

Antioxidant Capacity, Toxicity, Phytochemicals, Total Alkaloids and Phenolic Levels Bamboo Shoot Extract (*Bambusa vulgaris*)**Jurian Putra**

Universitas Tarumanagara, Jakarta, indonesia

jurianp93@gmail.com

KeywordsROS, Oxidative Stress, *Bambusa vulgaris*, Herb, phytochemicals, DPPH, BSLT.**Abstract**

The imbalance between reactive oxygen species (ROS) and endogenous antioxidants can cause various diseases, so exogenous antioxidants from plants such as bamboo shoots (*Bambusa vulgaris*) are needed. This study explored the antioxidant capacity, toxicity, and phytochemical content of bamboo shoot extracts. Using an in vitro experimental design, we analyzed bamboo shoot extracts for phytochemical content, antioxidant capacity using the DPPH method, and toxicity using the Brine Shrimp Lethality Test (BSLT). The research was conducted from December 2020 to June 2021 at the Biochemistry and Molecular Biology Laboratory, Tarumanagara University. Data were analyzed using Graph Pad Prism V.8 software with the level of significance set at $p < 0.05$. Phytochemical test results showed the presence of alkaloids, anthocyanins, betasianins, cardiac glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, terpenoids, and tannins. The bamboo shoot extract showed antioxidant capacity with IC₅₀ value of 673.54 µg/mL, total phenolic content of 2923.65 µg/mL, and total alkaloid content of 7.34 µg/mL. The toxicity test showed an LC₅₀ value of 362.16 µg/mL. The bamboo shoot extract showed potential as an antioxidant and anti-mitotic agent, although its antioxidant capacity was lower than that of ascorbic acid. The presence of various secondary metabolites contributes to its bioactive properties. Further research is needed to explore its potential applications in preventing diseases caused by free radicals. Bamboo shoot extract contains significant amounts of phenolic compounds and other phytochemicals, which exhibit antioxidant and toxic properties, making it a potential natural source for antioxidant agents.

Corresponding Author : Jurian Putra

E-mail: jurianp93@gmail.com**INTRODUCTION**

Healthy food is important to maintain a healthy body. Lack of public education about healthy eating patterns, Indonesian people often consume less healthy food (Sari, Widanti, Yani, Jehanara, & Prasetyo, 2023). A report from Riskesdas 2018 shows that 95.4% of the Indonesian population aged >5 years consumes less than 5 servings of vegetables and fruit per day a week. Consumption of foods and drinks high in sugar, high in salt, high in fat/cholesterol/fried foods is still high in Indonesia (Waris et al., 2018). Food that is processed by frying, baking, grilling, using preservatives and coloring substances can trigger free radicals. To prevent diseases that arise due to free radicals, antioxidants are needed (Singh, Singh, Singh, & Singh, 2019). Antioxidants can be obtained from plants which contain phenolic compounds such as flavonoids, carotenoids and there are also vitamin C, vitamin E, vitamin B, and others (Gulcin, 2020).

Antioxidants from plants are richer in nutritional content compared to commercially available supplements and natural plants do not contain additional chemicals that can damage the body (Zhang, Lin, & Ye, 2018). The nutritional contents most needed from plants to carry out body functions are vitamins, minerals and phytochemical compounds, namely

antioxidants (Tabiri, Agbenorhevi, Wireko-Manu, & Ompouma, 2016). One plant that is high in antioxidants is bamboo shoots or bamboo shoots. Bamboo shoots or bamboo shoots that are commonly consumed by Indonesian people are the types are Wulung bamboo (*Gigantochloa atroviolacia*), Betung bamboo (*Dendrocalamus asper* Backer), and Ampel bamboo (*Bambusa vulgaris*). Ample bamboo has high total phenolic bioactive compounds (Singhal, 2016). High total phenolic will influence the activity of antioxidants to be stronger (Iwansyah, Kumalasari, Darmajana, & Ratnawati, 2019).

Bamboo shoots are plants that originate from the provinces of Indochina and Yunan in South China, and are now widely cultivated in Southeast Asia (Akinlabi, Anane-Fenin, & Akwada, 2017). Apart from bamboo shoots, the leaves and stems are also rich in nutrients. However, there are still many people who don't know the benefits of bamboo shoots. In research conducted by Fitri et al in 2020, Ampel bamboo leaves contain carbohydrates, glycosides, saponins, alkaloids, flavonoids, phenolic and tannins , phytosterols and triterpenoids, fats (Fitri, Asra, & Rivai, 2020).

The content of these active compounds provides benefits for the body, namely lowering blood cholesterol levels, medicine for jaundice, (Nuari, Qowwiyah, & Eksyawati, 2019) antihyperuricemia, (Alen, Agresa, & Yuliandra, 2017) anti-microbial and anti-fungal, anti-inflammatory, protects nerves.

Indonesian people use bamboo shoots only as a vegetable for cooking. With the many nutritional contents in bamboo shoots which are beneficial for the body, bamboo shoots can be used to prevent diseases caused by free radicals in Indonesian society. However, until now scientific data regarding the bioactive compounds and antioxidant capacity of bamboo shoot extracts is still lacking in Indonesia.

This research aims to determine the antioxidant capacity and toxicity of bamboo shoot extract using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) and BSLT (Brine *Shrimp Lethality Test*) free radical capture methods. This research was completed with phytochemical tests and measurements of total alkaloid and total phenolic levels of bamboo shoot methanol extract.

RESEARCH METHODS

Research on phytochemical tests, antioxidant capacity, toxicity, alkaloid and total phenolic content of bamboo shoot extract (*Bambusa vulgaris*) using an experimental research design *in vitro* and *bioassays*. This research was carried out at the Laboratory of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University, Jalan Letjen S.Parman No 1 Jakarta 11440. The preparatory stage of this research was carried out in December 2020. Extraction process, phytochemical analysis, measurement of total phenolic and total alkaloids, antioxidant capacity test and toxicity tests were carried out from January 2021 to May 2021 and the preparation of the thesis was carried out from December 2020 to June 2021. This research used samples of bamboo shoots (*Bambusa vulgaris*) obtained from the Bogor Regency area, West Java. The variables that will be used in this research include several types, namely dependent variables, independent variables and control variables. The dependent variables in this study include antioxidant capacity, toxicity, and secondary metabolite levels of bamboo shoots. The independent variable used was the concentration of bamboo shoot methanol extract. Meanwhile, the control variables consist of methanol, temperature and maceration time.

The research equipment used in this study includes standard laboratory equipment such as a rotary evaporator, Genesys 30-Vis spectrophotometer, aluminium foil, maceration tube, analytical balance, test tube, vortex, micropipette, UV-light, water bath. , aerator, and incandescent lamps. The research materials used consisted of bamboo shoot extract (*Bambusa vulgaris*), distilled water, methanol solution, 1N NaOH, 2N NaOH, 5% FeCl₃, potassium iodine, chloroform, 2N H₂SO₄, Mayer's reagent, tannin standard solution, Follin Ciocalteu reagent, DPPH powder , 10% ammonium solution, *Artemia salina* shrimp eggs, and sea water. Data collection was obtained from laboratory test results which included phytochemical screening, measurement of total phenolic and total alkaloid levels, antioxidant capacity tests, and toxicity tests. Antioxidant capacity tests were carried out by

obtaining absorbance using a Genesys 30-Vis spectrophotometer, while phytochemical screening, total phenolic and total alkaloid tests, and toxicity tests were carried out by recording and collecting data in the form of reports. The data analysis method used in this research is the interpretation of data results presented in the form of tables, pictures and graphs. The software used for analysis is Graph Pad Prism V.8. All tests are based on a significance value of $p < 0.05$ through the Mann-Whitney comparative test and correlation test using the Pearson test and linear regression equation.

RESULTS AND DISCUSSION

Extraction results of bamboo shoots

1650 grams of bamboo shoots and 116 grams of simplicia and 35.62 grams of thick extract.

$$\begin{aligned} \text{Dry Weight Yield} &= \frac{\text{Jumlah berat ekstrak berupa pasta}}{\text{Jumlah berat kering}} \times 100\% \\ &= \frac{35,62}{116} \times 100\% = 30.71\% \end{aligned}$$

$$\begin{aligned} \text{Wet Weight Yield} &= \frac{\text{Jumlah berat ekstrak berupa pasta}}{\text{jumlah berat basah}} \times 100\% \\ &= \frac{35,62}{1650} \times 100\% = 2.16\% \end{aligned}$$

Phytochemical screening

The phytochemical tests for bamboo shoot extract were alkaloids, anthocyanins and betacyanins, cardio glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, steroids, terpenoids and tannins (Table 1).

Table 1 Phytochemical Content

Phytochemicals	Bamboo Shoot Extract	Reagent
Alkaloids	+	Mayer, Wagner
Anthocyanins and betacyanins	+	sodium hydroxide (NaOH)
Cardioglycosida	+	Legal tests
Coumarin	+	sodium hydroxide (NaOH)
Flavonoids	+	Alkaline
Glycosides	+	Borntrager
Phenolic	+	Folin Ciocalteu
Quinones	+	concentrated sulfuric acid (H ₂ SO ₄)
Saponins	+	Foam test
Steroids	-	Liebermann Burchard
Terpenoids	+	Copper acetate
Tannin	+	Gelatin test

Antioxidant Capacity Using the DPPH Method (*1,1-diphenyl-2-picrylhydrazil*)

1. Determination of Control Wavelength and Absorbance

The optimal absorption wavelength and absorbance of bamboo shoot extract obtained with a *Genesys 30-Vis spectrophotometer* are 516 nm and 0.62.

2. Bamboo Shoot Extract Test

For each different concentration of bamboo shoot extract (400 µg/mL , 500 µg/mL , 600 µg/mL , 700 µg/mL , 800 µg/mL) a *Genesys 30-Vis spectrophotometer* was used to determine the absorbance value and % inhibition (Table 2) where the X axis is the concentration of bamboo shoot extract and Y axis is % inhibition. Then, to find the IC₅₀ value , a linear equation curve for the DPPH test was created (Figure 1). In this research it was found that $Y = 0.075X - 0.5161$ and the value of $R^2 = 0.9674$. The calculation results show

that the IC₅₀ value of bamboo shoot extract is 673.548 µg/mL and has weak antioxidant properties.

Table 2 Concentration Data, % Inhibition and IC₅₀ Bamboo Shoot Extract

Bamboo Shoot Extract Concentration (µg/mL)	Inhibition Percentage (%)	IC ₅₀ (µg/mL)
400	26,935	673,548
500	39,516	
600	46,452	
700	50,645	
800	58,871	

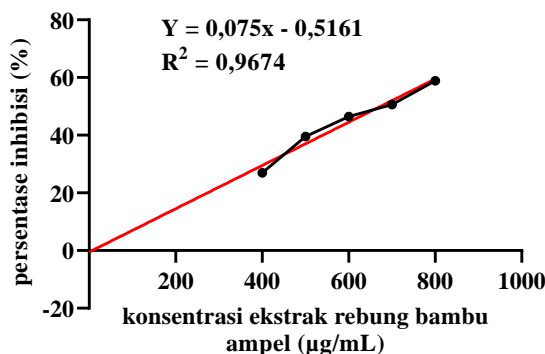


Figure 1 DPPH Test Curve for Bamboo Shoot Extract

3. Vitamin C Comparative Antioxidant Test

A *Genesys 30-Vis* spectrophotometer was used to measure the absorbance of each vitamin C concentration (2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL) and % inhibition (Table 3). Through these measurements, a standard curve for the linear equation of vitamin C was obtained (Figure 2). In this study the linear equation obtained is $Y = 6.934X + 12.52$ and $R^2 = 0.9988$, the X axis is vitamin C concentration and the Y axis is % inhibition. Then from these results it can be concluded that the IC₅₀ standard for vitamin C is 5.4 µg/mL.

Table 3 Concentration Data, % Inhibition and IC₅₀ Vitamin C

Vitamin C Concentration (µg/mL)	Inhibition Percentage (%)	IC ₅₀ (µg/mL)
2	26,85	5,4
4	39,11	
6	54,97	
8	67,87	
10	81,81	

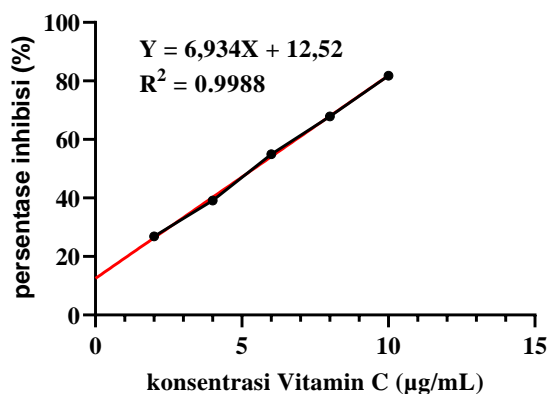


Figure 2 Vitamin C Standard Curve

Measurement of Total Phenolic Content

1. Tannin Standard Curve

Standard absorbance measurements for each tannin at different concentrations (300 µg/mL , 400 µg/mL , 500 µg/mL , 600 µg/mL , 700 µg/mL , 800 µg/mL) with a *Genesys 30-Vis spectrophotometer* to obtain absorbance at a wavelength of 765 nm. The tannin standard curve on the X axis is tannin concentration and the Y axis is absorbance (Figure 3). Then we get the results from creating a linear equation curve $Y = 0.0011X - 0.0311$ and $R^2 = 0.997$.

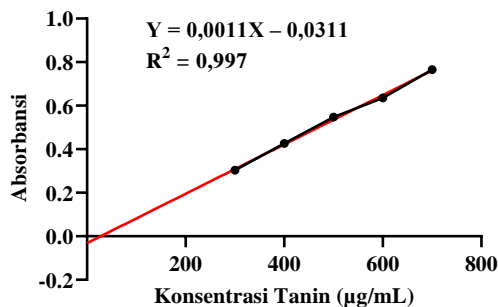


Figure 3 Tannin Standard Curve

2. Measurement of Total Phenolic Content of Bamboo Shoot Extract

To obtain the phenolic content of bamboo shoot extract, it is calculated using the linear equation $Y = 0.0011X - 0.0311$. The X axis is phenolic content and the Y axis is absorbance. In this study, absorbance was obtained using a 1:9 dilution and the phenolic content of bamboo shoot extract was 2923.6 µg/mL (Table 4).

Table 4 Absorbance Value and Phenolic Content of Bamboo Shoot Extract

Repetition	Absorbance	Phenolic Content (µg/mL)	Average Concentration (µg/mL)	Phenolic Content After dilution 10 times (µg/mL)
I	0.28	282.82	292.36	2923.6
II	0.301	301.91		

Measurement of Total Alkaloid Levels

1. Berberine Chlorid Standard Curve

Measurement of the standard absorbance and concentration of *berberine chloride* (2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL) with a *Genesys 30-Vis spectrophotometer* to obtain a linear equation at a wavelength of 415 nm. The standard curve for *berberine chloride* on the X axis is concentration and the Y axis is absorbance (Figure 4). Then we get the linear equation $Y = 0.0915X - 0.097$ and $R^2 = 0.9847$.

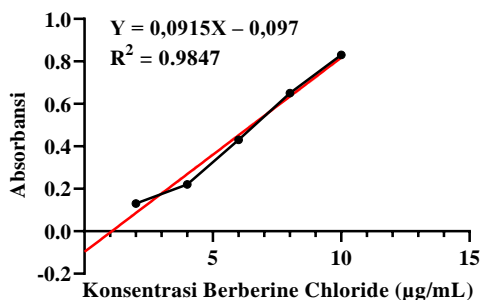


Figure 4 Berberine Chloride Standard Curve

2. Measurement of Total Alkaloid Content of Bamboo Shoot Extract

To obtain the alkaloid content of bamboo shoot extract, it was calculated using the linear equation $Y = 0.0915X - 0.097$ and $R^2 = 0.9847$. The X axis is alkaloid content and the Y axis is absorbance. In this study, the absorbance was obtained with a 1:1 dilution and the alkaloid content of bamboo shoot extract was 7.34 $\mu\text{g/mL}$ (Table 5).

Table 5 Absorbance Value and Alkaloid Content of Bamboo Shoot Extract

Repetition	Absorbance	Alkaloid Content ($\mu\text{g/mL}$)	Average Concentration ($\mu\text{g/mL}$)	Alkaloid content 2 times dilution ($\mu\text{g/mL}$)
I	0.224	3.51	3.67	7.34
II	0.254	3.84		

Toxicity Using the BSLT Method (*Brine Shrimp Lethality Test*)

Bamboo shoot extract was tested on *Artemia salina* larvae at different concentrations (50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, 800 $\mu\text{g/mL}$) to obtain the percentage of death of *Artemia salina* larvae (Table 6). Then the percentage of death of *Artemia salina* larvae was obtained for each concentration of bamboo shoot extract. The X axis is log concentration and the Y axis is % mortality (Figure 5). From the curve results, we get the linear equation $Y = 65.825X - 118.44$ and $R^2 = 0.9558$. Then the results obtained from the LC_{50} calculation were 362.165 $\mu\text{g/mL}$ which had toxic properties.

Table 6 Death of *A.salina* Larvae for Each Concentration

Bamboo Shoot Extract Concentration ($\mu\text{g/mL}$)	Concentration Log	Death Percentage (%)	LC_{50} ($\mu\text{g/mL}$)
50	1.7	1,449	362,165
100	2	7,547	
200	2,3	25,581	
400	2.6	52.5	
800	2.9	78,049	

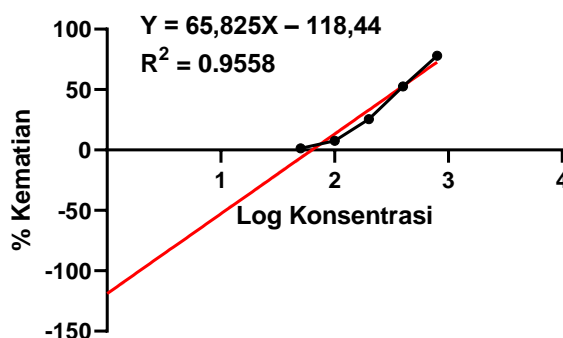


Figure 5 Toxicity Test Curve

Discussion

Phytochemistry and Extraction

The percentage of the weight of the extract produced in the form of a paste to the weight of the sample is called the yield. The resulting data obtained is important information and can be used for further research in procuring research materials. Bamboo shoot extract produced a yield of 30.71% by dry weight and 2.16 % by wet weight. The results of phytochemical tests on bamboo shoot extracts showed that they contained secondary metabolite compounds, namely alkaloids, anthocyanins and betacyanins, cardio glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, terpenoids and tannins (Table 1). The results of this phytochemical test study are in accordance with those conducted by previous researchers in detecting the content of secondary metabolite compounds, except for anthocyanin and betasianin secondary metabolite compounds, there have been no previous research reports on ampel bamboo. This research did not find any secondary metabolite compounds in the steroid class, this is in accordance with research conducted by researchers did not detect secondary metabolite compounds from the steroid group. Therefore, from the results of this research, bamboo shoots contain metabolite compounds and are effective as antioxidants because they have high phenolic levels.

Antioxidant Capacity Using the DPPH Method (*1,1-diphenyl-2-picrylhydrazil*)

The DPPH test results were calculated by calculating the IC₅₀ of bamboo shoot extract and ascorbic acid and then compared to assess the antioxidant capacity. The IC₅₀ value of bamboo shoot extract is 673.548 µg/mL and ascorbic acid is 5.4 µg/mL. According to research conducted by Iwansyah. et al bamboo shoot extract has an IC₅₀ value of 347.48 µg/mL, according to research conducted by researchers it was found that the IC₅₀ of ascorbic acid was 8.4 µg/mL. The results of this study show that the antioxidant capacity of bamboo shoot extract is low compared to ascorbic acid, requiring a lower concentration to inhibit 50% of DPPH free radicals. According to researchers excessive use of vitamin C can cause diarrhea while bamboo shoot extract does not .

Total Phenolic Content

Measurement of the total phenolic content of bamboo shoot extract was carried out using tannin standards. This research found that the phenolic content of bamboo shoot extract was 2923.6 µg/mL. Research conducted by Iwansyah. et al bamboo shoot extract has a phenolic content of 26.96 mg GEA/g. According to researchers phenolic is a secondary metabolite compound that has the capacity antioxidant by adding phenolic hydrogen atoms to free radicals so that they become more stable and autooxidation does not occur (Losada-Barreiro & Bravo-Diaz, 2017). It can be concluded that bamboo shoot extract has capacity good antioxidant.

Total Alkaloid Content

Measurement of the total alkaloid content of bamboo shoot extract was carried out using the *berberine chloride standard* (Milani et al., 2020). Determination of the alkaloid content of bamboo shoot extract was found to be 7.34 µg/mL. Research conducted by Adebayo. et al ⁴⁵ bamboo shoot extract had an alkaloid content of 3.52 mg AE/g sample. According to researchers alkaloids may provide protective effects against hydrogen peroxide-induced oxidative damage.

Toxicity Using the BSLT Method (*Brine Shrimp Lethality Test*)

Test the toxicity of bamboo shoot extract using the *Brine Shrimp Lethality Test method* . The results of the toxicity test for bamboo shoot extract showed that the LC₅₀ was 362.165 µg/mL with toxic properties. These results are in accordance with research conducted by Iwansyah. et al found that the LC₅₀ of bamboo shoot extract was 773.29 ppm (Yadav, Patel, Debbarma, Priyadarshini, & Priyadarshi, 2024). According to researchers samples were said to be toxic with an LC₅₀ value <1000 ppm and showed high levels of phenolic compounds and cyanogenic glycosides, so from the research results bamboo shoot extract was toxic.

CONCLUSION

Based on the results and discussion of the research above, it can be concluded that phytochemical screening of bamboo shoot extract shows that it contains alkaloids, anthocyanins and betacyanins, cardio glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, terpenoids and tannins. Bamboo shoot extract also has an antioxidant capacity of 673,548 $\mu\text{g/mL}$ with weak properties. Apart from that, this extract has an LC_{50} of 362.165 $\mu\text{g/mL}$ which is toxic, so it has the potential to be an antimetabolic agent. The phenolic content contained in bamboo shoot extract was 2923.6 $\mu\text{g/mL}$, while the alkaloid content contained was 7.34 $\mu\text{g/mL}$.

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