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Phytochemical Screening of Ater Bamboo Shoot Extract (Gigantochloa atter Kurz) with Different Solvents and Their Effect on Antioxidant Activity Using the DPPH Method

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Abstract

An imbalance of free radicals in the body can cause a health condition known as oxidative stress. Oxidative stress is a condition in which free radicals and antioxidants in the body are out of balance. Oxidative stress can be overcome with natural antioxidants. This study aims to determine the antioxidant activity indicated by the IC50 value of the extract of bamboo ater shoots (Gigantochloa atter Kurz) extracted with different solvent polarities. The extraction process was carried out by the maceration method using 96% alcohol solvent, n-Hexane, and ethyl acetate. Each extract was then subjected to phytochemical screening tests, including flavonoids, steroids, alkaloids, tannins, saponins, and terpenoids. Analysis of antioxidant activity in this study was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The results of phytochemical screening showed significant differences among each solvent, where the 96% alcohol solvent showed the most complete phytochemical screening results. Based on the results of the study, it can be concluded that bamboo ater shoots have antioxidant activity, and solvent polarities affect the IC50 value shown. The 96% alcohol extract of bamboo ater shoots has antioxidant activity with an IC50 value of 39.22 µg/mL, the extract of bamboo ater shoots with ethyl acetate solvent is 56.19 µg/mL, and the extract of bamboo ater shoots with n-Hexane solvent is 69.77 µg/mL.

Keywords: *Ater Bamboo Shoots, Phytochemical Screening, Antioxidants, DPPH*

INTRODUCTION

Oxidative stress resulting from an imbalance between free radicals (such as reactive oxygen species) and the body's antioxidant defenses has been widely implicated in the pathogenesis of numerous diseases, including Alzheimer's disease, diabetes mellitus, cancer, cardiovascular disorders, and neurodegenerative conditions (Wang et al., 2022; Silva & Santos, 2023). Free radicals, due to their unpaired electrons, can damage cellular components including DNA, proteins, and lipids (Liu et al., 2021). While endogenous antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) act to neutralize these radicals, when their activity is overwhelmed or external exposure increases (e.g., dietary, environmental, and physiological stressors), oxidative damage accumulates (Zhang et al., 2022). In recent years, there has been a rising interest in natural antioxidants derived from plants to counteract oxidative stress with fewer side effects compared to synthetic antioxidants (Nguyen et al., 2023; Kumar & Singh, 2024).

Bamboo shoots are one of the plant sources being explored for antioxidant potential due to their rich content of phytochemicals such as phenolics, flavonoids, saponins, and others, as well as favorable nutritional properties (fiber, vitamins, minerals) and low fat (Zhang et al., 2022; Adebayo et al., 2024). A recent review highlighted that bamboo shoots across multiple species show antioxidant, anti-inflammatory, and hypolipidemic activities, with variations depending on species, growing conditions, and processing (Food & Function, 2023; Adebayo et al., 2024). However, specific studies focused on *Gigantochloa atter* bamboo shoots remain scarce. Although related species like *Gigantochloa apus* have been shown to have significant antioxidant activity (Soesanto, 2016), whether *G. atter* has comparable potency, and how extraction solvents with different polarities affect its phytochemical profile and antioxidant capacity, are not well documented.

Several gaps can be identified in the existing literature. First, many studies on bamboo shoot extracts use a single solvent (often methanol or ethanol), which may extract only certain classes of phytochemicals while omitting non-polar or moderately polar compounds (Soesanto, 2016; Adebayo et

al., 2024). Second, there is limited information regarding *G. atter*, in particular its bioactive-metabolite content across different solvent types and how these influence antioxidant activity measured via standard assays such as DPPH. Third, although some works have reported IC50 values for bamboo shoot extracts (e.g. *Bambusa vulgaris* showing relatively high IC50, i.e. lower activity, under certain conditions) (Adebayo et al., 2024), comparisons across solvent systems for *G. atter* are not available, and many earlier studies are outdated or limited in methodological rigor in terms of replicates, characterization of extract composition, or statistical evaluation (Soesanto, 2016).

Therefore, this research aims to evaluate the phytochemical constituents and antioxidant activity of *Gigantochloa atter* bamboo shoots extracted using solvents of different polarities (e.g., 96% ethanol, ethyl acetate, n-hexane), by determining their phytochemical profiles and IC50 values using the DPPH assay. The urgency of this work lies in the need for natural antioxidant sources with high efficacy and low side effects, especially from under-studied species. The novelty resides in the comparative assessment of *G. atter* shoot extracts across three solvents, filling a gap in current literature by providing detailed solvent-based phytochemical screening correlated with antioxidant potency for this species, which, to our knowledge, has not been reported in studies from 2021 to 2025.

RESEARCH METHODS

Type and Method of Study

This research is a quantitative experimental laboratory study aimed at determining the phytochemical contents and antioxidant activity (using DPPH assay) of *Gigantochloa atter* bamboo shoots as influenced by different extraction solvents. Laboratory experimental designs are commonly used in pharmaceutical and natural product research to test how variations in controlled independent variables (such as extraction solvent polarity) affect dependent variables (e.g., antioxidant activity, phytochemical composition) (Hussain, Hassali, & Babar, 2019; Creswell, 2022). The study uses non-parametric screening tests for phytochemicals and quantitative measurement of antioxidant capacity (IC_{50} values) as outcome metrics.

Instruments and Data Analysis Techniques

The instruments include an analytical balance, a UV-Vis spectrophotometer, a rotary evaporator, thin-layer chromatography (TLC) equipment, an oven, a moisture analyzer, a water bath, a sample grinder (blender), and standard reagents (DPPH, quercetin, solvents, etc.). For phytochemical screening, both colorimetric/tube tests and TLC identification are used. For antioxidant activity, the DPPH free radical scavenging method is applied, measuring absorbance changes with UV-Vis spectrophotometry to calculate percentage inhibition and then IC_{50} through linear regression of concentration vs % inhibition. Data will be analyzed statistically for replicate measurement reliability, using, e.g., mean ± SD, and regression fitting. The method of determining IC_{50} follows standard practice in natural product antioxidant studies (Mphahlele et al., 2022; Nguyen et al., 2023).

Population and Sample

1. **Population**: All bamboo plants, particularly shoots of *Gigantochloa atter Kurz*, in the region of interest.
2. **Sample**: Young shoots (“tunas bambu ater”) of *Gigantochloa atter Kurz* collected from Desa Jlarem, Kecamatan Ampel, Kabupaten Boyolali. The number of samples collected will be sufficient to allow for extraction with each solvent, replicate assays, and screening tests. Sampling is purposive (non-random) since only *G. atter* species and shoots in good condition will be selected. This aligns with experimental sample selection in quantitative lab research (Sugiyono, 2022; Creswell, 2022).

Research Procedure

1. **Plant Identification / Determination**

   Fresh bamboo shoots identified as *Gigantochloa atter Kurz* will be verified at an institution formally authorized in plant taxonomy (e.g., UPF Pelayanan Kesehatan Tradisional Tawangmangu, Karanganyar).
2. **Sample Preparation**

   Shoots will be cleaned (removing dirt and extraneous parts), separated from their sheaths, then washed, drained, and sliced thinly. Slicing is followed by drying: sun-drying for 4-5 days

under shade with protection, or oven drying (~24 hours) until moisture is sufficiently reduced. The dried material is then ground into powder and sieved (mesh size ~40) to get a uniform solid sample.

3. **Standardization of Simplisia**
   A. **Loss on drying**: using 2 g of powder, heated at 105 °C until constant weight, to determine moisture loss percentage.
   B. **Moisture content**: using a moisture analyzer (e.g., heating at 105 °C for a fixed time, e.g., 15 minutes).
   C. **Total ash content**: 2 g sample incinerated in a porcelain crucible until charcoal burns off, cooled, and weighed to calculate ash percentage.
4. **Extraction**

   Three solvents of different polarity will be used: 96% ethanol (polar), ethyl acetate (semi-polar), and n-hexane (non-polar). Powder (~200 g) is macerated in each solvent (volume appropriate: e.g., 2000 mL for ethanol and hexane; for ethyl acetate proportionally) at room temperature away from direct sunlight, for 3×24 hours with periodic stirring, followed by secondary maceration (2×24 hours). Filtration and evaporation via rotary evaporator (40 °C, ~100 rpm), then concentration via water bath until viscous extracts are obtained. Extract yields (rendemen) will be calculated: (weight of extract/weight of dried powder) × 100%.
5. **Standardization of Extracts**

   Tests to ensure extract quality, including checking for residual solvent (especially ethanol), determining the moisture content of the extract, and loss on drying for the extract, following methods appropriate to natural product standards.
6. **Phytochemical Screening**

   Both qualitative tube tests and TLC (thin-layer chromatography) will be used to detect metabolite classes, including alkaloids, flavonoids, tannins, steroids/triterpenoids, and saponins. E.g., for flavonoids, tests with Mg + HCl; for alkaloids, reagents such as Dragendorff, Mayer, Wagner; for steroids/triterpenoids, acid-sulfuric reagents; saponin, frothing test. TLC identification will involve spotting extracts and standard compounds (e.g., quercetin), developing with an appropriate mobile phase (e.g., ethyl acetate: n-hexane ~7:3), visualizing under UV light (254 nm / 366 nm), and after derivatization (e.g., spraying with $AlCl_3$ for flavonoid fluorescence, $FeCl_3$ for phenolic detection, or others).
7. **Antioxidant Activity (DPPH Assay)**
   A. Prepare DPPH solution (e.g., 100 ppm) in methanol. Determine its maximum absorbance wavelength (~500-600 nm).
   B. Prepare standard antioxidant (quercetin) solutions with known concentrations (e.g., 5, 10, 15, 20, 25 ppm) to build a calibration curve.
   C. Prepare extract test solutions at concentrations (e.g., 10, 20, 30, 40, 50 ppm), mix with a fixed amount of DPPH solution, incubate for 30 minutes in the dark at room temperature, then measure absorbance.
   D. Calculate % inhibition via formula: %Inhibition=Ablank−AsampleAblank×100%\% \text{Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\%%Inhibition=AblankAblank−Asample×100%
   E. Determine IC_{50} (concentration causing 50% inhibition) by plotting concentration (X-axis) vs inhibition % (Y-axis), fitting linear regression, and solving for concentration at 50% inhibition.
8. **Data Analysis**

   Each experiment will be done in triplicate or more to ensure reproducibility. Data expressed as mean ± standard deviation. Regression analysis for IC_{50} estimation. Comparisons between solvents (e.g., which has a lower IC_{50}) will be made. If appropriate, analysis of variance (ANOVA) may be used to test differences in yields, phytochemical presence/semi-quantitative staining intensity, or antioxidant activity among solvents. Statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

RESULT

A. Determination Results

The first step that must be taken in a study using plants is to conduct a plant determination test. Plant determination is carried out to ensure the authenticity and truth of a plant to be used. The sample used in the determination of this plant consists of bamboo shoots, stems, twigs, and leaves. The determination of bamboo shoots (*Gigantochloa atter* Kurz*)* was carried out at UPF Tawangmangu Traditional Health Services, Karanganyar, Central Java. The results given from the determination test stated that the ater bamboo plant is included in the family *Poaceae* with the species name *Gigantochloa atter* (Hassk.) Kurz ex Munro. The results of the determination can be seen in Appendix 2.

B. Sample Preparation

The sample used in this study is a type of bamboo shoots obtained from Jlarem Village, Gladagsari District, Boyolali Regency, as much as 10,000 grams or 10 Kg. The simplest part of after bamboo shoots begins with wet sorting, which is separating after bamboo shoots from dirt such as soil, especially from the fine hairs that cover the surface of bamboo shoots, commonly known as lugut. Then washing is carried out with running water until it is clean. After washing is carried out, a sample of bamboo shoots is prepared until thin slices are obtained to facilitate the drying process. After the fermentation is carried out, the sample is dried for 5 days until a very dry sample is obtained, so that the sample does not rot easily, and is not overgrown with bacteria or mold that can reduce the quality of simplicity. During drying, the sample is covered with a black cloth so that it is not exposed to direct sunlight, which may damage the compounds in the dried material. After drying, pollination is carried out using *a blender.*

Table 1. Yield Percentage of Bamboo Bud Powder Yield

Wet Weight (Gram)	Dry Weight (Gram)	% Soak
10.000 gram	1.200 gram	12 %

Based on the Soak Result, simplesia powder was obtained; the wet weight of the sample was 10,000 grams, and the dry weight obtained was 1,200 grams. Based on this data, the percentage of Soak simplesia was obtained at 12%. Soak results meet the Soak simplesia standard, which is > 10% (Hasan *et al.,* 2022). The calculation of the percentage of Soak simplesia can be seen in Appendix 8.

A. Standardization of Simplicity

1. Shrinkage Drying

The determination of the drying shrinkage of the simplesia of ater bamboo *shoots (Gigantochloa atter* Kurz*)* is carried out by first heating the empty cross in the oven at a temperature of 105°C for 30 minutes, after which the cross is cooled on the decitor, recording the weight of the empty cross that has been cooled. Then add 2 grams of Siamensis powder of bamboo shoots (*Gigantochloa atter* Kurz*)* in krush and put it in the oven at a temperature of 105°C for 30 minutes, then the krush is cooled on a deciker, after which each replication is weighed. The results of the calculation of drying shrinkage can be seen in Table 2.

Table 2. Calculation Of Drying-drying Simplesia

Replication	Starting Weight	Final Weight	Result	Average
I	2 gram	1,91 gram	4,5 %	
II	2 gram	1,92 gram	4 %	4,1 %
III	2 gram	1,92 gram	4 %	

Based on Table 2, it can be seen that the drying shrinkage test was carried out with 3 Replications. The first Replication gave a Result percentage of 4.5%, the 2nd Replication gave a

Result of 4% and the 3rd Replication gave a Result of 4%. Of the three, the average result of drying shrinkage is 4.1%, which is in accordance with the drying shrinkage standard, which is < 10% (Ministry of Health of the Republic of Indonesia, 2017). The calculation of drying shrinkage can be seen in Appendix 9.

2. Moisture Content

Moisture content testing was carried out using *a moisture analyzer* with 3 replication tests. Using this tool, first turn on the tool by pressing the on button, menu, then set the time to be used, in this test use 15 minutes, after that determine the temperature to be used, which is 105°C, then put 2 grams of sample in the pan that has been provided evenly, close the tool then wait until the tool sounds after 15 minutes, then record the Results of the test. The results of the moisture content test can be seen in Table 3.

Table 3. Result Of Determination Of The Simplesia Moisture Content

Replication	Sample Weight (gram)	Result (%)	Average (%)
I	2,0	6,18	6,45
II	2,0	6,59	
III	2,0	6,60	

Based on the table above, the simplestia of bamboo shoots after *(Gigantochloa atter Kurz),* with 3 times of Replication, gave consecutive results, namely, 6.18%, 6.59%, and 6.60%, with the average test moisture content being 6.45%. The results given have met the standard of good moisture content of < 10%, too high water content can trigger the growth of microorganisms such as fungi and bacteria, and can also reduce the quality of simplesia (Djoko *et al.,* 2020).

3. Ash Content

Testing the ash content in the simplesia of ater bamboo *shoots (Gigantochloa atter* Kurz*)* is carried out by preheating the cross to be used in the oven for 15 minutes. Then 2 grams of simplesia is put into the cross, the cross is placed in the kiln at a temperature of 600°C for 4 hours until it becomes ashes. After that, wait for the temperature to decrease, then remove the cross from the kiln, and then weigh the test results. The results of the ash level test can be seen in Table 4.

Table 4. Hail Ash Rate Testing

Replication	Starting Weight (gram)	Ash Weight (gram)	Result (%)	Average (%)
I	2,0	0,12	6,0	6,66
II	2,0	0,15	7,5	
II	2,0	0,13	6,5	

Based on the data above, the ash content testing with 3 replications gave consecutive results, 6%, 7.5%, 6.5% with an average ash content test value of 6.66%. The results given are in accordance with the general standard of good total ash content according to the requirements of the Indonesian Herbal Pharmacopoeia (FHI), which is less than 10%. Testing the ash content in simplesia aims to determine the mineral content in the sample; the smaller the ash content, the higher the quality of purity of a sample. (Fauziah *et al.*, 2023). The calculation of ash content can be seen in Appendix 10.

B. Extract Manufacturing

The production of ater bamboo shoot *extract (Gigantochloa atter Kurz)* is carried out by maceration. This maceration method is carried out using 3 types of solvents with different polarities, namely 96% alcohol with polar properties, ethyl acetate with non-polar properties, and n-hexane with non-polar properties. Maceration was carried out in a ratio of 1:10. 200 grams of Siam bamboo shoots (*Gigantochloa atter* Kurz*)* were soaked in 2000 mL of each solvent in a glass jar covered with a black cloth, and placed in a place not exposed to light. Maceration is carried out for 3 days, with occasional

stirring, so that the solvent turnover is evenly distributed, so that the extraction process occurs more effectively (Octavia *et al.,* 2023). The remaceration process was carried out 2 times, each for 24 hours. After the maceration process is completed, sample screening is carried out using a flannel cloth. Then, to maximize filtering, filtering is carried out using *a Buchner* cup. After going through the filtering process, evaporation is carried out using *a rotary evaporator* at a temperature of 40°C at a speed of 100 rpm. After that, a thickening process was carried out to obtain a thick extract of ater bamboo shoots *(Gigantochloa atter* Kurz*)* using a *waterbath* device at a temperature of 70°C for ethyl acetate and alcohol solvents and a temperature of 60°C for n-Hexane solvents. The resulting condensed extract is then calculated for its Soak value. The results of the calculation of the Soak extract value can be seen in Table 5.

Table 5. Results Soak Extract Bamboo Shoots Ater

Solvent	Simplesia's Weight (gram)	Extract Weight (gram)	Result (%)
n-Heksan	200,0	5,23	2,61
Etil Asetat	200,0	8,93	4,46
Alkohol 96%	200,0	23,1	11,5

Based on the data above, the results of bamboo shoot extract *(Gigantochloa atter* Kurz*)* from each solvent gave a result with a significant difference, namely 2.61% for n-Hexane extract, 4.46% for ethyl acetate extract, and 23.1% for 96% alcohol extract. One of the factors that affects the difference in Soak values in extracts is the difference in polarity of the three solvents. The maceration process is based on the ability of the Solvent to filter phytochemical compounds (Arrofiqi et *al.,* 2024). The type of polarity of the Solvent greatly affects filtering; non-polar compounds will dissolve in non-polar solvents, and compounds with polar properties will be soluble in polar solvents (Kasminah, 2016). Solvent n-Hexane gives the lowest result, which shows that the content of non-polar compounds in bamboo shoots is relatively small. On the other hand, Solvent alcohol gives the highest result because alcohol is polar, which can attract polar compounds in bamboo shoots *(Gigantochloa atter* Kurz*).* The soak of an extract is greatly influenced by the type of solvent used; this is due to the ability of a solvent to absorb different active substances. The solubility of an extract will increase if the polarity of a solvent is higher; the more polar the solvent, the better the extraction process will be. This is based on the ability of a solvent to flow into the material cells, which will cause the protoplasm to swell, and the cell contents in the material will be dissolved according to the solubility. The polarity of the solvent and the polarity of the extracted material are related to the ability to filter the sample. (Wijaya & Satriawan, 2023). The calculation of the Soak k-strak value can be seen in Appendix 12.

C. Standardization of Extracts

1. Ethanol-Free Test

Ethanol-free tests are carried out to confirm whether or not ethanol is contained in the extract. Extracts that still contain ethanol may affect the test results and give inaccurate results. The test was carried out by putting the diluted condensed extract into the test tube, then adding 2 drops of H2SO4 and 2 drops of acetic acid, and then heating. Extracts that do not give an ester odor show their freedom from ethanol. (Tivani *et al.,* 2021). The test results can be seen in Table 8.

Table 6. Ethanol-Free Testing Results

Solvent	Intervention	Result	Description
n-Heksan	Ekstrak + 2 tetes asam asetat + 2 tetes asam sulfat, kemudian dipanaskan	(-)	Bebas etanol (Tidak memberikan bau ester)
Etil Asetat	Ekstrak + 2 tetes asam asetat + 2 tetes asam sulfat,	(-)	Bebas etanol (Tidak memberikan bau ester)

	kemudian dipanaskan		
Alkohol 96%	Ekstrak + 2 tetes asam asetat + 2 tetes asam sulfat, kemudian dipanaskan	(-)	Bebas etanol (Tidak memberikan bau ester)

Based on the data above, the extract of ater bamboo *shoots (Gigantochloa atter* Kurz*)* does not smell the ester that is typical of the smell of alcohol. The test gave the odor result of the sample resembling the smell of the extract, which showed that the extract of bamboo *atter shoots (Gigantochloa atter* Kurz) from all three solvents was free of ethanol. (Tivani *et al.,* 2021).

2. Water Content Test

Moisture content testing was carried out using *a moisture analyzer* tool. To use this tool, first turn on the tool by pressing the on button, then set the temperature to be used. Which is 105°C, then put 2 grams of the sample in the pan that has been provided evenly, close the tool, then wait until the alt sounds after 15 minutes, then record the results of the test. (Djoko *et al.,* 2020). The results of the moisture content test can be seen in Table 7.

Table 7. Extract Water Content Test Results

Sample Extract	Sample Weight (gram)	Result (%)
Ekstrak Alkohol Tunas Bambu Ater *(Gigantochloa atter* Kurz*)*	2,0	7,08%
Ekstrak n-Heksan Tunas Bambu Ater *(Gigantochloa atter* Kurz*)*	2,0	6,30%
Ekstrak Etil Asetat Tunas Bambu Ater *(Gigantochloa atter* Kurz*)*	2,0	7,58%

Based on the data above, the results given for testing the moisture content of alcohol extract are 7.08%, n-hexane extract is 6.30%, and ethyl acetate extract is 7.58%. This is in accordance with the general standard of extract moisture content according to Farakope Hebal Indonesia, which is not more than 10%. Moisture content testing on the extract aims to determine the amount of water contained in the extract. Too high a moisture content can affect the quality of an extract. High moisture content can accelerate the growth of microorganisms such as bacteria or fungi. (Djoko *et al.,* 2020).

3. Drying Shrinkage Test

The drying shrinkage test of extracts from three types of solvents in this study used *the moisturizer balance* tool, by activating the tool by pressing the on button, then selecting the drying shrinkage test by pressing the *"dryer" option* on the screen. After that, set the temperature to 105°C and the time to 15 minutes, then record the result. The results of the drying shrinkage test can be seen in Table 8.

Table 8. Extract Drying Shrinkage Test Results

Sample Extract	Starting Weight (gram)	Final Weight (gram)	Result (%)
Ekstrak Alkohol Tunas bambu ater	2,515	2,337	7,08

(Gigantochloa atter Kurz*)*			
Ekstrak N- Heksan Tunas bambu ater *(Gigantochloa atter* Kurz*)*	2,142	2,007	6,30
Ekstrak Etil Asetat Tunas bambu ater *(Gigantochloa atter* Kurz*)*	2,124	1,963	7,58

The drying shrinkage test is intended to determine the maximum limit on the amount of compounds lost in the drying process. The parameter of this test is the measurement of the remaining substance after drying at a temperature of 105°C, expressed in percent values. Based on the above test data, it can be seen that the test results of the drying shrinkage of extracts from each solvent are 7.08% for Solvent alcohol, 6.30% for Solvent n-Hexane, and 7.58% for Solvent ethyl acetate. The results given are in accordance with the general standard set by the Indonesian Herbal Pharmacopoeia (FHI), which is no more than 10% (Utami *et al.,* 2020). The results of the drying shrinkage calculation can be seen in Appendix 13.

4. Screening Phytochemistry

Phytochemical screening is carried out to determine the type of secondary metabolite compounds contained in the extract of ater bamboo *shoots (Gigantochloa atter* Kurz*).* The secondary metabolite compounds tested in this study were flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids. Phytochemical screening tests were carried out on three extracts with polarity differences, namely alcohol extract, ethyl acetate extract, and n-hexane extract. The results of the identification of secondary metabolite compound content from the extract of ater bamboo *shoots (Gigantochloa atter* Kurz*)* can be seen in Table 9.

Table 9. Phytochemical Screening Results of Ater Bamboo Bud Extract

Test Sample	Chemical Compounds	Result Description	Result
Ekstrak Alkohol Tunas Bambu Ater *(Gigantochloa atter)*	Alkaloid	• Wagner : terbentuk endapan coklat muda. • Mayer : terbentuk endapan putih kekuningan. • Dragendrof : terbentuk endapan coklat tua.	(+) (+) (+)
	Flavanoid	Terbentuk warna orange jingga	(+)
	Tanin	Terbentuk warna hijau kehitaman	(+)
	Saponin	Terbentuk busa yang stabil	(+)
	Steroid	Terbentuk warna biru kehitaman (biru tua)	(+)
Ekstrak Etil Asetat Tunas	Alkaloid	• Wagner : terbentuk endapan coklat muda. • Mayer : terbentuk endapan putih kekuningan.	(+) (+) (+)

Bambu Ater *(Gigantochloa atter)*		• Dragendrof : terbentuk endapan coklat tua.	
	Flavanoid	Terbentuk warna jingga	(+)
	Tanin	Terbentuk warna hijau	(+)
	Saponin	Terbentuk busa yang stabil	(+)
	Steroid/Terpenoid	Tidak terjadi perubahan warna	(-)
Ekstrak n-Heksan Tunas Bambu Ater *(Gigantochloa atter)*	Alkaloid	• Wagner : terbentuk endapan coklat muda • Mayer : tidak terbentuk endapan • Dragendrof : terbentuk endapan merah kecoklatan	(+) (-) (+)
	Flavanoid	Tidak terjadi perubahan warna	(-)
	Tanin	Terbentuk 2 fase larutan coklat tua, dan coklat kekuningan	(-)
	Saponin	Tidak terbentuk busa	(-)
	Triterpenoid	Terbentuk cicin coklat	(+)

Alkaloid testing was carried out by first acidifying the sample with the addition of hydrochloric acid reagent, followed by three tests with Dragendorff, Mayer, and Wagner reagents. The formation of a reddish-brown to orange precipitate indicates the presence of alkaloid compounds in the Dragendrof tube, while the formation of a yellowish-white precipitate in the Mayer tube and a brown precipitate in the Wagner tube indicates the presence of alkaloids. According to (Yeti & Yuniarti, 2021), a sample is positive for alkaloids if it shows changes in the form of turbidity or precipitation in at least 2 of the 3 tests.

Flavonoid testing is carried out by adding Mg powder and concentrated HCL. These reagents are added to reduce the glycosidic bonds of plants with flavonoids, which must be broken by reducing these bonds. Usually, a positive result will show a color change to yellow, orange, or red (Rahmasiahi *et al.,* 2023).

Tannin testing is done by adding 1% $FeCl_3$ reagent, which will turn green if the sample is positive for tannin. The green color is formed because the $FeCl_3$ solution reacts with the hydroxyl groups present in the tannin compound (Dewi *et al.,* 2021).

Saponin testing is carried out by adding water and then shaking it until foam forms if the sample is positive for saponin. Saponin is a compound that has hydrophilic and hydrophobic groups. The hydrophilic group binds with water while the hydrophobic group binds with air, which causes saponin compounds to form foam when shaken (Dewi et al., 2021).

Triterpenoid and steroid compounds are tested by adding anhydrous acetic acid and concentrated sulfuric acid. The addition of concentrated sulfuric acid through the wall of the test tube causes the anhydrous acetic acid to react, forming a carbocation on the C atom of the anhydride. The carbocation then reacts with the O atom in the –OH group present in the triterpenoid compound. The formation of a brown ring indicates the formation of an ester compound by the triterpenoid compound with acetic anhydride, which is called the esterification reaction (Dewi et al., 2021). In the steroid test, the addition of chloroform aims to dissolve the steroid compound in the sample. The steroid compound will undergo dehydration with the addition of concentrated sulfuric acid and form ions that produce a number of color reactions. The color change that occurs is caused by an oxidation reaction in the steroid group through the formation of conjugated double bonds. Usually, the sample will turn blue or green if it is positive for steroid compounds (Sholikhah, 2016).

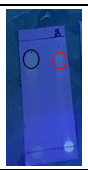
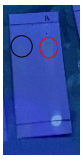
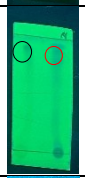
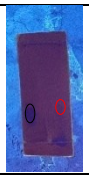
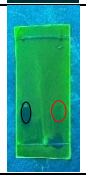
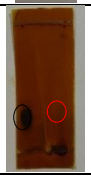
Based on the data above, the results of the identification of metabolite content in bamboo shoot extract *(Gigantochloa atter* Kurz*)* from alcohol solvent are positive for alkaloids, flavonoids, tannins, saponins, and steroids. This is in line with the research conducted by (Yeti & Yuniarti, 2021), who tested the metabolite compounds in bamboo grass extract *(Lopatherum gracile Brongn)*, which has the same *family* as ater bamboo shoots *(Gigantochloa atter* Kurz*)*. Then, the ethyl acetate extract positively contained alkaloids, flavonoids, tannins, and saponins. This is in accordance with the research conducted by (W. S. Putri et al., 2018), which found that the use of ethyl acetate solvent could extract several metabolite compounds such as alkaloids, tannins, flavonoids, and saponins. Then, n-hexane extract can only extract alkaloids and triterpenoids contained in ater bamboo shoots (Gigantochloa atter Kurz). This is due to the polarity of n-hexane, which is a nonpolar solvent. Metabolite compounds dissolve based on their polarity. Alkaloids have nitrogen bases in their cyclic chains and contain various substituents, so alkaloids can be semipolar, which can also dissolve in nonpolar solvents. Triterpenoids have polar and nonpolar parts. These compounds consist of a long C_{30} hydrocarbon chain, which causes them to be non-polar, and also have a hydroxyl group, which causes some triterpenoids to be polar (W. S. Putri *et al.*, 2018).

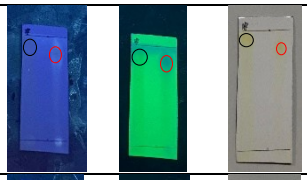
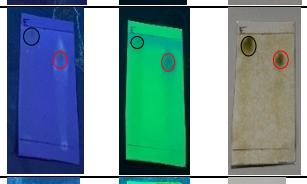
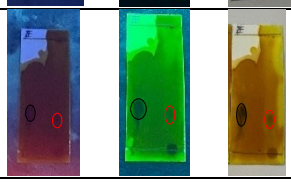
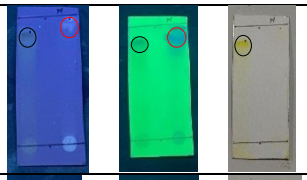
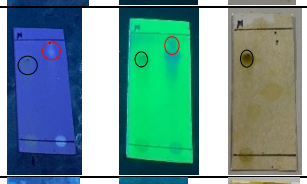
D. Thin-layer chromatography (KLT) testing

The identification of the compound content of bamboo ater bud *extract (Gigantochloa atter* Kurz*)* was carried out by observing samples under UV 254 and UV 366 light. Thin-layer chromatography is a simple analysis used to confirm the Chemical Compounds contained in plants. The KLT Test aims to determine the purity of the compounds contained in a sample. The working principles of KLT are adsorption, desorption, and elution. Adsorption occurs when the sample is softened in the silent phase. The components in the sample will be adsorbed in the silent phase. Then desorption occurs when the adsorbed component is pushed by the motion phase or eluent, and the elution phase occurs when the component is carried by the eluent or motion phase. (Muhsin & Ramandha, 2023).

The silent phase used in this study is silica gel GF254 with a size of P x L, which is 5 x 2 cm. The selection of the motion phase is based on the ability of the motion phase to trace the components in the sample. N-hexane: Ethyl acetate with a ratio (3:7) was used in this study because N-hexane, which is non-polar, and Ethyl acetate, which is semi-polar, can be regulated in polarity. The extract is pressed on the KLT plate and then put in a chamber containing a combination of eluents that have been saturated beforehand. The results of the identification of compounds using KLT can be seen in Table 10.

Table 10. KLT Test Results Of Atter Bamboo Bud Extract

Compounds	Result			Rf	Test Sample
	UV 366	UV 254	Sinar Tampak		
Flavonoid				Pembanding : 0,90 Ekstrak : 0,87	Ekstrak tunas bambu ater *(Gigantochloa atter* Kurz*)* dengan Solvent alkohol 96%
Fenol				Pembanding : 0,85 Ekstrak : 0,80	
Alkaloid				Pembanding : 0,32 Ekstrak : 0,3	

Flavanoid		Pembanding : 0,82 Ekstrak : 0,87	Ekstrak tunas bambu ater *(Gigantochlos atter* Kurz*)* dengan Solvent Etil asetat
Fenol		Pembanding : 0,87 Ekstrak : 0,82	
Alkaloid		Pembanding : 0,32 Ekstrak : 0,25	
Flavanoid		Pembanding : 0,85 Ekstrak : 0,93	Ekstrak tunas bambu ater *(Gigantochloa atter* Kurz*)* dengan Solvent n-Heksan
Fenol		Pembanding : 0,75 Ekstrak : 0,90	
Alkaloid		Pembanding : 0,35 Ekstrak : 0,3	

1. Flavonoid

The flavonoid compound in the KLT separation of bamboo shoots extract *(Gigantochloa atter* Kurz*)* in Solvent alcohol gave an Rf value of 0.87, and a Rf value of 0.90 was obtained in the quercetin comparator. The Rf value in the sample that is close to the Rf of tilapia in the comparator shows the similarity of characteristics, or it can be a marker that the sample has the same content of quercetin, which is a flavonoid. (Muhsin & Ramandha, 2023).

Testing on the Ethyl Acetate Extract Sample gave an Rf value of 0.82, and on quercetin comparators gave an Rf value of 0.87. Similar to alcohol extracts, ethyl acetate extract is also suspected to have similar characteristics to the comparator compound, namely quercetin.

The test on the n-Hexane Sample Extract gave an Rf value of 0.92, and on the quercetin comparator stain gave an Rf value of 0.85, the result of both the Rf value on the sample stain and the quercetin stain gave a very different result, this shows that the stain on the sample does not have the same characteristics as the comparator stain, namely quercetin.

The results of the flavonoid compound test from the three extracts were corroborated by spraying the AlCl3 reagent 5%; the stain visible in the light appears to give a yellow color change to the stain if the sample contains flavonoids. (Rusmawijayanto & Luliana, 2019). Stains on ethyl acetate and alcohol extracts give a yellow stain, meaning both extracts contain flavonoids. While the stains on n-Hexan extract do not show yellow stains, this indicates that the flavonoid compound content is not found in the n-Hexan Extract Sample.

2. Phenol

Testing on a Sample Extract of bamboo shoots with Solvent alcohol and ethyl acetate gave Rf values of 0.80 and 0.82, respectively. The Rf values given from the quercetin comparator

stain are 0.85 and 0.87, respectively. Both give the same Rf value close to the Rf value of the comparator, showing the similarity of the characteristics of the compound content in the sample and the compound content in the comparator. Testing on visible light with FeCl3 spraying will give the result of green, red, purple, blue, or black stain color (Rusmawijayanto & Luliana, 2019). The result given on the 2 stains from ethyl acetate extract and alcohol is blackish-green, indicating that the 2 positive extracts contain phenols.

Pengujian pada sampel n-Heksan memberikan Result nilai Rf pada noda sampel yaitu 0,90 dan noda pada pembanding Kuersetin yaitu 0,75. Kedua nilai Rf yang diResultkan memberikan selisih nilai yang cukup signifikan, hal ini menunjukan ketidak samaan antara sampel dan pembanding kuersetin, Hal ini juga diStrongkan dengan ketidak munculan noda hijau, merah, ungu, biru, atau hitam setelah penyemprotan $FeCl_3$ 10 %.

3. Alkaloid

Testing on a Sample Extract of bamboo shoots with 3 solvents, alcohols, ethyl acetate, and n-Hexane gave consecutive Rf values of 0.30, 0.25, and 0.30. In the Caffeine comparator, the Rf values given are 0.32, 0.35, and 0.32, respectively. The Rf value given is at the vulnerable Rf value for alkaloid compounds, which is 0.07 – 0.62 (Kapondo *et al.,* 2020). This shows the presence of alkaloid compounds in the Sample Extract with the 3 Solvents. The test was strengthened by spraying a dragendrof reagent, which will give a brown-orange color. (Taupik *et al.,* 2023) In this test, the stain given was a faint reddish brown, indicating the presence of alkaloid compounds in the three Sample extracts of ater bamboo *shoots (Gigantochloa atter* Kurz*).* The calculation of the Rf value can be seen in the appendix.

E. Antioxidant Activity Test of Ater Bamboo Shoot Extract

1. Determination of the Maximum Wavelength of DPPH

Determination of DPPH wavelength is the first stage that must be done before testing the antioxidant activity of ater bamboo *shoots (Gigantochloa atter* Kurz*).* The determination of the maximum wavelength is carried out to determine the highest absorption using a DPPH solution, at which wavelength the inhibition of DPPH free radicals can be carried out. In this test, methanol is used as a blank solution, and the determination of the maximum wavelength is done in the range of 500-600 nm. The maximum wavelength measurement result was obtained at a wavelength of 514 nm with an absorbance value of 0.628.

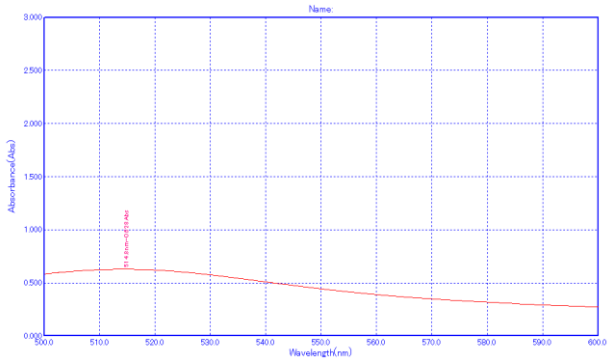


Figure 1. Curve Result of DPPH wavelength determination

Antioxidant testing can be carried out by various methods; in this study, the testing of the antioxidant activity of bamboo ater *shoots (Gigantochloa atter* Kurz*)* was carried out using the DPPH ***(2,2-diphenyl-1-pyrylhydrazyl) method***. This method is based on the principle of the interaction of antioxidants with DPPH by electron transfer. If all the electrons in the free radical have been paired, then it will be marked by a change in color from purple to yellow. (Mulangsri *et al.,* 2017). The results of the determination of wavelengths can be seen in Appendix 18.

2. Determination *of Operating Time* (OT) of DPPH Solution

The determination *of operating time* is carried out to determine the most appropriate time or to determine the length of good incubation time for a solution in warding off free radicals of DPPH. *Operating time* is used to show that the reaction between a solution and free radicals has

been fully incubated. The determination of this *operating time* is based on the time when the absorbance value given begins to stabilize (Putri, 2023).

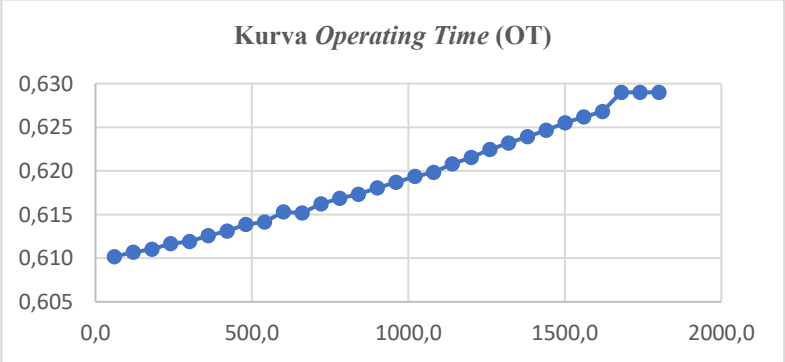


Figure 2. Result Curve from Determining Operating Time

Based on the curve above, it can be seen that the measurement of *operating time* starts from minute 0 to minute 30 at a maximum wavelength of 514 nm. The curve in Figure 4.2 shows that the measurement of the operating time of the DPPH solution was obtained at 30 minutes at an absorbance of 0.629. This indicates that the most appropriate time for incubating the DPPH solution is 30 minutes. Within 30 minutes, the solution will react well with DPPH (Putri, 2023).

F. Quercetin Antioxidant Activity Test Results (Comparator)

The selection of quercetin as a comparison in the antioxidant activity test of ater bamboo shoots *extract (Gigantochloa atter* Kurz*)* is because quercetin is a flavonoid group that shows several biological activities with good free radical inhibition capabilities. Besides that, quercetin, which is a flavonoid group, is also often found in plants (Hasanah *et al.,* 2023).

The predetermined wavelength is then used to measure the absorbance of quercetin to determine the IC50 value. IC50 is a value used to show the value of antioxidant concentrations to ward off 50% of free radical activity. This test was carried out by making a 100 ppm quercetin parent solution by dissolving 2 mg of quercetin in 20 ml of methanol, then making a raw solution from the parent solution with concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. The dilution of these various concentrations is carried out in order to determine the right concentration range to see the effects of antioxidants at various levels. The results of the antioxidant activity test on the quercetin comparator can be seen in Table 11.

Table 11. Quercetin Antioxidant Activity Test Results

Test Solution	Concentration (ppm)	Absorbantion Average	% Inhibition	IC_{50} Value	Category
Kuersetin	5	0,345	44,957	10,62	Very Strong
	10	0,312	50,318		
	15	0,294	53,078		
	20	0,263	57,749		
	25	0,236	62,314		

Based on the data above, it can be seen that the antioxidant activity of the quercetin comparator is classified as very strong, with a Result IC50 of 10.62 µg/mL. The smaller the IC50 value shown, the stronger its antioxidant activity. The calculation of the inhibition percentage of quercetin can be seen in Appendix 21.

3. Results of Testing the Antioxidant Activity of Atter Bamboo Shoots Extract

Testing of antioxidant activity aims to determine the ability of bamboo ater *(Gigantochloa atter* Kurz*)* bud extract in capturing free radicals. In this study, the DPPH *(2,2-diphenyl-1-pyrrylhydrazyl)* method was used. This method was chosen because it is a commonly used method, in addition to being easy to do, simple, and fast, and requires only a small sample

to identify the antioxidant activity of a natural material. The DPPH method is based on the principle of a change in color intensity. DPPH free radicals that do not have electron pairs will give a purple color; the color will begin to turn yellow when the electrons begin to pair. This change in the intensity of the purple color occurs due to the immersion of free radicals by hydrogen compounds released by the sample compound molecules, causing the formation of *diphenyl pyrryl hydrazine* compounds, which make the purple color change to yellow in DPPH free radicals. (Farah *et al.,* 2019).

In this study, the measurement of antioxidant activity used the IC50 parameter, which is defined as the concentration of extracts that can ward off free radicals by 50%. If the IC50 value obtained in the test gives a small value, this shows the high antioxidant activity provided by the sample. (Nirmalasari *et al.,* 2024). The results of the antioxidant activity test on the extract of bamboo shoots of ater *(Gigantochloa atter* Kurz*)* showed a change in the color of the dissolved DPPH from purple to yellow. This indicates the presence of antioxidant activity in the extract of bamboo shoots of ater *(Gigantochloa atter Kurz)*.

Table 12. Results of Antioxidant Activity Test of Ater Bamboo Shoots Extract

Test Solution	Concentration (ppm)	Absorbantion Average	% Inhibition	IC_{50} Value	Category
N-Heksan	10	0,574	8,545	69,77	Strong
	20	0,526	16,188		
	30	0,475	24,256		
	40	0,434	30,785		
	50	0,407	35,191		
Etil Asetat	10	0,494	21,284	56,19	Strong
	20	0,455	27,441		
	30	0,395	37,048		
	40	0,375	40,180		
	50	0,345	45,063		
Alkohol 96%	10	0,463	26,220	39,22	Sangat Strong
	20	0,409	34,872		
	30	0,345	44,957		
	40	0,316	49,681		
	50	0,263	58,067		

In this study, the preparation of sample solutions referred to the study (Handayani *et al.,* 2019), with several modifications, where sample absorbance measurements were performed at a wavelength of 514 nm, and the sample concentrations used were 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. According to (Susiloningrum & Mugita Sari, 2021), a compound is considered to have very strong antioxidant activity if it gives an IC_{50} value < 50 ppm, strong if IC_{50} is 51-101 ppm, moderate if the IC50 value is 101-150 ppm, weak if the IC50 value is greater than 151 ppm (> 151), and if the IC50 value is greater than 500 ppm (> 500), it is considered inactive, or the sample has no potential to counteract free radicals. The calculation of the percentage of antioxidant activity can be seen in Appendix 24

. Based on the data above, it can be seen that each solvent has different IC_{50} values, with the alcohol solvent giving the highest value of 39.22 µg/mL. This shows that bamboo shoot extract *(Gigantochloa atter* Kurz*)* with alcohol solvent has a strong ability to ward off free radicals. Alcohol is a solvent with a polarity level of 5.2, ethyl acetate has a polarity level of 4.1, and n-hexane has a polarity level of 0. This indicates that 96% alcohol has the highest polarity level among the three solvents used, so it can attract more polar antioxidant compounds such as flavonoids (Hasanah et al., 2023). These compounds play a very important role in antioxidant activity through their mechanism of donating hydrogen atoms to free radical compounds. Flavonoids are polyphenolic compounds that can provide antioxidant activity in free radical neutralization reactions or in stopping chain reactions (Handayani *et al.,* 2019).

Bamboo shoot extract *(Gigantochloa atter* Kurz*)* with ethyl acetate solvent produced an IC_{50} value of 56.19 µg/mL. Ethyl acetate is a semi-polar solvent that can dissolve both polar and non-polar compounds. Polar compounds such as flavonoids, alkaloids, and saponins extracted in ethyl acetate from ater bamboo shoots (Gigantochloa atter Kurz) are likely to be compounds that provide antioxidant activity. Flavonoids can donate hydrogen atoms to free radicals, thereby breaking the chain reaction. The types of alkaloids that play a role in antioxidant activity include the indole and quinolone groups, which can suppress free radicals and protect cells from the effects of radiation. In addition, saponin compounds can also act as antioxidants by suppressing superoxide through the formation of hydroxyperoxide intermediates, thereby preventing biomolecular damage caused by free radicals (Farah et al., 2019).

CONCLUSION

The findings of this study demonstrate that the ethanolic extract of *Gigantochloa atter* bamboo shoots contains the highest diversity of phytochemicals and exhibits the strongest antioxidant activity compared to ethyl acetate and n-hexane extracts. The variation in solvent polarity significantly influenced the yield and type of secondary metabolites obtained, with ethanol proving to be the most effective in extracting flavonoids, tannins, and other bioactive compounds. These results suggest that *G. atter* bamboo shoots have considerable potential as a natural source of antioxidants and may be developed into functional food ingredients or pharmaceutical candidates aimed at mitigating oxidative stress-related disorders.

Despite these promising findings, this research has certain limitations as it relied solely on in vitro antioxidant assays and did not isolate or characterize the specific bioactive compounds responsible for the observed activity. Future studies should employ advanced analytical techniques such as HPLC, LC-MS, or NMR to identify and quantify the active constituents, as well as in vivo models to confirm efficacy and safety. Further investigation into formulation development, optimal dosage, and stability testing would also provide practical insights for potential industrial applications. Overall, this study not only contributes to the growing body of knowledge on bamboo shoot bioactivity but also highlights its practical implications for the development of natural antioxidant products in pharmaceutical and nutraceutical industries.

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