Jurnal Ners Volume 9 Nomor 3 Tahun 2025 Halaman 4667 - 4672
JURNAL NERS
Research & Learning in Nursing Science
http://journal.universitaspahlawan.ac.id/index.php/ners

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF SEVERAL BAMBOO LEAVES SPECIES FROM GARUT INDONESIA

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Abstrak

Tumbuhan kaya akan senyawa antioksidan yang memberikan perlindungan terhadap berbagai penyakit yang berkaitan dengan radikal bebas. Senyawa antioksidan tersebut umumnya disintesis sebagai metabolit sekunder. Salah satu tumbuhan yang memiliki aktivitas antioksidan adalah bambu. Beberapa spesies bambu yang tumbuh di daerah Garut antara lain bambu kuning, bambu aur, dan bambu tali. Penelitian mengenai metabolit sekunder dan aktivitas antioksidan dari daun bambu yang berasal dari Garut belum ada. Oleh karena itu, penelitian ini bertujuan untuk mengkaji kandungan metabolit sekunder dan aktivitas antioksidan dari daun ketiga spesies bambu tersebut. Penapisan fitokimia dilakukan dengan menggunakan beberapa pereaksi identifikasi, sedangkan uji aktivitas antioksidan menggunakan metode DPPH (2,2-difenil-1-pikrilhidrazil). Hasil penapisan fitokimia menunjukkan bahwa serbuk simplisia dan ekstrak daun bambu kuning, bambu aur, dan bambu tali mengandung senyawa fenol, saponin, dan steroid/triterpen. Hasil uji aktivitas antioksidan menunjukkan bahwa ekstrak daun bambu kuning, bambu aur, dan bambu tali memiliki aktivitas antioksidan yang kuat, dengan nilai IC50 masing-masing sebesar 51,64; 82,04; dan 76,67 µg/ml. Penelitian ini menunjukkan bahwa daun bambu kuning, bambu aur dan bambu tali yang berasal dari Garut memiliki potensi besar sebagai sumber antioksidan alami yang dapat membantu mencegah dan mengobati penyakit yang berkaitan dengan stres oksidatif.

Kata Kunci: *Antioksidan, Bambusa vulgaris, DPPH, Penapisan Fitokimia, Gigantochloa apus*

Abstract

Plants are rich in antioxidants that provide protection against diseases linked to free radicals. These antioxidant compounds are predominantly synthesized as secondary metabolites. One of the plants that possesses antioxidant activity is bamboo. Several bamboo species grow in Garut including yellow bamboo, rope bamboo, and aur bamboo. There is no research regarding the phytochemistry and antioxidant activity of bamboo leaves collected in Garut. Therefore, this study aims to investigate the secondary metabolite content and antioxidant activity of the leaves from the three bamboo species. Phytochemical screening was carried out using several identification reagents, while the antioxidant activity test used the DPPH (2,2 diphenyl-1-picrylhydrazyl) method. The results of the phytochemical screening showed that the dry powder and leaf extracts of yellow bamboo, aur bamboo, and tali bamboo contain phenolic compounds, saponins, and steroids/triterpenes. The results of the antioxidant activity test showed that the leaves extracts of yellow bamboo, aur bamboo, and tali bamboo exhibited strong antioxidant activity, with IC50 values of 51.64, 82.04, and 76.67 µg/ml, respectively. This study indicates that the leaves of yellow bamboo, aur bamboo, and rope bamboo from Garut have great potential as natural antioxidant sources that may help prevent and treat diseases related to oxidative stress

***Keywords*:** *Antioxidant, Bambusa vulgaris, DPPH, Screening phytochemistry, Gigantochloa apus*

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INTRODUCTION

Aging and chronic degenerative diseases are characterized by elevated reactive oxygen species (ROS), oxidative stress, and mitochondrial impairments. Excess ROS leads to cellular damage, which the body mitigates through the production of endogenous antioxidants (Leyane et al., 2022).

When the levels of free radicals and reactive species exceed the capacity of endogenous antioxidants, the body requires additional antioxidant intake from external sources. Plants are rich in antioxidants that provide protection against diseases linked to free radicals. These antioxidant compounds are predominantly synthesized as secondary metabolites (Nwozo et al., 2023).

One of the plants that possesses antioxidant activity is bamboo. The antioxidant activity in bamboo plants is attributed to the presence of flavonoids, phenols, phenylpropanoids, and alkaloids (Benjamin et al., 2023). Parts of the bamboo plant that exhibit antioxidant activity include the leaves. For example, the leaves of *Gigantochloa pseudoarundinaceae* show antioxidant activity with an IC_{50} value of 42.02 µg/ml (Mamay et al., 2022).

Several bamboo species grow in Garut including yellow bamboo, rope bamboo, and aur bamboo. There is no research regarding the phytochemistry and antioxidant activity of bamboo leaves collected in Garut. Therefore, this study aims to investigate the secondary metabolite content and antioxidant activity of the leaves from the three bamboo species.

METHODS

Equipment

The equipment used in this study includes a UV-Vis spectrophotometer, cuvettes, a water bath, a test tube rack, a mortar and pestle, and various glassware.

Materials

The primary materials used in this study include DPPH (2,2-diphenyl-1-picrylhydrazyl), vitamin C obtained from Sigma, various reagents, and solvents. The plant samples consisted of the leaves of yellow bamboo, rope bamboo, and aur bamboo, collected from Cintaasih village, Samarang district, Garut. Plant identification (determination) was conducted at the School of Life Sciences and Technology, Institut Teknologi Bandung, to confirm the species used. The plant materials were washed, chopped, dried, and ground into powder.

Extraction

A total of 200 grams of dried leaves powder from yellow bamboo, aur bamboo, and rope bamboo were each macerated with 96% ethanol, with the solvent replaced every 24 hours over a period of 3 days. The resulting filtrates from the maceration were then filtered and concentrated using a vacuum evaporator to obtain thick extracts

Screening Phytochemistry

Phytochemical screening was carried out to determine the presence of secondary metabolites or phytochemical constituents in the dry powders and the extracts. The analysis focused on detecting several secondary metabolites, including alkaloids, flavonoids, saponins, quinones, tannins, phenols, steroid/triterpenes. Specific reagents were used for identification: Dragendorff's reagent for alkaloids, aluminum chloride ($AlCl_3$) for flavonoids, gelatin solution for tannins, ferric chloride ($FeCl_3$) for phenols, and the Liebermann-Burchard reagent for steroids/triterpenes. (Farnsworth, 1966)(Harbone, 1998). Each reagent solution was prepared in accordance with the guidelines stated in the Indonesian Herbal Pharmacopoeia (Kementerian Kesehatan Republik Indonesia, 2017).

Alkaloid identification

Each of 2 grams of dry powder and extract was moistened by adding 5 mL of 25% ammonia, then ground in a mortar. A total of 25 mL of chloroform was added to the mixture. The mixture was then filtered, and the filtrate was dropped onto filter paper and treated with Dragendorff's reagent. A positive result is indicated by a red or orange color change on the filter paper. The residue was re-extracted using 10% HCl solution and filtered. The filtrate was then treated with Mayer's and Dragendorff's reagents. A positive result is indicated by the formation of a white precipitate with Mayer's reagent and a brick-red precipitate with Dragendorff's reagent.

Flavonoid identification

Each of 1 gram of dry powder and extract was added to 100 mL of hot water, then boiled for 15 minutes and filtered. Magnesium powder and 2 mL of an alcohol-hydrochloric acid solution (1:1) were added to 5 mL of the filtrate, followed by the addition of amyl alcohol. The mixture was shaken vigorously and allowed to separate. A positive result is indicated by the formation of a red, orange, or yellow color in the amyl alcohol layer.

Phenol identification

Each of 1 gram of dry powder and extract was added to 100 mL of hot water, then boiled for 15 minutes, cooled, and filtered. A total of 5 mL of the filtrate was placed into a test tube, followed by the addition of a few drops of 1% $FeCl_3$ solution. A positive result is indicated by the formation of a purple or dark bluish-colored solution.

Quinone identification

Each 1 gram of dry powder and extract was added to 100 mL of hot water, then boiled for 15 minutes and filtered. A few drops of NaOH solution were added to 5 mL of the filtrate. A positive result was indicated by the formation of a red-colored solution.

Saponin identification

Each 1 gram of dry powder and extract was added to 100 mL of hot water, then boiled for 15 minutes and filtered. A total of 10 mL of the filtrate was placed into a test tube, shaken vertically for 10 seconds, and then allowed to stand for 10 minutes. A positive result was indicated by the formation of stable foam after the addition of a few drops of dilute HCl

Tanin identification

Each 1 gram of dry powder and extract was added to 100 mL of hot water, then boiled for 15 minutes, cooled, and filtered. A total of 5 mL of the filtrate was added with a few drops of 1% gelatin solution. A positive result was indicated by the formation of a white precipitate.

Steroid/triterpene identification

Each 1 gram of dry powder and extract was macerated with 15 mL of ether for 2 hours, then filtered. A total of 5 mL of the filtrate was evaporated using an evaporating dish over a water bath, and the residue was then added with a few drops of Liebermann-Burchard reagent. A positive result was indicated by the appearance of red, blue, green, or violet coloration.

Antioxidant activity test

The antioxidant activity was evaluated using the DPPH method, with several modifications from the procedure developed by Andriani et al. Various concentrations of each extract and vitamin C were prepared. For each test, 1 ml of the sample solution was mixed with 2 ml of DPPH solution (50 μg/ml) in a vial. The mixture was then homogenized and incubated in a dark environment for 30 minutes. Absorbance was recorded at a wavelength of 516 nm using a UV-Vis spectrophotometer, with ethanol serving as the blank. For control measurements, 4 ml of DPPH solution (50 μg/ml) were used and also kept in the dark for 30 minutes. All experiments were conducted in triplicate. The percentage of inhibition was calculated after absorbance readings using the following formula:

Inhibition % = [(CA-SA)/(CA)] x 100%

CA: Control Absorbance

SA: Sample Absorbance

The IC_{50} value, which represents the concentration required to inhibit 50% of DPPH activity, was determined from the percentage of inhibition. This value was obtained by applying linear regression analysis to the relationship between the inhibition percentage and the various concentrations of the sample (Andriani & Murtisiwi, 2020).

RESULTS AND DISCUSSIONS

The results of the sample identification indicated that the plant samples consisted of *Bambusa vulgaris* Schard ex. J.C. Wendl (yellow bamboo), *Bambusa vulgaris* ex J.C. Wendl (aur bamboo), and *Gigantochloa apus* (Schult.f.) Kurz ex Munro (rope bamboo). Yellow bamboo and aur bamboo are the same plant species but belong to different varieties.

The materials used in this study were the leaves of yellow bamboo, aur bamboo, and rope bamboo. Each material underwent wet sorting, followed by washing under running water to remove any adhering dirt, and was then dried. After drying, dry sorting was performed, and the materials were ground into powder to increase the surface area and enhance the efficiency of the extraction process.

The extraction procedure used was maceration, a cold extraction technique. This method offers the advantage of being simple while also preventing the degradation of compounds that could occur due to heat exposure. The solvent used for maceration was 96% ethanol because it is a universal solvent capable of extracting various types of secondary metabolite compounds.

Phytochemical screening aims to identify the secondary metabolites present in the samples. The results of the phytochemical screening of both dry powders and extracts are presented in Table 1. The dry powders and extracts from all bamboo leaves contained phenols, saponins, and steroids. While, alkaloids, flavonoids, tannins, and quinones were not detected in either the dry powders or extracts of any of the bamboo leaves. In previous studies, alkaloids, terpens, flavonoids and tannins were identified in the leaves of *Bambusa vulgaris*

(Tripathi et al., 2015). While, flavonoids and triterpens were identified in the leaves of *Gigantochloa apus* (Adrianto et al., 2024). This difference may be attributed to the variation in the source of the crude drug (simplisia) used. Different growing environments can influence the content of secondary metabolites in plants.

Tabel 1. The Results of Phytochemistry Screening of Dry Powder and Extracts

Phtochemical compound	Yellow bamboo		Aur bamboo		Rope bamboo	
	DP	E	DP	E	DP	E
Alkaloid	-	-	-	-	-	-
Flavonoid	-	-	-	-	-	-
Phenol	+	+	+	+	+	+
Quinone	-	-	-	-	-	-
Saponin	+	+	+	+	+	+
Tannin	-	-	-	-	-	-
Steroid/ Triterpen	+	+	+	+	+	+

Note: DP: dry powder
E: extract
+: detected
-: not detected

Antioxidant activity was evaluated using the DPPH method and a UV-Vis spectrophotometer, based on the color change observed in DPPH free radicals (Abdullah et al., 2022). A DPPH solution in ethanol shows a strong purple absorption at a wavelength of 516 nm. The compounds present in the sample can donate hydrogen atoms, leading to a reaction that transforms the purple DPPH radicals into yellow-colored, non-radical DPPH molecules, causing a visible color change. The absorbance measured corresponds to the remaining DPPH solution that did not react with the antioxidant compounds in the sample (Kedare & Singh, 2011).

The antioxidant test results are shown in Table 2. Vitamin C, used as the standard, exhibited an IC_{50} value of 3.46 µg/ml, while the IC_{50} values for the extracts of yellow bamboo, aur bamboo, and rope bamboo were 51.64, 82.04, and 76.67 µg/ml, respectively. The IC_{50} (Inhibition Concentration) value refers to the concentration of an antioxidant required to inhibit 50% of free radicals. The lower the IC_{50} value, the higher the antioxidant activity. The yellow bamboo leaf extract exhibited the highest antioxidant activity among the samples, although it was still lower than vitamin C as standard.

Table 2. Regretion Equation and IC_{50} Samples

	Vitamin C	Yellow bamboo	Aur bamboo	Rope bamboo
Regretion equation	Y=13.14 6 +4.538	Y=0.669 x - 15.564	Y=0.568 x +3.329	Y=0.619 x -2.466
IC_{50} (µg/ml)	3.46	51.64	82.04	76.67

The antioxidant potency of a sample is determined by its IC_{50} value, with values less than 50 µg/ml indicating very strong activity, between 50 and 100 µg/ml classified as strong, 100 to 250 µg/ml as moderate, 250 to 500 µg/ml as weak, and values above 500 µg/ml regarded as inactive (Wulansari, 2018). Based on this classification, the antioxidant activity of the extracts from yellow bamboo, aur bamboo, and rope bamboo leaves are categorized as strong antioxidants.

Based on the results of the phytochemical screening, phenols were detected in the extracts of yellow bamboo, aur bamboo, and rope bamboo leaves. Several studies have successfully isolated phenolic compounds from bamboo. Among them are p-coumaric acid and 4-methoxynamic acid, which were isolated from *Bambusa beecheyana* (Nuzul et al., 2022). It suggested that phenol content contribute to the bamboo leaf's antioxidant activity.

Phenols are a group of secondary metabolites in plants that possess strong antioxidant activity. The antioxidant mechanism of phenolic compounds is primarily related to their ability to neutralize free radicals, including through free radical scavenging, where phenolic compounds donate a hydrogen atom or electron to the free radicals, thereby stabilizing them and preventing oxidative damage to biomolecules such as lipids, proteins, and DNA. The hydroxyl groups (-OH) in the phenolic structure play a crucial role in this process by providing hydrogen to reduce the free radicals (Rice-Evans et al., 1997).

Based on Vuolo's publication, phenolic compound antioxidants exert their effects mainly via two mechanisms: electron donation and neutralization of free radicals. In the first mechanism, the antioxidant (ArOH) transforms into a radical itself after donating a hydrogen atom, which is taken up by the free radical (R*)

$$R^{\cdot} + ArOH \rightarrow RH + ARO^{\cdot}$$

In the second mechanism, the free radical is converted into a cation radical as a result of

receiving an electron from the antioxidant (Vuolo et al., 2019)

$$R^{\cdot} + ArOH \rightarrow RH^{-} + ARO^{\cdot +}$$

CONCLUSION

The results of the phytochemical screening showed that the dry powder and leaf extracts of yellow bamboo, aur bamboo, and rope bamboo contain phenols, saponins, and steroids/triterpenes.

The results of the antioxidant activity test showed that the leaves extracts of yellow bamboo, aur bamboo, and rope bamboo exhibited strong antioxidant activity, with IC_{50} values of 51.64, 82.04, and 76.67 µg/ml, respectively.

This study indicates that the leaves of yellow bamboo, aur bamboo, and rope bamboo from Garut have great potential as natural antioxidant sources that may help prevent and treat diseases related to oxidative stress.

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