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In Vitro Anti-inflammatory Activity of Bamboo Tali Leaf (*Gigantochloa apus*) Ethanol Extract

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Abstract

Inflammation is a physiological process that serves as a defense mechanism for the body against foreign substances, bacteria, or irritants. Inflammation can be cured with anti-inflammatory drugs. One of the plants that has the potential to be an anti-inflammatory agent is the bamboo leaf. This research aims to analyze the content of secondary metabolites, determine the inhibition value and IC_{50} value of the anti-inflammatory activity of the ethanol extract of bamboo tali leaves. Bamboo tali leaves were macerated using 96% ethanol and subjected to phytochemical screening. The extract was then tested for anti-inflammatory activity in vitro with the Bovine Serum Albumin (BSA) protein denaturation inhibition method. Bamboo tali leaf ethanol extract contains flavonoids, alkaloids, saponins, and phenols. The anti-inflammatory activity of the ethanol extract of bamboo tali leaves with concentrations of 28, 42, 56, 70, and 84 ppm had an inhibition percentage value of 23.14 ± 0.008%; 34.30 0.026%; 54.51 0.060%; 69.07 ± 0.006%; and 87.02 ± 0.021% with an IC_{50} value of 52.991 ppm. These results indicate that the ethanol extract of bamboo tali leaves has the potential to be an anti-inflammatory with a strong IC_{50} value below 100 ppm.

Keywords: Anti-inflammatory; Bamboo tali; Phytochemicals; Protein denaturation; Secondary Metabolites.

INTRODUCTION

A physiological process called inflammation includes a living thing's immune system. Inflammation serves as a physiological defense against certain diseases, such as cancer, by shielding the organism from microbial infection (Bouyahya *at al.*, 2022). There are several signs of inflammation in the body, including redness, fever, edema, and loss of tissue function (Novika *at al.*, 2021). Inflammation can be treated with synthetic medications such ibuprofen, aspirin, diclofenac sodium, and celecoxib. Long-term use of these medications may result in gastrointestinal adverse effects (Izzany *at al.*, 2018). Research into anti-inflammatory medications manufactured from natural substances, sometimes known as herbal medicines, is therefore required.

In Indonesia, herbal remedies use is one way to utilize of the country's natural resources. One of the bamboo species found in Indonesia, bamboo tali, has been extensively used in the medical field. One component of rope bamboo, called bamboo tali leaves, is not appropriate for use because it is regarded as waste for the environment (Fitriani, 2018). Extraction of the bioactive chemicals present in bamboo leaves is one strategy that can be used to make use of them (Romansyah *at al.*, 2019). In bamboo tali (*Gigantochloa apus*) leaf extract macerated with 70% ethanol, bioactive substances including alkaloids, saponins, tannins, phenols, flavonoids, triterpenoids, steroids, and glycosides were discovered (Setiawan & Yusransyah, 2018). secondary metabolites with anti-inflammatory effects, including as phenols, saponins, and tannins. These metabolites work to suppress the production of inflammatory mediators and increase free radical production (Armadany *at al.*, 2020). Consequently, the bamboo tali's leaves can be utilized as an anti-inflammatory medication.

Through the approach of preventing protein denaturation, anti-inflammatory activity can be tested. This is due to the fact that protein denaturation is one of the causes of tissue inflammation (Farida, Rahmat, & Amanda, 2018). The protein bovine serum albumin (BSA) was utilized in this study. The way the sample solution interacts with BSA demonstrates the theory behind protein denaturation inhibition. Free radical generation causes body proteins to be more susceptible to denaturation. The outcome of the inflammatory process is the release of inflammatory mediators (Shalihah *at al.*, 2022).

MATERIALS AND METHODS

Materials and Tools

The tools used in this study were volume pipettes, stir bars, spatulas, thermometers, test tubes, test tube racks,

Erlenmeyer (IWAKI), porcelain cups, volumetric flasks (100 mL, 100 mL, 25 mL, and 10 mL) (IWAKI), vials, cuvettes, dark glass bottles, pH meter, analytical balance (AND), blender (COSMOS), Centrifuge (PLC SERIES), 1 set of distillation apparatus, one set of vacuum rotary evaporator, and UV-Vis spectrophotometry instrument (ORION AQUAMATE 8000).

The juvenile leaves of *Gigantochloa apus*, a species of rope bamboo, were the materials employed in this investigation. In this study, additives with proanalytical quality were employed. 96% technical ethanol, distilled water, Mg, HCl, Wagner reagent, $FeCl_3$, bovine serum albumin (BSA), tris buffer saline (TBS), NaCl (Merck), and CH_3COOH are the other ingredients.

Methods

Making powder from bamboo tali leaf *(Wahyuni at al., 2020)*

Bamboo leaves are removed, cleaned under running water, and allowed to air dry for about five days. Using a blender, the little bits of dried leaves were mixed into a powder.

Figure 1. Bamboo tali leaf.

Calculation of Bamboo Tali Leaf Powder's Moisture Content *(Fitri & Anita, 2014)*

Two grams of powdered rope bamboo leaf were baked for 30 minutes at 105 degrees. It was then placed in a desiccator to chill for 15 minutes. After cooling, the resulting weight is weighed to ascertain the water content**.**

$$\%\, Moisture\ content\ = \frac{b-(c-a)}{b} \qquad \text{(Eq. 1)}$$

Information:

a : weight of the cup (g)
b : sample weight (g)
c : cup weight + sample (g)

Producing Bamboo tali Leaf Extract from Tali leaf *(Wahyuni at al., 2020)*

Bamboo tali leaf powder was macerated with 96% ethanol that has undergone a 1:5 (w/v) distillation ratio. This procedure is continued until the combination turns a light shade of clear, provided that it is filtered and the solvent is changed once every 24 hours in order to acquire the ethanol extract and determine the yield level.

$$\%Yield = \frac{\boldsymbol{Extraxt\ weight}}{\boldsymbol{Simplicia\ weight}} 100\% \qquad \text{(Eq. 2)}$$

Phytochemical Screening Test

Flavonoid *(Romansyah at al., 2019)*

Two milliliters of the sample in total, heated for 5 minutes. The material is heated before being combined with five drops of concentrated HCl and 0.1 g of Mg metal. Positive reaction is indicated by a yellowish-orange to reddish solution.

Alkaloid *(Wahid & Safwan, 2020)*

Wagner's reagent was added in three separate drops to the 2 mL extract sample. If a brown hue shows up, the sample is positive for alkaloids.

Saponin *(Wahid & Safwan, 2020)*

A test tube containing 3 mL of the extract sample was then filled with 10 mL of hot water, cooled, and vigorously shaken for 10 seconds. One drop of pure HCl was then added after that. The positive sample includes saponins if the foam that forms does not vanish.

Tannin

The extract sample, a total of 3 mL, was heated for 5 minutes before being combined with 5 drops of 1% FeCl3. According to Romansyah et al. (2019), the development of dark blue or blackish green is a sign that tannins are present.

Phenol *(Putri, 2018)*

Two milliliters of the extract sample were mixed with five drops of 1% FeCl3. A positive sample that contains phenol is indicated if a solid black or bluish green color is produced.

An anti-inflammatory effect Test

TBS (Tris Buffer Saline) production *(Barr at al., 2006)*

900 mL of distilled water was added after 8.7 g of NaCl and 1.21 g of tris base were weighed in total. After then, the pH was maintained at 6.2–6.5 by adding glacial CH3COOH. A 1000 mL volumetric flask is filled with a stable NaCl and tris base mixture, and the pH is then raised to the desired level using distilled water.

BSA (bovine serum albumin) production 0.2% *(Williams at al., 2008)*

0.2 g of BSA was added to a 100 mL volumetric flask, and the volume was subsequently raised to 100 mL with TBS solution.

Positive Controls Creation *(Rusli & Setiawan, 2020)*

Two milliliters of 0.2% BSA solution were combined with 1.5 mL of TBS and 1.5 mL of distilled water to

create a 5 mL volumetric flask. Without any additional processing, the mixture's absorbance value was instantly determined using a UV-Vis spectrophotometer at the optimized wavelength of 289 nm. Duplicate runs of the experiment were conducted.

Negative Control Creation (Rusli & Setiawan, 2020)
Two milliliters of 0.2% BSA solution, 1.5 mL TBS, and enough distilled water to reach the boundary mark should be added to the 5 mL volumetric flask. The mixture was cooked for 30 minutes at 37°C and then for another 10 minutes at 72°C. Following the completion of the heating phase, the mixture was centrifuged at 6,000 rpm for 10 minutes while maintaining a temperature of 32 °C. At a wavelength of 289 nm, the absorbance value was measured twice (duplo). A 5 mL volumetric flask was filled to the boundary mark with distilled water, 1.5 mL TBS, and 2 mL of 0.2% BSA solution. The mixture was cooked for 30 minutes at 37°C and then for another 10 minutes at 72°C. Following the completion of the heating phase, the mixture was centrifuged at 6,000 rpm for 10 minutes while maintaining a temperature of 32 °C. At a wavelength of 289 nm, the absorbance value is measured twice.

Developing the primary and test solutions *(Muliati, 2014)*
In order to create mother liquor, a 10 mL volumetric flask containing up to 70 mg of an ethanol extract of bamboo tali leaves was combined with 10 mL of ethanol. This resulted in a concentration of 7,000 ppm. Following that, the mother liquor concentration was adjusted to 840, 700, 560, 420, and 280 ppm.

Testing for Anti-Inflammatory *(Novika at al., 2021)*
To a mixture of 2 mL of 0.2% BSA solution, 1.5 mL of TBS, and distilled water, a total of 0.5 mL of the test solution at various concentrations was added. Concentration changes from the solution were 84, 70, 56, 42, and 28 ppm. The temperature of each concentrated solution was raised for 10 minutes at 72°C after 30 minutes at 37°C. Following the completion of the heating operation, the mixture's temperature was maintained at 32°C for 30 minutes before being centrifuged at 6,000 rpm for 10 minutes. A UV-Vis spectrophotometer was used to measure the absorbance, which was repeated twice (duplo). (Shalihah *at al.,* 2022). Once the absorbance value has been read, each solution's percent inhibition value has to be calculated. If a natural product's % inhibition value is greater than 20%, it is considered to have anti-inflammatory activity.

$$\%Inhibition = \frac{test\ absorbance - negative\ control\ absorbance}{positive\ control\ absorbance - negative\ control\ absorbance} \times 100\% \quad \text{(Eq. 3)}$$

RESULTS AND DISCUSSION

Sample preparation
Using a blender, the dried bamboo leaves were chopped and ground into a fine, light-weight powder that was light green in color. Bamboo tali leaf powder has a moisture level of 3.42%. 10% of the sample must be water-free to maintain sample quality (Wijaya & Noviana, 2022). These facts demonstrate that the sample of rope bamboo leaves satisfies the water content requirements. The sample's water content has a significant impact on its quality and shelf life, making it more susceptible to damage and microbial contamination (Hutauruk *at al.,* 2014).

Obtainable Yield
At a temperature of 60 °C and a rotational speed of 70 rpm, the macerated filtrate was concentrated. Because ethanol has a high boiling point (79.37°C), using a temperature of 60°C during the evaporation process aims to prevent harming the bioactive components. A small amount of oil and a dark, viscous extract are produced by the evaporation process. 12.34 g of the extracted viscous material had a yield value of 12.34%. To estimate the value of bioactive components that can be extracted using solvents, the percent yield is calculated (Dewatisari *at al.,* 2018).

Phytochemical ScreeningTest
As can be seen in table 1, the phytochemical screening of the ethanol extract of bamboo leaves revealed the presence of flavonoids, alkaloids, saponins, and phenols (Table. 1).

Table 1. Results of Phytochemical Testing.

Bioactive Substances	Description of Phytochemical Test Results	Information
Flavonoid	orange-yellow	+
Alkaloid	Brown with developed black clumps	+
Saponin	Formed Stable Foam	+
Tannin	Brown	-
Phenol	Solid Black	+

Information: (+) : Has bioactive ingredients, (-) : Does not include any bioactive ingredients

Inflammatory-Reduction Capacity

The BSA protein denaturation inhibition method was used to assess the ethanol extract of bamboo tali leaves for anti-inflammatory effects. BSA is a protein derived from cow albumin. BSA works in enzymatic processes to stabilize proteins. BSA has a pH between 6.0 and 8.5 (Borzova *at al.,* 2016). Because BSA is simple to produce, easily soluble, affordable, and minimizes the use of live specimens in the drug development process, it is used in this study (Novika *at al.,* 2021). One of the reasons why the cell tissues of the body get inflamed is protein denaturation. Protein denaturation is the process by which pressure or chemicals from the outside result in an alteration of the tertiary and secondary structure of proteins or nucleic acids. The approach of protein denaturation inhibition was chosen because it is non-acidic, inexpensive, and simple to use (Aditya *at al.,* 2015).

Table 2. results of Bamboo tali leaf extract's % BSA inhibition.

No	Concentration (ppm)	%Inhibition
1.	28	23,14±0,008
2.	42	34,30±0,026
3.	56	54,51±0,060
4	70	69,07±0,006
5.	84	87,02±0,021

Table 3. Results of % inhibition of BSA by diclofenac sodium.

No	Concentration (ppm)	%Inhibition
1.	10	26,38%±0,006
2.	20	35,77%±0,001
3.	30	44,54%±0,000
4	40	56,31%±0,001
5.	50	66,73%±0,001

When BSA protein is heated, protein denaturation takes place (Fitriyani & Fatahillah, 2022). The protein's molecules travel quicker and sustain damage as a result of the rising temperature's increased kinetic energy (Shalihah *at al.,* 2022). As a buffer solution, BSA was dissolved in Tris Buffer Saline (TBS). BSA's ideal pH is 7, so TBS solution was selected because it has a pH of 6.25 to 6.5 (Abidin *at al.,* 2020). Percent inhibition is used to express the protein denaturation activity. When the proportion of inhibition is greater than 20%, anti-inflammatory action is present (Farida, Rahmat, & Widia Amanda, 2018). Tables 2 and 3 show the findings of research on the anti-inflammatory effects of diclofenac sodium and an ethanol extract of bamboo tali leaves.

The percentage of inhibition for each extract concentration is shown in Table 2. The % inhibition values for concentrations of 28, 42, 56, 70, and 84 are known to already meet the criterion for % inhibition of protein denaturation, namely > 20%, based on Table 2. The cell membrane will be harmed by protein denaturation, which will allow the phospholipase enzyme to convert phospholipids into arachidonic acid. Cyclooxygenase (COOX) enzymes will then break down arachidonic acid to create prostaglandins, which can activate pain receptors and increase capillary permeability (Fitriyani & Fatahillah, 2022). Secondary metabolites have the ability to limit the synthesis of prostaglandins. Bamboo tali leaf extract has anti-inflammatory properties due to the presence of secondary metabolites including flavonoids, alkaloids, saponins, and phenols.

Through the action of the phospholipase enzyme, flavonoids prevent the synthesis of prostaglandins (Bustanul & Sanusi, 2018). Because of their capacity to suppress the creation of cytokines that promote inflammation, alkaloids have the potential to be anti-inflammatory medications. Saponins' anti-inflammatory mechanism, which involves preventing the breakdown of glucocorticoids and the release of inflammatory mediators (Mohammed *at al.,* 2014). Phenol is a secondary metabolite with OH groups, which can protect membranes, prevent the production of inflammatory mediators, and deactivate free radicals (Novika *at al.,* 2021).

The anti-inflammatory efficacy of the dicoflenac sodium medication is shown in Table 3. According to the data in table 3, all of the variants employed satisfied the requirements for % protein denaturation inhibition. Diclofenac sodium was chosen since it is one of the most effective anti-inflammatory medications (Novika *at al.,* 2021). Diclofenac sodium is a heteroarylacetate salt of sodium (2,6-dichlorophene) (Fitri Yani & Reynaldi, 2021). Figure 2 depicts the structure of diclofenac sodium. Diclofenac sodium can inhibit the cyclooxygenase COX-1 and COX-2 enzymes, lowering prostaglandin, prostacyclin, and thromboxane synthesis (Altman *at al.,* 2015).

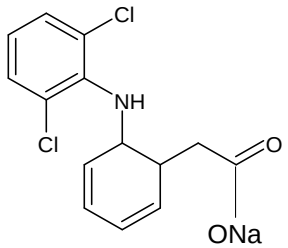

Figure 2. Diclofenac Sodium Structure.

After acquiring data on the percentage inhibition value, the IC_{50} value is calculated. The anti-inflammatory test results are interpreted using the IC_{50} value. The IC_{50} value denotes the concentration of the test substance that can reduce inflammation by 50%. As illustrated in Figures 3 and 4, the obtained % inhibition value data is entered into the linear regression equation with the extract concentration (ppm) as the x-axis and the % inhibition value as the y-axis.

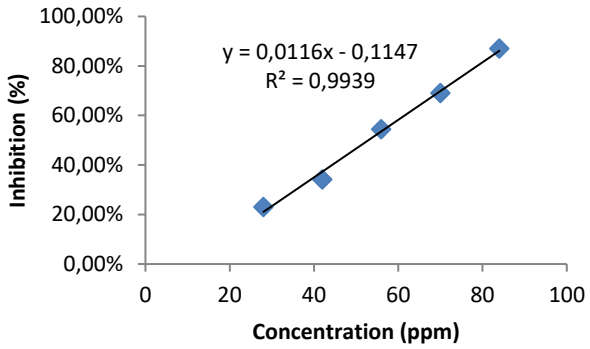

Figure 3. Linear Regression Curve of Anti-inflammatory Activity of Bamboo Tali Leaf Ethanol Extract.

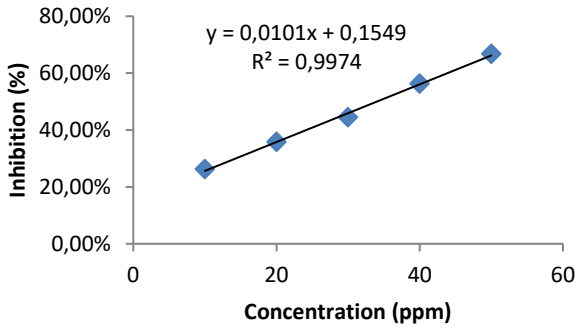

Figure 4. Linear regression curve of diclofenac sodium extract anti-inflammatory efficacy.

Figure 3 displays the linear regression curve of the ethanol extract of Bambu Tali leaves, yielding y = 0.0116x-0.1147 and R^2 = 0.9939. The obtained correlation r value is excellent. In the region 0.80-1.00, where the correlation of the variables grows and the line has a positive slope, the correlation coefficient is quite strong. The IC_{50} value is calculated using the curve. According to the curve analysis results, each rise in concentration appears to be proportional to an increase in the value of % inhibition. The curve line producing a linear line for each rise in the logarithm of concentration demonstrates this. The IC_{50} value is a metric that indicates the extract's efficiency in suppressing protein denaturation with a percentage value of up to 50%. Bamboo tali Leaf Extract has an IC_{50} value of 52.991 ppm. This value indicates that at a concentration of 52.991 ppm, the ethanol extract of bamboo tali leaves inhibited protein denaturation by 50%.

Figure 4 depicts the linear regression curve for diclofenac sodium's anti-inflammatory efficacy. The curve yields the result y = 0.0101x + 0.1549 with R^2 = 0.9974, allowing the IC_{50} value to be calculated. Diclofenac sodium has an IC_{50} value of 34.168 ppm. The IC_{50} value of diclofenac sodium demonstrated more anti-inflammatory efficacy than the ethanol extract of bamboo tali leaves. This is due to diclofenac sodium's potent anti-inflammatory properties. Furthermore, dicloofenac sodium has a faster absorption and pain alleviation than other nonsteroidal anti-inflammatory medicines like ibuprofen or naproxen (Altman *at al.*, 2015).

CONCLUSIONS

Bamboo tali leaves (*Gigantochloa apus*) ethanol extract contains secondary metabolites including flavonoids, alkaloids, saponins, and phenols. The anti-inflammatory activity of the ethanol extract of bamboo tali leaves (*Gigantochloa apus*) at concentrations of 28, 42, 56, 70, and 84 ppm was 23.14%, 34.48%, 55.12%, 69.30%, and 87.41%, respectively, with an IC_{50} value of 52.991 ppm.

Competing Interests: The authors declare that there are no competing interests.

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