and lipids. Deficiency or dysfunction of key antioxidants, like the catalase enzyme, exacerbates oxidative stress and has been linked to age-related degenerative diseases, including diabetes, hypertension, and Alzheimer's disease<sup>4</sup>. These insights highlight the importance of exploring natural antioxidant sources, such as soursop leaves, to mitigate oxidative stress and its harmful effects.

Oxidative stress plays a critical role in the progression of various diseases, emphasizing the need for effective antioxidant interventions. Tea, the second most consumed beverage globally, is highly valued for its antioxidant properties, largely attributed to its phenolic content. Research shows that free phenolic compounds exhibit significantly higher antioxidant activity compared to bound phenolic compounds. Additionally, studies on spiced herbal tea blends highlight the importance of ingredient selection and combinations in enhancing antioxidant benefits<sup>5</sup>. These findings emphasize the potential of herbal teas, such as soursop leaf tea, as functional beverages with health-promoting properties.

Soursop (Annona muricata Linn.), a member of the Annonaceae family, is widely used in traditional medicine due to its diverse pharmacological properties, including anticancer, antiulcer, antidiabetic, antibacterial, antiviral, and wound-healing effects. These effects are attributed to its bioactive compounds, such as acetogenins, alkaloids, flavonoids, phenolics, and vitamins. A study has demonstrated the antioxidant potential of soursop leaf extracts, with IC<sub>50</sub> values of 14.48 µg/mL, depending on extraction methods<sup>6</sup>. Recent research has further optimized extraction conditions, achieving strong free radical scavenging activity with an IC IC<sub>50</sub> value of 35.51  $\mu$ g/mL. The presence of flavonoids, tannins, and saponins in soursop leaves underscores their potential for use in herbal medicine formulations<sup>7</sup>. Although soursop leaves have yet to be widely incorporated into specific food products, tea products offer a promising avenue for their utilization. The rich bioactive composition of soursop leaves makes them a valuable ingredient for herbal teas, aligning with the growing interest in functional beverages with health-promoting properties. This has led researchers to explore the feasibility of producing tea with soursop leaves as the primary ingredient. The study is motivated by the public's growing preference for herbal alternatives in treatment. Researchers aim to analyze the phytochemical compounds, vitamin C levels, and antioxidant activity of soursop leaf tea powder prepared under varying drying conditions.

## **MATERIALS AND METHODS**

## Type and Design

This study employed a descriptive research design based on laboratory testing. The research involved preparing soursop leaf tea drinks with varying drying durations: 0-hour (P0), 1-hour (P1), 3 hours (P2), 6 hours (P3), and 12 hours (P4). Testing was conducted in duplicate to evaluate antioxidant activity, vitamin C levels, and phytochemical composition.

#### Location and Time

The study on the effect of drying time on the phytochemical compounds, vitamin C levels, and antioxidant activity of soursop leaf tea powder was conducted from November 23 to 29, 2022, at the Research Laboratory of the Faculty of Mathematics and Natural Sciences, Tadulako University.

#### **Tools and Materials**

The materials used for sample preparation were soursop leaves, which were dried for varying durations: 0-hour, 1-hour, 3-hour, 6-hour, and 12-hour. Materials for phytochemical screening included soursop leaf sample solution, magnesium powder, concentrated HCl, distilled water, 5% ferric chloride solution, Dragendorff reagent, glacial acetic acid, and concentrated sulfuric acid. For vitamin C analysis, the materials used were soursop leaf samples, 25 mL distilled water, 0.01 N iodine solution, and 1% starch indicator, and ascorbic acid standard. The antioxidant activity analysis utilized soursop leaf sample solutions, ethanol, 96% alcohol, DPPH (2,2-diphenyl-1-picrylhydrazyl) solution, and **ascorbic acid**. The tools used for sample preparation included an oven, blender, aluminum foil, and containers. Tools used in phytochemical screening included Petri dishes, dropper pipettes, 10 mL measuring cups, test tubes, tube racks, spatulas, 50 mL beakers, and aluminum foil. For determining vitamin C levels, the tools utilized were an oven, 100 mL measuring cup, ten 50 mL Erlenmeyer flasks, aluminum foil, scales, a shaker, funnel, filter paper, container, spatula, 10 mL measuring cup, dropper pipette, 5 mL measuring cup, titration apparatus (50 mL burette), and a 50 mL beaker. The tools used for antioxidant activity analysis included an oven, scales, 100 mL Erlenmeyer flask, 100 mL measuring cup, aluminum foil, filter paper, funnel, spatula, 10 mL measuring cup, 10 mL beaker, a 100-1000 µL dropper pipette, a 20–200 µL dropper pipette, and a UV-Vis spectrophotometer (for absorbance measurement at 517 nm). Gloves, goggles, and lab coats were used during the experiments to ensure safe handling of chemicals, including hydrochloric acid (HCI), sulfuric acid, and iodine, and to maintain a safe laboratory environment.

#### Stages

The initial stages of the research involved sample preparation, which included drying the samples for varying durations (0-hour, 1-hour, 3-hour, 6-hour, and 12-hour). Subsequent stages included phytochemical screening, determination of vitamin C levels, and analysis of antioxidant activity.

## Working Procedure

#### Sample Preparation

The procedure followed in this study was based on the research conducted by Adri et al. (2013)<sup>8</sup>. Fresh soursop leaves were picked directly from the tree, selecting the third to fifth leaves from the base of the stem. The leaves were collected in the morning to ensure optimal phytochemical content, as metabolite levels are typically higher during this time. After harvesting, the leaves were washed under running water until clean, drained, and air-dried. To remove excess surface moisture, the leaves were air-dried in the shade for 30 minutes before being subjected to oven drying. Each sample was then dried in a 50°C oven for varying durations (0-hour, 1-hour, 3-hour, 6-hour, and 12-hour). Once dried, the samples were ground into a fine powder using a blender, which was thoroughly cleaned between samples to prevent crosscontamination and ensure the integrity of the powdered samples. The powdered soursop leaf samples were stored in airtight containers, kept away from light, and maintained at room temperature to preserve their integrity and prevent the degradation of bioactive compounds.

## Phytochemical Compound Screening

Phytochemical screening involves a color test reaction using specific color reagents. In this study, qualitative phytochemical screening was performed to identify the presence of flavonoids, alkaloids, tannins, terpenoids, and saponins in soursop leaf tea powder. Flavonoid Testing: A 1.0 mL sample of the alcoholic solution was placed in a test tube, followed by the addition of a small amount of magnesium powder and a few drops of concentrated HCl (Shinoda reagent). A positive reaction was indicated by the appearance of an orange, pink, or red coloration in the solution. Saponin Testing: A 2.0 mL sample solution was mixed with an equal volume of distilled water in a test tube and shaken thoroughly. A positive result was indicated by the appearance of foam that remained stable for at least 10 minutes. Ttkan annin Testing: One milliliter of the sample solution was placed in a test tube and mixed with a 5% ferric chloride solution. A positive reaction was indicated by the formation of a dark greenish-black or brown precipitate 9.

**Alkaloid Testing:** A 1.0 mL sample was placed into a test tube and mixed with 2–3 drops of Dragendorff's reagent. The reagent was freshly prepared and stored under appropriate conditions to ensure its activity and reliability during the alkaloid test. Dragendorff's reagent was prepared by dissolving 8.0 grams of bismuth nitrate (Bi(NO<sub>3</sub>)<sub>3</sub>·H<sub>2</sub>O) in 30% (w/v) nitric acid (HNO<sub>3</sub>). Separately, 4.15 grams of potassium iodide (KI) were dissolved in 50 mL of distilled water. The two solutions were combined and allowed to stand for 24 hours to ensure complete reaction. Following this, the mixture was filtered using filter paper to remove any precipitates, and the filtrate was di-

luted with distilled water to a final volume of 100 mL. A positive reaction was indicated by the formation of an orange precipitate, confirming the presence of alkaloids in the sample<sup>10</sup>. **Terpenoid Testing:** To test for the presence of terpenoids, 1.0 mL of the extract was mixed with 5 drops of glacial acetic acid in a test tube. Following this, 1.0 mL of concentrated sulfuric acid was carefully added to the mixture. The test tube was observed for any color change. A positive result was indicated by the formation of a violet or brownish red color in the solution, confirming the presence of terpenoids<sup>11</sup>.

Notes: The sample volumes used in the tests (1.0 mL, 2.0 mL) were carefully measured to ensure proper mixing and clear visualization of the results. Reagents such as concentrated HCl, sulfuric acid, and ferric chloride were stored in airtight containers and handled with care under controlled conditions to maintain their quality and ensure accurate results. Control samples, including blank solutions and samples known to lack specific phytochemicals, were used to validate the specificity of observed color changes or precipitate formation during the tests. For validation, standard solutions of known phytochemicals, such as quercetin for flavonoids and tannic acid for tannins, were tested alongside the samples to ensure accuracy and reliability of the results.

#### Determination of Vitamin C Levels

The levels of vitamin C in the sample were measured using iodometric redox titration, employing a starch indicator solution. An iodine (I<sub>2</sub>) solution of known molarity was gradually added to the sample until the equivalence point was reached. The endpoint was indicated by a color change to dark blue, which occurred due to the formation of the iodine-starch complex. The vitamin C content was determined by calculating the volume of iodine solution required to reach the endpoint, based on the known concentration of iodine<sup>12</sup>.

#### Antioxidant Activity Analysis

The antioxidant activity of the concentrated extract was evaluated using the spectrophotometric method with the DPPH reagent (2,2-diphenyl-1-picrylhydrazyl). A solution of the extract was prepared, and an aliquot was mixed with a DPPH solution. The reaction mixture was incubated for a specified period of time, and the decrease in absorbance was measured at a suitable wavelength, typically at 517 nm, using a spectrophotometer. The antioxidant activity was determined by comparing the absorbance of the sample to that of a control, with a greater decrease in absorbance indicating higher antioxidant activity<sup>13</sup>.

#### Data Processing

**Phytochemical Screening:** The presence of flavonoids, alkaloids, tannins, terpenoids, and saponins in the sample

was indicated by a positive result, marked as (+). Conversely, the absence of these compounds was indicated by a negative result, marked as (-). Determination of Vitamin C Levels: The results indicated that the highest vitamin C concentration was obtained from one of the drying variations. Antioxidant Activity Analysis: The antioxidant activity was quantitatively measured using the DPPH method, and the results were expressed as  $IC_{50}$  values. A lower  $IC_{50}$  value indicates greater free radical scavenging activity. Antioxidant compounds were categorized based on their IC<sub>50</sub> concentrations as follows: Very strong:  $IC_{50} < 50$  ppm; Strong:  $IC_{50}$  between 50–100 ppm; Moderate: IC<sub>50</sub> between 101–150 ppm; and Weak: IC<sub>50</sub> between 151-200 ppm. Data from the antioxidant activity analysis, including the percentage of inhibition and IC<sub>50</sub> values obtained through UV-Vis spectrophotometry, were analyzed using Microsoft Excel. The mean IC<sub>50</sub> values from the initial and subsequent measurements were used to determine the deviation.

The percentage of inhibition was calculated using Equation 1:

$$\%inhibition = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where:

 $A_{control}$  = Absorbance of the control sample.

 $A_{sample} = Absorbance of the test sample.$ 

A linear plot of percentage inhibition (% inhibition) versus concentration was analyzed using Equation 2:

$$y = a + bx$$

Where:

x = Concentration of the measured substance.

y = Percentage of inhibition.

The  $IC_{50}$  value was determined as the x value corresponding to a y value of 50% in this equation<sup>13</sup>.

## Data Analysis

Phytochemical screening was analyzed descriptively. Statistical analyses were performed for vitamin C levels and antioxidant activity. The data for vitamin C levels showed a normal distribution, with a p-value of 0.341, while the data for antioxidant activity also demonstrated a normal distribution, with a p-value of 0.451. Both p-values ( $\geq 0.05$ ) confirm that the data are normally distributed. Further analysis of normally distributed data was conducted using the ANOVA (Analysis of Variance) test, with a significance threshold of p-value < 0.05.

## RESULTS

## Phytochemical Compounds Screening

The phytochemical screening results in Table 1 provide an overview of the presence of key bioactive compounds in soursop leaf tea powder samples, with varying drying times. All five samples (P0 to P4) were tested for five different phytochemicals: flavonoids, saponins, tannins, alkaloids, and terpenoids. These results suggest that drying time does not significantly affect the presence of these phytochemicals in soursop leaf tea powder, as all compounds were detected across all drying times (from 0 hour to 12 hours). This consistency highlights the potential of soursop leaf tea powder as a source of various bioactive compounds with therapeutic properties.

## Vitamin C Levels

Table 2 demonstrates that increased drying time generally leads to higher vitamin C content in soursop leaf tea powder, particularly between P2 (3-hour) and P3 (6-hour) of drying. However, the P4 (12-hour) drying time did not show a significant improvement compared to the 3-6 hour drying times and even showed a slight reduction in vitamin C content. Based on this data, it appears that drying times between 3 and 6 hours are optimal for preserving or enhancing the vitamin C levels in soursop leaf tea powder. Statistical analysis indicates a signifi-

Table 1. Phytochemical	Screening Results of	Soursop Leaf Tea	Powder Samples
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Phytochemical	Sample						
type	P0 (0-hour)	P1 (1-hour)	P2 (3-hour)	P3 (6-hour)	P4 (12-hour)	Description	
Flavonoids	+	+	+	+	+	Showed a red color	
Saponins	+	+	+	+	+	Formed stable foam for 10 minutes	
Tannins	+	+	+	+	+	Changed to a blackish-green color	
Alkaloids	+	+	+	+	+	Formation of orange precipitates	
Terpenoids	+	+	+	+	+	Showed a brownish-red color change	

(+) Indicates the compound is present in the sample.

Table 2. Vitamin C levels of soursop leaf tea powder samples per 100 g  $\,$ 

Sample	Drying time	Average of vitamin C Level ± standard deviation (mg/100g)	p-value
P0	0 hour	$16.9 \pm 0.04$	
P1	1 hour	$17.1 \pm 0.02$	
P2	3 hours	34.3 ± 0.28	0.000
P3	6 hours	34.5 ± 0.09	
P4	12 hours	34.2 ± 0.14	

cant effect of drying time at 50°C on the Vitamin C levels of soursop leaf tea powder, with a p-value < 0.05. The p-value for the comparison between the samples is 0.000, indicating a statistically significant difference in vitamin C content as drying time increases. These findings suggest that longer drying times may help retain or enhance the vitamin C content in soursop leaf tea powder. The comparison of Vitamin C levels among soursop leaf samples is presented in Table 2 and illustrated in Figure 1.

The graph in Figure 1 indicates that longer drying times result in higher vitamin C levels, up to a specific threshold (3 hours), after which the levels stabilize.

## Antioxidant Activity

Table 3 shows that the  $IC_{50}$  values decrease as the drying time increases up to 6 hours (P3), indicating an im-

Sample	Average of IC <sub>50</sub> ± SD (ppm)	Antioxidant Activity Classification	p-value
P0 (0-hour)	59.4 ± 0,6	Strong	
P1 (1-hour)	50.8 ± 0,4	Strong	
P2 (3-hour)	42.9 ± 0,08	Very Strong	0.000
P3 (6-hour)	38.3 ± 0,21	Very Strong	
P4 (12-hour)	47.3 ± 0,73	Very Strong	

Table 3. IC<sub>50</sub> Value (ppm) and antioxidant activity of sample

provement in antioxidant activity. The decrease in  $IC_{50}$  reflects better free radical scavenging activity, as a lower  $IC_{50}$  value signifies a stronger antioxidant. At 12 hours (P4) of drying, while the antioxidant activity remains classified as "Very Strong", there is a slight increase in  $IC_{50}$  compared to the 6-hour (P3) sample, suggesting a potential reduction in antioxidant potency with prolonged drying time. The statistical significance of the results (p-value = 0.000) confirms that the changes in  $IC_{50}$  values are reliable and not due to random fluctuations, further strengthening the conclusion that time influences antioxidant potential in this sample. The antioxidant activity of the samples is presented in Table 3 and illustrated in Figure 2.

Figure 2 shows that as the drying time increases, the  $IC_{50}$  value strengthens up to 6 hours, after which it decreases with further drying.

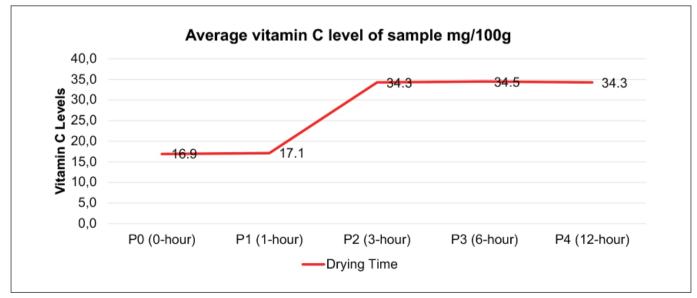


Figure 1. Graph of average vitamin C levels in soursop leaf tea powder sample

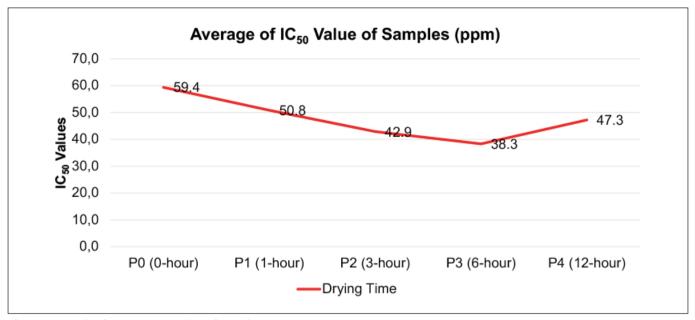


Figure 2. Graph of average IC<sub>50</sub> value of sample

#### DISCUSSION

Flavonoids are one of the key chemical constituents found in soursop leaves, known for their medicinal properties. Flavonoids are a diverse group of polyphenolic compounds found in plants, classified into six primary categories: flavones (e.g., apigenin and luteolin), flavonols (e.g., guercetin and myricetin), flavanones (e.g., naringenin and hesperidin), catechins or flavanols (e.g., epicatechin and gallocatechin), anthocyanidins (e.g., cyanidin and pelargonidin), and isoflavones (e.g., genistein and daidzein). These compounds can exist as aglycones (without attached sugars) or as glycosides (with attached sugar molecules). Flavonoids are recognized for their diverse biological activities, including anti-inflammatory, antioxidant, antibacterial, antiviral, antiallergic, cytotoxic, and anticancer effects. They are also utilized in the treatment of neurological disorders and possess vasodilatory properties. Additionally, flavonoids have been shown to inhibit the activity of various enzymes, such as hydrolases, hyaluronidase, alkaline phosphatase (ALP), arylsulfatase, cAMP phosphodiesterase, lipase, and alpha-glucosidase kinase<sup>c</sup>.

Saponins are a diverse group of naturally occurring plant secondary metabolites found in various foods, including grains, pulses, green leaves, and sea creatures. They consist of a hydrophilic sugar moiety linked to a lipophilic aglycone, resulting in an amphiphilic nature and unique functional properties. Saponins have been reported to exhibit a wide variety of biological activities, including anti-inflammatory, antioxidant, antibacterial, antiviral, antiallergic, cytotoxic, and anticancer effects. They can induce apoptosis in cancer cells, inhibit tumor cell proliferation, and suppress angiogenesis. Saponins also inhibit the production of pro-inflammatory cytokines and enzymes, thereby reducing inflammation and alleviating symptoms in conditions like arthritis and inflammatory bowel disease. They serve as adjuvants and immunostimulants, enhancing the immune response to infections and improving vaccine efficacy. Additionally, saponins possess antioxidant activity, helping to neutralize free radicals and oxidative stress in the body. They can form non-soluble compounds with cholesterol and other sterols, inhibiting their absorption and leading to a reduction in blood cholesterol levels, specifically LDL cholesterol. Saponins exhibit antimicrobial effects against a wide range of pathogens, including bacteria, viruses, fungi, and protozoa, by disrupting microbial cell membranes and interfering with their replication<sup>15</sup>.

Tannins are convoluted, constrictive, and deliquescent polyphenols, with bioavailability that reduces intestinal absorption. Tannins have a range of health implications. While they are known for their anti-nutritional effects, decreased digestibility, mutagenic and carcinogenic potential, and hepatotoxic activity, they may also act as co-promoters of various diseases. However, recent studies have highlighted their numerous health benefits, including antioxidant, anti-cancer, anti-allergy, anti-inflammatory, anti-parasitic, and antimicrobial properties. Despite their astringent nature, which limits their use in the food industry, tannins have extensive applications in the pharmaceutical field<sup>16</sup>.

Alkaloids are nitrogen-containing compounds commonly found in plants and are characterized by their heterocyclic structures. Alkaloids exert significant biological effects on both humans and animals. They interact with a- and  $\beta$ -re-

ceptors, providing antipsychotic and antihypertensive effects, blocking presynaptic alpha-2 adrenergic receptors, and exhibiting mild anti-diuretic properties. Additionally, alkaloids function as antineoplastic agents and demonstrate anti-inflammatory, demulcent, ganglionic blocking, anti-spasmodic, insecticidal, and hepatoprotective properties<sup>17</sup>.

Terpenoids are a very prominent class of natural compounds produced in diverse genera of plants, fungi, algae and sponges. Terpenoids possess a wide range of biological activities, including cancer prevention, antimicrobial, antifungal, and antiviral properties. They also demonstrate the ability to lower blood sugar levels, reduce inflammation, combat parasites, and improve memory. Furthermore, terpenoids exhibit significant therapeutic potential against diseases such as cancer, malaria, tuberculosis, and various viral and bacterial infections<sup>18</sup>.

The results of this research align with those of Nuryani et al. (2018), where the extract from boiling *Daruju* leaves tea was found to contain phytochemical compounds, including flavonoids, alkaloids, phenols, steroids, tannins, and saponins<sup>19</sup>. Similarly, these findings are consistent with research conducted by Iskandar (2020), in which phytochemical tests on *Uncaria tomentosa* leaves, used as an ingredient for making tea, revealed the presence of alkaloids, flavonoids, polyphenols, triterpenoids, steroids, saponins, and tannins through qualitative analysis<sup>20</sup>.

## Vitamin C levels

The drying process can degrade soursop leaf tea powder because vitamin C is highly susceptible to oxidation and destruction at elevated temperatures. In this study, oven drying was carefully controlled, with the temperature maintained at 50°C and not exceeding 60°C. Research by Saputera et al. (2012) demonstrated that drying temperature significantly affects vitamin C levels in roselle leaf tea (p < 0.05). Their study found that drying roselle leaves at 50°C resulted in a vitamin C content of 27.926 mg/100 g <sup>21</sup>. This value is lower than the vitamin C levels observed in the current study, which were 34.5  $\pm$  0.09 mg/100 g. Similarly, Puspitasari et al. (2017) reported a continuous decline in the vitamin C levels of kombucha tea after the seventh day of fermentation<sup>22</sup>. On the other hand, Guerra et al. (2023) reported the vitamin C content of ripe Shiraca fruit at 108.35 mg/100 g <sup>23</sup>.

## Antioxidant activity

The concentration of an extract that reduces DPPH activity by 50% is known as the  $IC_{50}$  value. Antioxidant activity increases as the  $IC_{50}$  value decreases. The findings of this study align with those of Harningsih and Wimpy (2018), who reported that a 2:1 blend of cherry and soursop leaves exhibited very strong antioxidant activity, with an  $IC_{50}$  value of 6.9126 ppm when tested using the DPPH method<sup>24</sup>. In this study, the strongest antioxidant activity was observed during

drying in P3 (6 hours). However, in P4 (12 hours), antioxidant activity declined significantly, suggesting that prolonged drying weakens antioxidant effects. Drying also impacts the water content of the sample, which plays a crucial role in microbial growth. Managing water activity and storage conditions can help reduce microbial contamination and aflatoxin production in medicinal herbs. Sorption isotherms (adsorption analysis) can identify optimal drying conditions to reduce water activity, control *Aspergillus flavus* growth, and prevent damage from excessive drying. This approach also minimizes costs by reducing the drying time to an ideal level<sup>25</sup>.

Antioxidants are stable molecules capable of donating electrons to free radicals, thereby neutralizing their harmful effects. They primarily mitigate cellular damage by scavenging free radicals. Low molecular weight antioxidants interact with free radicals without being consumed, halting chain reactions before essential biomolecules are damaged. Research by Anggorawati et al. (2016) demonstrated that avocado leaves, when processed as herbal tea, exhibited the highest antioxidant activity at a drying time of 30 minutes, with an  $IC_{50}$  value of 24.863 µg/ml <sup>26</sup>. Similarly, Adri et al. (2013) found that antioxidant activity in soursop leaf tea was influenced by drying variations. At a drying time of 150 minutes, the antioxidant activity was 82.16  $\mu$ g/ml, and the IC<sub>50</sub> value decreased as drying time increased. Furthermore, Veliz et al. (2025) reported in their study that dried hibiscus flowers have an antioxidant capacity indicated by values ranging from 13.60 mg mL-1 - 14.22 mg mL-1 <sup>27</sup>.

Oxidative stress occurs when there is an imbalance between free radical production and the body's antioxidant defenses. This condition is linked to damage to various molecules, such as lipids, proteins, and nucleic acids<sup>28</sup>. Oxidative stress can arise from trauma, infections, heat injury, hyperoxia, toxins, or excessive exercise. Injured tissues generate enzymes like xanthine oxidase, lipoxygenase, and cyclooxygenase, activate phagocytes, release free iron and copper ions, or interfere with oxidative phosphorylation's electron transport chain, leading to an overproduction of reactive oxygen species (ROS).

Oxidative stress, resulting from the excessive intracellular accumulation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and other free radical species, contributes to the onset and progression of various diseases, including diabetes, obesity, diabetic nephropathy, diabetic neuropathy, and neurological diseases, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD). Oxidative stress is also implicated in cardiovascular disease and cancer<sup>29</sup>. An excess of oxidative stress may oxidize lipids and proteins, altering their structure and function. Antioxidants function as free radical scavengers, hydrogen and electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, and metal-chelating agents. Both enzymatic and non-enzymatic antioxi

dants operate in intracellular and extracellular environments to eliminate ROS. Antioxidants work through two primary mechanisms. The first involves breaking the chain reaction by donating electrons to free radicals. The second disrupts chain-initiating catalysts, effectively neutralizing ROS and reactive nitrogen species. Antioxidants also influence biological systems by donating electrons, chelating metal ions, acting as co-antioxidants, or regulating gene expression<sup>30</sup>.

#### **CONCLUSION AND RECOMMENDATION**

In conclusion, the results highlight soursop leaves as a promising source of natural bioactive compounds, and further investigation into the optimal processing methods for maximizing their therapeutic potential is warranted. The study indicates that drying soursop leaves at 50°C for 3 to 6 hours optimally preserves vitamin C content and enhances antioxidant activity, as evidenced by decreased  $IC_{50}$  values. However, extending the drying time to 12 hours may lead to a slight reduction in these beneficial properties. Statistical analyses confirm that drying time significantly affects both vitamin C levels and antioxidant activity in soursop leaf tea powder.

Further research is needed to explore the optimal processing methods for preserving other bioactive compounds and enhancing the therapeutic potential of soursop leaves.

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