

# Antioxidant Activity and Vitamin C Concentration Analysis of Gandaria (*Bouea macrophylla Griff*) Ethanol Extract Using Spectrophotometry UV Vis

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## Abstract

Antioxidants are chemical compounds that donate one or more electrons to free radicals to inhibit free radical reactions. One of the potential antioxidant sources is gandaria fruit (*Bouea Macrophylla Griff*). This research aims to determine the antioxidant activity and vitamin C content of the gandaria fruit extract. Gandaria fruit was macerated with ethanol as a solvent. Furthermore, the rotated extract was carried out for the phytochemical screening. The results of the phytochemical screening of the gandaria fruit extract showed a positive of flavonoids, terpenoids, tannins, saponins, and phenolics. The gandaria fruit extract was tested for antioxidants and quantitative analysis of vitamin C levels to obtain IC<sub>50</sub> values and vitamin C levels using spectrophotometry UV-Vis at a wavelength of 517nm with vitamin C as a positive control. The results of spectrophotometric measurements revealed that the gandaria fruit extract had an IC<sub>50</sub> value of 5.72 g/ml, and vitamin C had an IC<sub>50</sub> value of 2.260 g/ml, indicating that the gandaria fruit extract and vitamin C had very strong antioxidants. The value of vitamin C levels was 0.526 mg.

**Keywords:** antioxidants; gandaria fruit; spectrophotometry UV-Vis; vitamin C

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## INTRODUCTION

Oxidative stress is a state with a high level The territory of Indonesia has various natural potentials/assets that benefit Indonesian people's lives. However, many natural assets in Indonesia have not been fully explored and extracted optimally. Indonesia is the third largest wealth of biological resources after Brazil and Zaire. According to WHO records, only about 20,000 plant species have been used as medicinal ingredients.<sup>1</sup>

Antioxidants are compounds that can neutralize the bad effects of free radicals in the body. Based on the source, there are two kinds of antioxidants: natural and synthetic (artificial). Natural antioxidants are more desirable than synthetic antioxidants as they are considered safer or harmless. Synthetic antioxidants such as BHT (butylated hydroxytoluene) and BHA (butylated hydroxy anisole) have been questioned as they have big side effects and can cause liver damage. It makes natural antioxidants an alternative needed by today's society.<sup>2</sup>

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Since ancient times, Indonesian people have used natural resources to survive, one of them is as medicine. Indonesian people have used plants as medicines to solve health problems. The knowledge and skills of the Indonesian people to use plants as medicine has become an ancestral heritage from generation to generation that remains useful today.<sup>3</sup>

One kind of traditional medicinal plant that the medical industry has begun to look at is gandaria (*Bouea macrophylla Griff*). Gandaria is the most abundant plant in Malaysia, Sumatra, West Java, Ambon and Banten. This plant, known as exotic fruit, is generally spread on the island of Ambon. In Indonesia, this plant is called gandaria, jatake (Sunda). Some research showed gandaria (*Bouea macrophylla Griff*) has antibacterial, anticancer and antiaging activity. Gandaria fruit (*Bouea macrophylla Griff*) contains high vitamin C.<sup>1</sup> Vitamin C is a water-soluble vitamin. Insufficient consumption of vitamin C will cause some effects such as weakness, shortness of breath, muscle spasms, sore bones and joints and reduced appetite, dry, rough and itchy skin, and dry lips. Some research revealed that gandaria has several pharmacological activity such as anticancer, antiphotaging, antimicrobial, anti-hyperglycemic, and improved vegetable intake and blood beta carotene concentration. This research focuses on analyzing antioxidant activity and vitamin C concentration in gandaria fruit.

## METHOD

### Extraction

Gandaria fruit was obtained from Pandeglang, Banten, Indonesia. Fresh fruit was washed and dried. 400 g of powder was extracted in ethanol (1:7) for 3 days and macerated 3 times. The ethanol

extracts were filtered and evaporated with a rotary evaporator.

## Phytochemical Screening

### Alkaloid Test

2 ml of extract was added to Mayer and Dragendorf reagent through the wall of the test tube. The presence of a white precipitate indicated the presence of alkaloids.

### Phenolic Test

The extract was dissolved in water, then added to a lead acetate solution (10%). A white precipitate indicated the presence of phenolic compounds.

### Steroid Test

Acetic anhydride was added to 0.5 mL crude extract of plant sample with H<sub>2</sub>SO<sub>4</sub>. The color change from violet to blue or green in the sample indicated the presence of steroids.

### Flavonoid Test

The Extract was dissolved in ethanol 96%, then 0.1 g magnesium and HCl were added. The yellow or violet coloration disappeared on standing.

### Saponin Test

Approximately 3 ml of Gandaria extract was added to 3 ml of aqua dest and shaken vigorously. Stable, persistent froth formation was taken as a positive test for saponin.

### Tannin Test

The gandaria extract was added to 1% pb(CH<sub>3</sub>COOH) solution. Tannins are present a yellow precipitate formed during the reaction.

### Steroid Test

Approximately 2 ml of chloroform and H<sub>2</sub>SO<sub>4</sub> were added to 5 ml of gandaria

extract. A red layer indicated the presence of steroids in the low chloroform.

### Antioxidant Activity Analysis

The method used to determine antioxidant activity by 1,1-Di[phenyl-2-picrylhydrazyl (DPPH) and Spectroscopy Uv-Vis. A volume of 2 mL DPPH solution, freshly prepared in 96% ethanol, was mixed with 2 mL gandaria fruit extract, and the maximal absorbance was measured at 517 nm. The antioxidant activity (IC<sub>50</sub>) was analyzed using a calibration curve using  $y=ax+b$ . The value of Y has been replaced with 50. The IC<sub>50</sub> value was classified by Blois in table 1

### Determination of Vitamin C concentration

A standard 100 ppm ascorbic acid solution was prepared by dissolving 0.5 mg of ascorbic acid and diluted to 25 mL with aqua dest. Next, the solution was determined to analyze maximal absorbance at 200–400 nm. The next step was calibration curve analysis. 5 different concentration (1 ppm, 2 ppm, 4 ppm, 8 ppm and 16 ppm) was prepared and measured at 216 nm.

**Table 1. parameter of antioxidant activity based on the value of IC<sub>50</sub>**

No	Value of IC <sub>50</sub>	Antioxidant Properties
1	<50 ppm	Very strong
2	50-100 ppm	Strong
3	101-150 ppm	Moderate
4	151-200 ppm	Weak
5	>200 ppm	Very weak

## RESULTS AND DISCUSSION

Gandaria fruit was observed to determine its plant identity. This plant was determined at the biology learning laboratory of the faculty of applied science and technology Ahmad Dahlan University (UAD). The determination result showed that the sample used was *Bouea Macrophylla* griff.

Extraction is the process of separating the active compound using a certain solvent. The extraction process aims to obtain the active compounds of a sample.<sup>4</sup> The extraction results can be seen in table 2.

**Table 2. Extract Yield**

Gandaria Fruit Simplicia	Extract Weight	Yield	Extract Characteristics		
			Appearance	Color	Scent
3 kg	74.5gram	2.483%	Thick	Brown	Unique

Table 2 shows that the Simplicia weight of gandaria fruit (*Bouea macrophylla* griff) is 3 kg with a total solvent is 3.5 liters. After being concentrated with a rotary evaporator, the gandaria extract is thick and brown in color, weighing 74.5 grams with a yield of 2.483%.

The results of the phytochemical screening test aim to identify the content of secondary metabolites that can potentially have antioxidant activity. The results of the phytochemical screening carried out can be seen in Table 3.

**Table 3. Gandaria fruit Phytochemical Screening Results (*Bouea macrophylla Griff*)**

Examination	Reactant	Observation	Results
Alkaloid	Sample+ammonia+chloroform+mayer	No white sediment	-
Flavonoid	Sample + alcohol + hydrochloric acid	The color changes to orange	+
Tanin	Sample + FeCl <sub>3</sub>	Color change	+
Steroid& terpenoid	Sample + H <sub>2</sub> SO <sub>4</sub> + acetic anhydrous	The color changes to dark red	+
Saponin	Sample + aqua dest	Foam doesn't disappear	+

Description:

(+) Contains the test compound

(-) Does not contain the test compound

Based on table 3, the results of the phytochemical screening conducted, it is known that the extract of gandaria fruit (*Bouea macrophylla Griff*) contained secondary metabolites, including alkaloids, flavonoids, tannins, triterpenoids, and saponins,<sup>5</sup> which states that gandaria fruit contained secondary metabolites including flavonoids, triterpenoids, and saponins. We used the metal reagent Mg and concentrated HCl for the flavonoid test, reducing the benzopyran core in the flavonoid structure and forming an orange flavilium salt. The results of the flavonoid sample test and the *Bouea macrophylla griff* extract did not contain flavonoids (negative) by showing a red-orange color change in the extracted sample. The reaction that occurs is between flavonoid compounds with HCl and Mg metal.

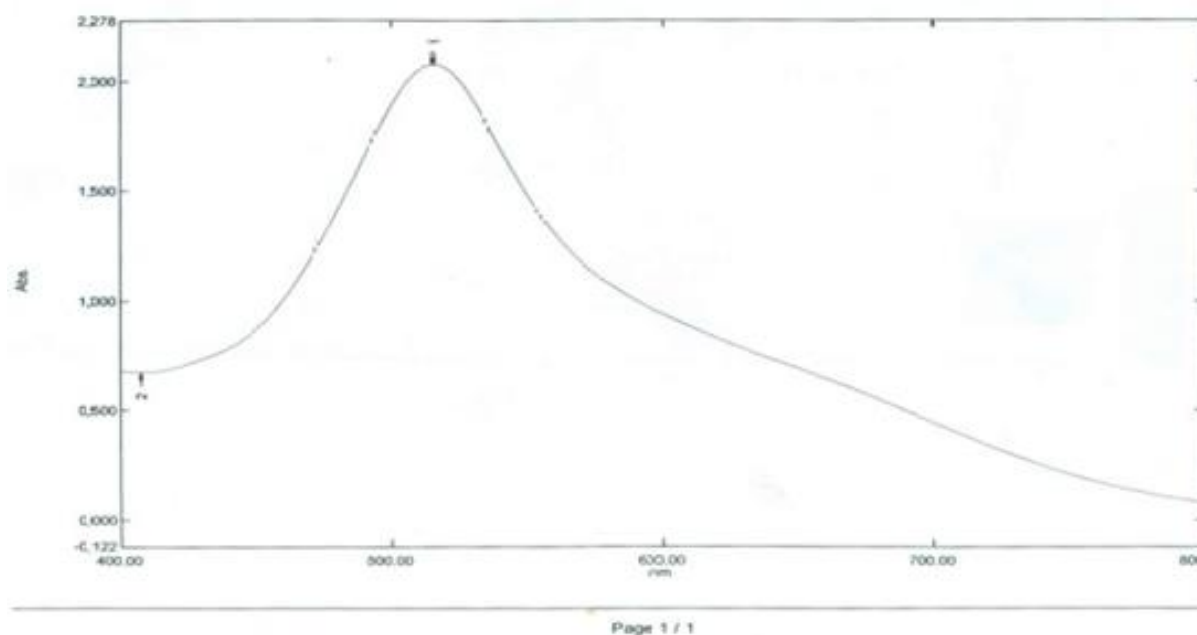
Tannins are chemical compounds of the phenol group soluble in water and polar solvents. Testing for the existence of tannins in the ethanol extract was carried out with a FeCl<sub>3</sub> reagent. After the addition of FeCl<sub>3</sub>, the color of the solution became blackish-green. The formation of a green-black color was caused by tannins

forming a complex with Fe<sub>3</sub><sup>+</sup>. Catechol tannins were green-black after adding FeCl<sub>3</sub>.<sup>5</sup>

The results of the tannin test on the *Bouea macrophylla griff* extract did not contain tannins (negative) by showing a blackish-green color change so that the type of tannin contained in the *Bouea macrophylla griff* extract was catechol tannin.

For the saponin glycosides test, it can form foam in water when stirred. The saponin test was carried out based on the Forth test by adding distilled water to the ethanol extract and then shaking it with Vortex. If the *Bouea macrophylla griff* extract contains saponins, then after shaking, it will form a foam.<sup>5</sup> The *Bouea extract macrophylla griff* test results revealed that it did not contain saponins, showing the presence of foam after shaking.

In this study, the antioxidant activity of the extract of Gandaria fruit *Bouea macrophilia Griff* was tested using the DPPH method quantitatively using a UV-Vis spectrophotometer at a wavelength of 517 nm.



Picture 3. Absorbance curve Wavelength DPPH

The purpose of determining the maximum wavelength is to determine the absorption area that can be produced in the form of the absorbance value of the gandarua fruit extract dissolved in ethanol and then measure the wavelength of 200-600 nm.

Testing of antioxidant activity was carried out using the DPPH method. The DPPH method is a method that can be used to determine antioxidant activity in the sample to be tested by looking at its ability to ward off DPPH free radicals. This method was chosen as it was a simple, easy, fast, and sensitive method and only required a small sample. It is easy to apply

as the DPPH radical compound is relatively stable compared to other methods.<sup>6</sup>

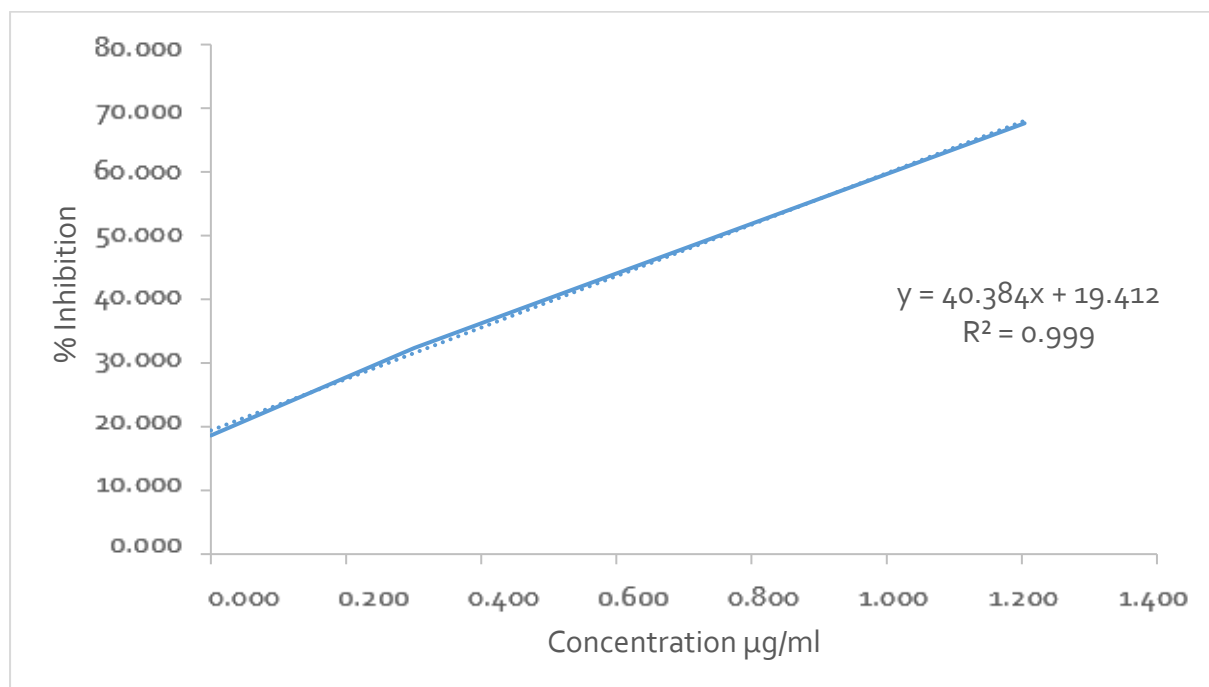
The absorbance measurement in this study was carried out at a maximum wavelength of 517 nm DPPH solution with an absorbance of (2,000). The wavelength is in accordance with the maximum wavelength range that can be used for measurements with the DPPH method stated by Londo,<sup>5</sup> namely, from 517 nm to 522 nm, which can be seen in Table 4.

Table 4. Gandaria fruit *Bouea macrophilia Griff* antioxidant test results

Concentration (ppm)	Average of Absorbance (A) = 517 nm Inhibitory	Activity (%) Linear	linear regression equation	IC <sub>50</sub> value (µg/ml)
1	0.012	18.627	y = 40.384x + 19.412	5.72
2	0.016	32.353		
4	0.020	44.118		
8	0.024	55.882		
16	0.073	67.647		
Blanko	0.035			

The antioxidant activity of the fruit of gandaria *Bouea macrophylla Griff* was measured at 5 dilution concentrations. Analysis of antioxidant activity was carried out three times. It can be seen from the data in table 4 that all sample concentrations showed a reduced

absorbance value, and % DPPH radical inhibition (% inhibition) increased with increasing concentration. It indicated a reduction in the concentration of DPPH after being reacted with the sample, which can be seen in Figure 4.



**Figure 4. Linear Regression Curve of Gandaria Fruit Extract (*Bouea macrophylla Griff*)**

The regression equation between the concentration of the extract of gandaria fruit (*Bouea macrophylla Griff*) on the x-axis and the percent (%) of antioxidant activity on the y-axis is  $y =$

$40,384x+19,412$  with a correlation coefficient value of 0.999. The positive correlation coefficient value illustrated that increasing the concentration of the extract of gandaria fruit (*Bouea macrophylla Griff*) also raises the antioxidant activity.

The parameter used to show antioxidant activity is *Inhibition Concentration* (IC<sub>50</sub>) which is the concentration of an antioxidant substance that can cause 50% of DPPH to lose its radical character or the concentration of an antioxidant substance

that gives 50% inhibition. The smaller the IC<sub>50</sub> value is, the higher the antioxidant activity will be. The IC<sub>50</sub> value of the sample extract was obtained based on the calculation of the linear regression equation from the absorbance curve of the sample.

In this research, the antioxidant test utilized a comparison of vitamin C since the compounds contained in vitamin C could reduce or ward off free radicals, which were very good and are widely used by research as a comparison to other compounds, especially in antioxidants of gandaria fruit. The measurement of the antioxidant activity of vitamin C is shown in Table 5.

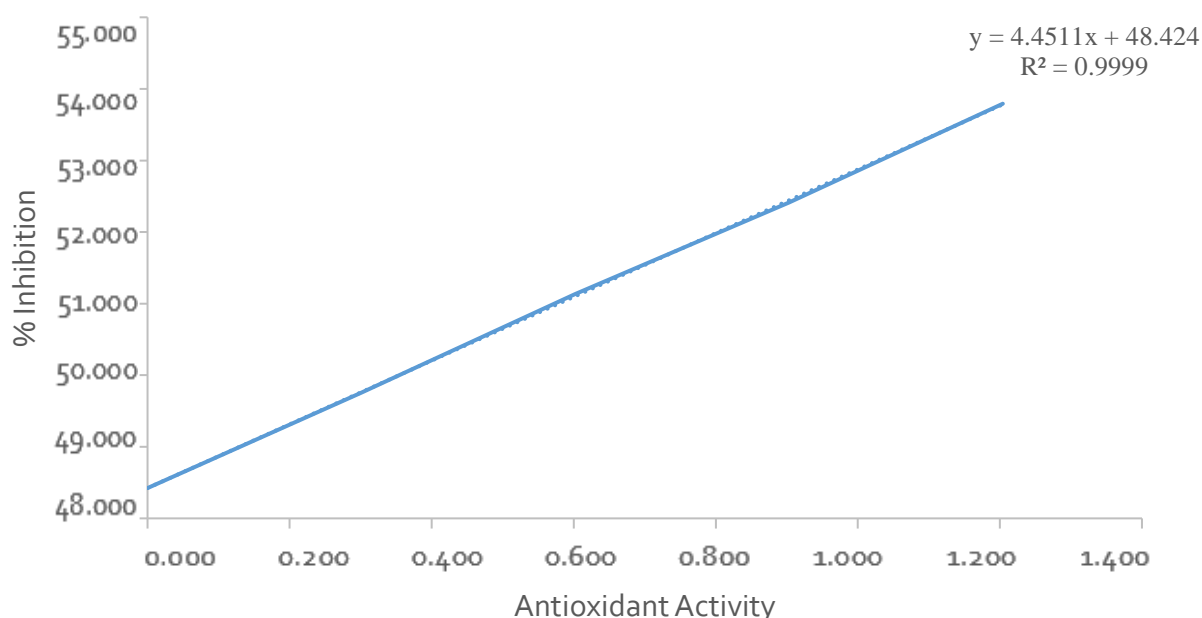
**Table 5. Vitamin C antioxidant test results**

Concentration (ppm)	Average of Absorbance (A) = 50nm Inhibitory	Activity (%)	Linear regression equation	IC <sub>50</sub> value (µg/ml)
1	0.329	48.424	$y = 4.4511x + 48.424$	2.260
2	0.338	49.754		
4	0.347	51.133		
8	0.356	52.414		
16	0.365	53.793		
Blanko	0.678			

In measuring the antioxidant activity of vitamin C, the largest percentage at a concentration of 1 ppm revealed a free radical inhibition value of 48.424%. In comparison, the largest percentage at a concentration of 16 ppm showed a free radical inhibition value of 53.793%, so it can be seen that vitamin C had a strong activity from the large value—free radical inhibition from the smallest concentration to the highest concentration. Analysis of

antioxidant activity was carried out three times.

The data from the antioxidant activity test results were then associated with the concentration of vitamin C on the absorbance curve so that a linear equation was obtained to achieve the IC<sub>50</sub> value. The absorbance curve of vitamin C can be seen in Figure 5.

**Figure 5. Linear Regression Curve of vitamin C**

Gandaria fruit *Bouea macrophylla griff* contains secondary metabolites, namely phenol. Antioxidant activity in gandaria fruit is due to the presence of phenol components. In the human body, phenolic compounds can bind to active oxygen. In this case, these phenolic compounds act as antioxidants and prevent the effects of active oxygen, which can damage biological components such as proteins, lipids, vitamins and DNA.

A compound is classified as a very strong antioxidant if its IC<sub>50</sub> value is less than 50 g/ml, strong if its IC<sub>50</sub> value is between 50-100 g/ml, moderate if its IC<sub>50</sub> value is between 100-150 g/ml, and weak if the IC<sub>50</sub> value is between 150-200 g/ml. The IC<sub>50</sub> value of Gandaria fruit *Bouea macrophylla griff* is 5.72 µg/ml, which is classified as a strong antioxidant agent. However, vitamin C's antioxidant activity

(IC<sub>50</sub> value 2.260 µg/ml) has more potency than Gandaria fruit *Bouea macrophylla griff*. Gandaria fruit *Bouea macrophylla griff* is an extract with several components in small concentrations. At the same time, vitamin c is an isolated compound and single component with high concentration. Determination of vitamin C concentration in the juice of Gandaria fruit flesh was carried out using the UV Vis spectrophotometry method. The linearity of vitamin C compounds was determined by making a standard series of 5 concentrations in the range of 0.25 ppm to 4 ppm at a wavelength of 265 nm. The following is a table and graph of the linear equation of standard vitamin C with distilled water at a wavelength of 265 nm for low concentrations (1 ppm to 16 ppm), as shown in Table 6.

**Table 6. Absorbance Analysis of Vitamin C Standard Curve**

Concentration (ppm)	Average of Absorbance	Linear Regression Equation
1	0.042	y= 0.0122+ 0.0577x R <sup>2</sup> = 0.9326
2	0.085	
4	0.128	
8	0.171	
16	0.242	

Based on the data above, the absorbance value was in the allowable linear range, namely  $y = 0.0122 + 0.0577x$ , with a correlation coefficient of  $R^2 = 0.9326$ , indicating the linearity of the equation. In determining vitamin C levels, the results obtained were 0.526 mg/g. The value of vitamin C content of gandaria fruit indicated the measurement's accuracy level. Accuracy is a measure that shows the degree of correspondence between individual test results and the average; if the procedure is repeated, the value used is 2%.<sup>7</sup>

## CONCLUSION

This study revealed phytochemical compounds of gandaria (*Bouea Macrophylla griff*) were flavonoid, tannin, steroid, terpenoid, and saponin. The IC<sub>50</sub> value of the extract of gandaria (*Bouea Macrophylla griff*) was 5.720 µg/ml, classified as a strong antioxidant agent. The vitamin C concentration of gandaria (*Bouea Macrophylla griff*) was 0.526 mg/ml.



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