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COMPARISON OF ANTIOXIDANT ACTIVITY OF FRESH AND DRIED BANGUN-BANGUN (*Plectranthus Amboinicus* L.) LEAF DECOCTIONS USING THE DPPH METHOD BY UV–VIS SPECTROPHOTOMETRY

Sofia Eliasari Br Bangun [1] , Sulasmi [2] , Fitri Wahyu Dhini [3] , Nischayanty Hulu [4]
Deli Husada Institute of Health, Delitua Street No. 77, Deli Tua Tim., Deli Tua District, Deli Serdang Regency, North Sumatra 20355
e-mail: sofiaelia8@gmail.com, sulasmistore@gmail.com, fitriwayudini@gmail.com , nischayanty@gmail

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Abstract: Bangun-bangun (*Plectranthus amboinicus* L.) is an ethnobotanical plant traditionally used by the people of North Sumatra, especially for postpartum mothers. This plant contains secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and steroids, which have antioxidant potential. Antioxidants play an important role in neutralizing free radicals that can cause oxidative stress and degenerative diseases. This study aimed to determine the antioxidant content, IC_{50} value, and antioxidant activity of fresh and dried bangun-bangun leaf decoctions based on IC_{50} values. This research used a quantitative experimental design. Antioxidant activity was analyzed using the DPPH method with a spectrophotometer, showing a maximum DPPH wavelength of 517 nm. Phytochemical screening of bangun-bangun leaf powder confirmed the presence of alkaloids, tannins, saponins, flavonoids, and steroids. The results showed that the fresh leaf decoction had an IC_{50} value of 94.622 µg/mL, classified as strong antioxidant activity, while the dried leaf decoction had an IC_{50} value of 102.33 µg/mL, classified as moderate antioxidant activity. Vitamin C, used as a positive control, showed very strong antioxidant activity with an IC_{50} value of 10.291 µg/mL

1. INTRODUCTION

Bangun-bangun (*Plectranthus amboinicus* Lour Spreng) is one of Indonesia's native plants that has been known for a long time. Bangun-bangun leaves, commonly known among the Batak people, are one of Indonesia's ethnobotanical plants that have been used for generations by the people of North Sumatra as a daily vegetable dish and are especially served to new mothers. The origin of this plant is unknown. Its stem is round and slightly hairy, rarely flowers (purple and white), but is very easy to propagate by cuttings and can be quickly pulled out of the ground (Silitonga, 2021).

The bangun-bangun leaf plant has potential for utilization. In addition to its nutritional content, it also has pharmacoseutical properties. The pharmacoseutical benefits of the bangun-bangun leaf plant (*Plectranthus amboinicus*) include antibacterial, lubricant, dye, solvent, stabilizer, and antioxidant properties (Kaban & Yusmarlisa, 2018).

According to research conducted by Anggreini et al. (2024), bangun-bangun leaves (*Plectranthus amboinicus* L.) contain secondary metabolites such as alkaloids, tannins, saponins, flavonoids, and steroids.

Secondary metabolites in plants play an important role in providing pharmacological effects, one of which is as antioxidants. Antioxidant compounds function to ward off free radicals that can cause oxidative stress and various degenerative diseases. Several groups of compounds known to have high antioxidant activity are flavonoids, phenolics, alkaloids, and tannins. The mechanism of action of these antioxidants involves the donation of electrons or hydrogen to neutralize free radicals and enhance the body's antioxidant defense system. The

antioxidant potential of plants is attracting increasing attention in pharmacological research, particularly in the development of natural-based therapies to counteract the negative effects of oxidative stress (Arviani., 2023).

Free radicals are molecules or atoms that have one or more unpaired electrons, making them highly reactive and unstable. The presence of free radicals in the body can trigger chain reactions that damage important biological molecules such as DNA, proteins, and lipids, contributing to various degenerative diseases, including cancer, cardiovascular disorders, and premature aging. The main sources of free radicals come from environmental factors such as pollution, cigarette smoke, pesticide exposure, medication consumption, and oxidative stress due to unhealthy eating habits. To reduce the negative effects of free radicals, the body needs antioxidants, which are compounds that can counteract oxidative effects by donating electrons and stabilizing free radicals. Thus, consuming sufficient sources of antioxidants is an important strategy in preventing various diseases caused by oxidative stress (Trimadianti et al., 2022).

Previous research conducted by Kaban & Yusmarlisa (2018) tested the antioxidant activity of bangun-bangun leaves (*Plectranthus Amboinicus*) using ultraviolet-visible spectrophotometry, which found that bangun-bangun leaves (*Plectranthus Amboinicus*) contain antioxidants with a very strong IC_{50} category of <50 ppm.

This research provides a strong basis for my research entitled "Comparison of the Antioxidant Activity of Boiled Bangun-Bangun Leaves (*Plectranthus amboinicus* L.) Fresh and Dried Using the DPPH Method by UV-Vis Spectrophotometry," in which the researcher will explore and compare the antioxidant activity of fresh and dried bangun-bangun leaf decoctions in inhibiting free radicals by calculating the IC_{50} value. In general, people often consume fresh bangun-bangun leaf decoction, which is believed to increase breast milk production. Currently, there are also products made from dried bangun-bangun leaves that are sold on e-commerce platforms. Researchers want to see whether fresh and dried bangun-bangun leaf decoctions contain antioxidants and whether there are differences in antioxidant activity between fresh and dried conditions, as no research has been conducted on the antioxidant activity of fresh and dried bangun-bangun leaf decoctions. Given the importance of antioxidants in counteracting free radicals that can cause various degenerative diseases, the selection of the optimal dosage form is a crucial factor in the utilization of herbal plants, so that a scientific evaluation of its effectiveness is necessary. Thus, this study will serve as a reference in the utilization of bangun-bangun leaves as a natural source of antioxidants in functional foods or the development of pharmaceutical products.

2. RESEARCH METHODS

2.1 Tools and Ingredients

2.1.1 Tools

The tools used in this study include an analytical balance, UV-Vis spectrophotometer, vortex mixer, micropipette, aluminum foil, stirring rod, dropper pipettes, glass beakers, parchment paper, cuvette tissues, boiling pans, water baths, test tubes, test tube racks, porcelain dishes, filter paper, spatulas, funnels, Erlenmeyer flasks, measuring cups, volumetric pipettes, stopwatches, measuring flasks, hot plates, blenders, and dark glass bottles.

2.1.2 Ingredients

The ingredients used in this study include fresh and dried bangun-bangun leaves, 2N hydrochloric acid, Mayer, Bouchardat, Dragendorff, magnesium powder, concentrated HCL, potassium iodide, iodine, mercury (II) chloride, amyl alcohol, 10% FeCl3, methanol, DPPH powder (2,2-diphenyl-1-picrylhydrazyl), distilled water, and Vitamin C.

3. RESEARCH PROCEDURE

3.1 Sample Collection

The sample in this study is in the form of the Baktiraja subdistrict, Humbang Hasundutan district, North Sumatra province. The sampling method used was purposive sampling without comparing samples from other areas. ‘’’/Sampling was carried out purposively, without comparing with similar samples from other locations

3.2 Sample preparation

Fresh bangun-bangun leaves were collected, separated from stems and debris, washed

thoroughly, and then chopped into small pieces. The samples were divided into two groups: fresh leaves were used immediately, while dried leaves were dried in a drying cabinet using incandescent lamps without exposure to sunlight to prevent degradation of active compounds, then stored in airtight containers. The dried samples were blended into simplisia powder for phytochemical characterization and screening.

3.3 Simplicia Characterization Test

3.3.1 Determination of The Moisture Content

The moisture content is determined using a moisture analyzer. Five grams of the crude drug powder is weighed and spread evenly in the initial container. The container is closed and left until the device beeps. The moisture content displayed on the device is recorded. The standard moisture content must be below 10% *(Depkes RI*,1995).

3.3.2 Determination of water-soluble pollen levels

5 grams of powder is macerated for 24 hours in 100 ml of water-chloroform (2.5 ml chloroform/liter of water) in a separating funnel, shaken occasionally for the first 6 hours, then filtered. Evaporate 20 ml of the filtrate to dryness and heat the residue at 105°C until the weight remains constant. The soluble extract content is calculated based on the dry material *(Depkes RI*,1995).

3.3.3 Detemination of Soluble Essence Levels in Ethanol

Macerate 5 grams of powder for 24 hours in 100 ml of 96% ethanol in a stoppered bottle, shaking occasionally for the first 6 hours, then filter. Evaporate 20 ml of the filtrate to dryness, heat the residue at 105°C until a constant weight is reached. Calculate the soluble extract content based on dry matter *(Depkes RI*,1995).

3.3.4 Determination of Total Ash Content

A total of 2 grams of powder is placed in a porcelain crucible that has been incinerated and weighed, then slowly incinerated until the charcoal is completely burned. If charcoal remains, hot water is added, filtered, and the residue is incinerated until a constant weight is reached. The ash content is calculated based on dry matter *(Depkes RI*,1995).

3.3.5 Determination of Acid Insoluble Ash Levels

The total ash is boiled with 25 ml of dilute HCl for 5 minutes. The insoluble ash is filtered, washed with hot water, incinerated until a constant weight is obtained, and calculated as a percentage by weight of the test material *(Depkes RI*,1995).

3.4 Phytochemical Screening

3.4.1 Alkaloi d

Add 1 ml of 2 N HCl and 9 ml of distilled water to 0.5 g of crude drug powder, heat for 2 minutes in a water bath, cool, and filter. Test the filtrate with Mayer's, Bouchardat's, and Dragendorff's reagents. Alkaloids are positive if a precipitate forms in two or three experiments *(Depkes RI*,1995).

3.4.2 Flavonoi d

Add 0.1 g of Mg, 1 ml of concentrated HCl, and 2 ml of amyl alcohol to 0.5 g of powder, shake, and allow to separate. A yellowish-red or orange color in the amyl alcohol layer indicates the presence of flavonoids *(Depkes RI*,1995).

3.4.3 Saponin

Place 0.5 g of powder in a test tube, add 10 ml of distilled water, heat for 2–3 minutes, cool, and shake vigorously for 30 seconds. Foam 1–10 cm high that is stable for at least 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl indicates the presence of saponins *(Depkes RI*,1995).

3.4.4 Tannin

Add 2–3 drops of 10% FeCl3 to 0.5 g of powder. The formation of a blackish blue or blackish green color indicates the presence of tannin *(Depkes RI*,1995).

3.4.5 Terpenoid

Add 20 ml of n-hexane to 1 g of sample for 2 hours, filter, and evaporate the filtrate. Add 2 drops of anhydrous stearic acid and 1 drop of concentrated H2SO4 to the residue. The formation of a blue to green color indicates the presence of steroids *(Depkes RI*,1995).

3.5 Extraction Process

Extraction was carried out using the decoction method with water. Ten grams each of fresh and dried bangun-bangun leaves were boiled for 15 minutes with 100 ml of water at a temperature of 70°C, as this temperature is sufficient to extract bioactive compounds without damaging their components. After boiling, the solution is filtered using cloth to separate the leaf residue, producing a decoction extract with a concentration of 100,000 ppm (Trimadianti et al., 2021).

3.6 Research Procedure

3.6.2 Preparation of DPPH Stock Solution

Twenty milligrams of DPPH powder were weighed, placed in a 100-ml volumetric flask, and dissolved with methanol to the mark, resulting in a stock solution concentration of 200 µg/ml (Trimadianti et al., 2021).

3.6.3 Preparation of DPPH LIB II Solution

5 ml of DPPH stock solution was pipetted into a 25 ml volumetric flask, filled with methanol to the mark to obtain a LIB II solution with a concentration of 40 µg/ml (Trimadianti et al., 2021).

3.6.4 Determination of Maximum Wavelength

The maximum wavelength of the 40 µg/ml DPPH solution was determined by measuring the absorbance in the range of 400–800 nm using a UV-Vis spectrophotometer to find the wavelength that produced the highest absorbance (Trimadianti et al., 2021).

3.6.5 Determination of Operating Time

The absorbance of the 40 µg/ml DPPH solution was read every 5 minutes for 60 minutes to determine the time at which the absorbance began to stabilize, which was used as the operating time in the antioxidant measurement (Trimadianti et al., 2021).

3.6.6 Preparation of Vitamin C Reference Standard Solution

Weigh 50 mg of vitamin C powder and dissolve it in a 50 ml volumetric flask of methanol to achieve a concentration of 1000 µg/ml (Leo & Daulay, 2022).

3.6.7 Preparation of Vitamin C LIB II Solution

5 ml of 1000 ppm vitamin C solution was pipetted into a 50 ml volumetric flask and supplemented with methanol to obtain a concentration of 100 ppm (Leo & Daulay, 2022).

3.6.8 Preparation of Vitamin C Concentration Solutions

Vitamin C solutions at 100 ppm were prepared as lower concentration solutions by pipetting 0.75, 1.25, 1.75, 2.25, and 2.75 ml into a 25 ml volumetric flask to obtain concentrations of 3, 5, 7, 9, and 11 ppm, then mixed with methanol and homogenized (Leo & Daulay, 2022).

3.7 Preparation of Test Solution

3.7.1 Measurement of Sample Antioxidant Activity

0.2–1 ml of the sample stock solution was pipetted into a 10 ml measuring flask to obtain concentrations of 20, 40, 60, 80, and 100 ppm. then 2 ml of 40 ppm DPPH solution was added, made up with methanol, homogenized, and incubated for 30 minutes before measuring the absorbance at the maximum wavelength (Trimadianti et al., 2021).

3.7.2 Measurement of Vitamin C Antioxidant Activity

Vitamin C solutions with concentrations of 3–11 ppm were pipetted into a measuring flask, 2 ml of 40 ppm DPPH solution was added, methanol was added to make up the volume, homogenized, and incubated for 30 minutes before measuring the absorbance at the maximum wavelength as a control (Leo & Daulay, 2022).

3.7.3 Measurement of Blank Solution

A 40 ppm DPPH solution was used as a blank and its absorbance was measured at the maximum wavelength for reference (Trimadianti et al., 2021).

3.7.4 Analysis of DPPH Radical Quenching Percentage

The DPPH radical quenching percentage was calculated by comparing the absorbance of the control without sample and the absorbance of the solution after adding the sample, to assess

antioxidant activity (Susiloningrum & Mugita Sari, 2021).

3.7.5 Determination of IC_{50}

IC_{50} was calculated to determine the sample concentration that inhibited 50% of DPPH radical activity. The inhibition percentage data from various concentrations were entered into a linear regression with the sample concentration as the X-axis and the inhibition percentage as the Y = x + ab.

4. RESULTS AND DISCUSSION

4.1 Sample Identification Results

The identification of the plant was carried out at the Medanese Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), University of North Sumatra. The plant sample was identified as bangun-bangun leaves (*Plectranthus amboinicus* (Lour.) Spreng) from the family *Lamiaceae*

4.2 Results of Taking and Making Simplicia

The results of collecting bangun-bangun leaf samples taken from the Baktiraja District, Humbang Hasundutan Regency, weighing 1 kg, which had been cleaned and washed thoroughly to remove dirt and chopped. The chopped fresh leaves were immediately processed. Some of the fresh leaves were dried to obtain 500 grams of dry simplisia, and some of the dried bangun-bangun leaves were ground using a blender into simplisia powder for characterization testing and phytochemical screening.

4.3 Results of Simplicia Characterization Examination

Table 1. Results of Simplicia Characterization Examination

No.	Characterization checks	Results	Requirements (MMI, 1989)	Conclusions
1	Determination of the moisture content	5,12 %	<10%	MS
2	Determination of water-soluble pollen levels	30,09 %	>29%	MS
3	Determination of Soluble Essence Levels in Ethanol	8,88%	>5%	MS
4	Determination of total ash content	1,99%	<2%	MS
5	Determination of acid insoluble ash levels	0,92%	<1%	MS

This study used bangun-bangun leaves (*Plectranthus amboinicus* L.) obtained from Baktiraja District, Humbang Hasundutan Regency, North Sumatra, which had been identified at the Medanese Herbarium, University of North Sumatra, and belonged to the Lamiaceae family. The simplisia characterization process showed good quality and met the 1989 Indonesian Materia Medica (MMI) standards. The recorded moisture content of the simplisia was 5.12%, which is below the maximum limit of 10%, indicating that this simplisia has good storage stability. The water-soluble extract content (30.09%) and ethanol extract content (8.88%) meet the specified requirements (>29% and >5%), indicating good extraction of active compounds. Additionally, the total ash content (1.99%) and acid-insoluble ash content (0.92%) are also within acceptable limits (<2% and <1%), indicating low inorganic contamination in the sample.

4.4 Phytochemical Screening Results

Table 2. Phytochemical Screening Results

Compoud Group	Reagent	Results	Conclusion
Alkaloid	1 ml HCl 2N + 9 ml water + Mayer's reagent 2 drops, Bouchat's reagent 2 drops, Dragendr off's reagent 2 drops	1. Mayer (white to yellow) 2. Bouchart (Reddis brown precipit ate, dark brown) 3. Dragendrof f (orange precipitate)	(+)
Flavonoid	0.1 g of serbxuk Mg + 1 ml HCl + 2 ml Amyl alcohol	Forms a yellow layer on the amyl alcohol layer	(+)
Tannin	2 drops of $FeCl_3$	Forms a dark blue color	(+)

Saponin	Heat solution – shake + 2 drops of 2N HCl	Forms foam / lather	(+)
Steroid	Anhydrous acetic acid + Liberman bourchart	Blue color	(+)

Phytochemical screening of bangun-bangun leaf simplisia identified various secondary metabolites with potential biological activity. Alkaloid testing showed positive results with the formation of yellow (using Mayer's reagent), brown (Bouchardat's reagent), and brick red (Dragendorff's reagent) precipitates. The presence of flavonoids was indicated by the formation of an orange-yellow layer on the amyl alcohol layer. Saponins were identified by the formation of a stable foam 1 cm high. Tannins were detected by a greenish-black color change after the addition of $FeCl_3$, which indicated the formation of a complex with Fe3+ ions. In addition, the steroid test also gave positive results with the formation of a bluish-green color. The presence of these compounds supports the potential of bangun-bangun leaves as a source of natural antioxidants.

4.5 Maximum Wavelength Measurement Results of DPPH

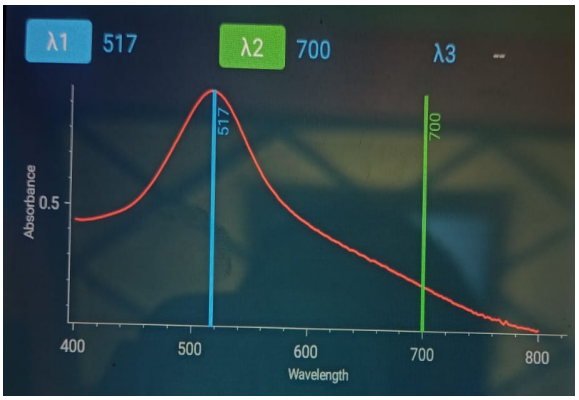

Figure 1. Data results of maximum wavelength measurement of DPPH

Antioxidant activity testing using the DPPH method requires the determination of maximum wavelength and operating time to ensure testing accuracy and efficiency. The maximum wavelength for DPPH 40 µg/ml in methanol was found to be 517 nm with an absorbance of 0.957, which is the optimal absorption point. The determination of the operating time showed that the DPPH solution reached a stable absorbance between 30 and 40 minutes, with an absorbance value of 0.943. This result is in line with the general recommendation that the effective operating time for DPPH is around 30 minutes, which ensures that the free radical scavenging reaction has reached equilibrium.

4.6 Results of Antioxidant Activity Analysis of Test Solutions

Table 3 Results of DPPH Radical Scavenging Activity Test on Fresh Bangun-Bangun Leaf Decoction

Concentration (µg/mL)	Average Absorbance	Average % Inhibition
0	0.956	0.00
20	0.853	10.74
40	0.723	24.34
60	0.637	33.40
80	0.555	41.95
100	0.463	51.60

Table 4. Results of DPPH Radical Scavenging Activity Test on Dried Bangun-Bangun Leaf Decoction

Concentration (µg/mL)	Average Absorbance	Average % Inhibition
0	0.956	0.00
20	0.804	15.93
40	0.755	21.03
60	0.650	32.01
80	0.594	37.87
100	0.488	48.95

Table 5. Results of DPPH Radical Scavenging Activity Test on Fresh Bangun-Bangun Leaf Decoction

Concentration (µg/mL)	Average Absorbance	Average % Inhibition

0	0.956	0.00
3	0.806	15.66
5	0.735	23.15
7	0.635	33.54
9	0.531	44.46
11	0.445	53.42

4.7 IC_{50} Value Analysis Results

Table 6. Results of Linear Regression Equations for Fresh and Dried Bangun-Bangun Leaf Extracts and Vitamin C Solution

Test Solution	Regression Equation	IC_{50} (µg/mL)
Fresh Bangun-bangun Leaf Decoction	Y = 0.5153x + 1.2408	94.62
Dried Bangun-bangun Leaf Decoction	Y = 0.4593x + 2.9996	102.33
Vitamin C	Y = 4.852x + 0.0674	10.29

Based on the results obtained, it can be seen that the higher the concentration, the higher the antioxidant activity, as it exceeds 50%. The percentage inhibition (% inhibition) describes the ability of antioxidant compounds in the sample to capture free radicals at the test solution concentration. Fresh bangun-bangun leaf extract has an IC_{50} value of 94.622 µg/ml, which is classified as having strong antioxidant activity, while dried bangun-bangun leaf extract has an IC_{50} value of 102.33 µg/ml, which is classified as having moderate antioxidant activity. Meanwhile, based on the antioxidant activity data, vitamin C has an IC_{50} value of 10.291 µg/ml, which is classified as a very strong antioxidant compared to the samples. The results are in accordance with the literature, which states that vitamin C is one of the most powerful antioxidant compounds and is used as a comparison in this study because it is a commercial preparation, proven to have good free radical scavenging ability, and is relatively inexpensive. According to Molyneux (2004), the IC_{50} value is inversely proportional to antioxidant activity; the higher the antioxidant activity, the lower the IC_{50} value.

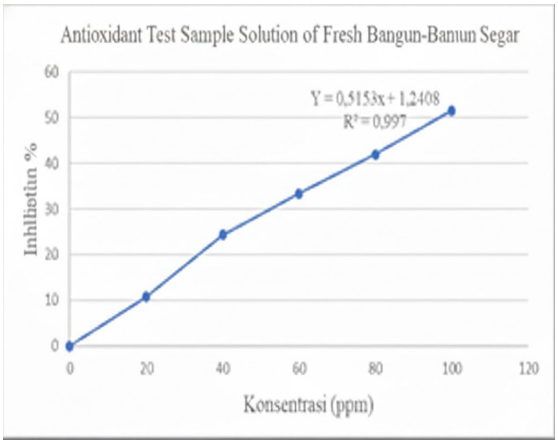

Figure 2. Antioxidant Analysis Graph of Fresh Bangun-Bangun Leaves Test Solution

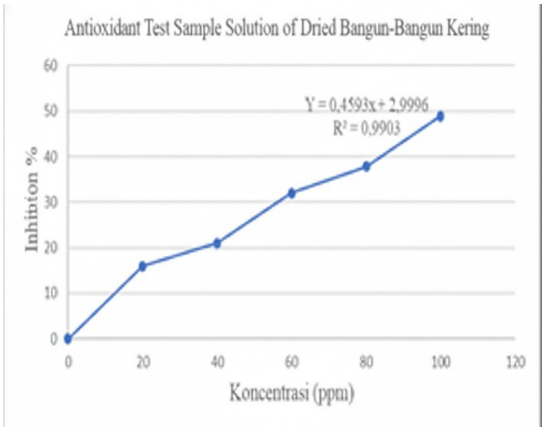

Figure 2. Antioxidant Analysis Graph of Dried Bangun-Bangun Leaves Test Solution

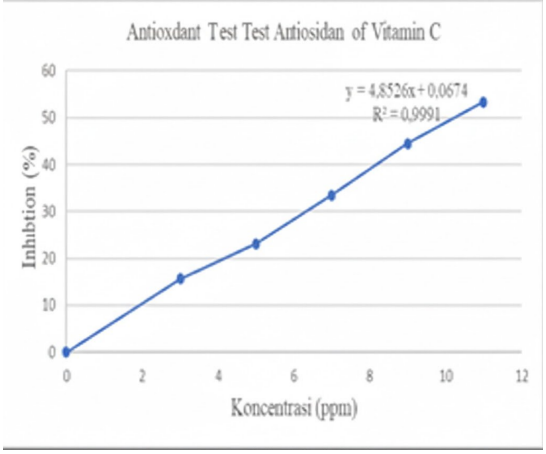

Figure 3. Antioxidant Analysis Graph of Vitamin C Test Solution

Antioxidant activity analysis shows that both fresh and dried bangun-bangun leaf decoctions have the ability to scavenge DPPH free radicals, with an increase in inhibition percentage as the concentration increases. Fresh leaf decoction has an IC_{50} value of 94.622 µg/ml, placing it in the category of antioxidants with strong activity (range 50-100 µg/ml). In contrast, dried leaf decoction has an IC_{50} value of 102.33 µg/ml, indicating moderate activity (range 101-150 µg/ml). For comparison, Vitamin C exhibits very strong antioxidant activity with an IC_{50} value of 10.291 µg/ml (<50 µg/ml), confirming Vitamin C as a highly effective antioxidant. This comparison shows that fresh bangun-bangun leaves have slightly stronger antioxidant activity than dried leaves, although both still exhibit significant antioxidant potential.

5. CONCLUSIONS AND SUGGESTION

5.1 Conclusions

1. The results of the antioxidant activity test show that both fresh and dried bangun-bangun leaf decoctions, tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, have significant antioxidant activity.
2. Based on the results of the antioxidant activity test using the DPPH method, the decoction of fresh bangun-bangun leaves showed antioxidant activity with an IC_{50} value of 94.622 µg/ml, while the decoction of dried bangun-bangun leaves showed antioxidant activity with an IC_{50} value of 102.33 µg/ml.
3. The strength of the antioxidant activity of fresh bangun-bangun leaf decoction is classified as strong (with an IC_{50} between 50-100 µg/ml), while dried bangun-bangun leaf decoction is classified as moderate (with an IC_{50} between 101-150 µg/ml).

5.2 Suggestions

Based on these findings, it is recommended that further research be conducted using other methods to measure the antioxidant activity of fresh and dried bangun-bangun leaf decoctions. The research can also be expanded to explore other parts of the plant, such as the stem or other parts.

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