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Research Article

Antibacterial Activity of Tali Bamboo (*Gigantochloa apus*) Leaf Extract to Inhibit *Escherichia coli* and *Salmonella typhimurium*

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Abstract: Bamboo leaves contain phytochemical compounds that have the potential to act as antimicrobial agents in inhibiting pathogenic bacteria. This study aimed to evaluate the potential of tali bamboo (*Gigantochloa apus*) leaves extracted with different solvents to inhibit the growth of *Escherichia coli* and *Salmonella typhimurium*. The tali bamboo leaves were extracted using ethanol and methanol with a ratio of 1:10 between tali bamboo leaf and solvent. The antibacterial activity of bamboo leaf was carried out using the agar diffusion method at varying concentrations (0% - 0.08%), with cotrimoxazole was used as the positive control. Phytochemical analysis confirmed the presence of tannins, steroids, saponins, and flavonoids in bamboo tali leaf extract. The inhibition zone test showed that methanol extract of *G. apus* did not inhibit the growth of *E. coli* and *S. typhimurium*, whereas ethanol extract had weak antibacterial activity (clearing zone < 5 mm) with a minimum inhibitory concentration of 0.2% for *E. coli* and 0.3% for *S. typhimurium*. Based on the results, it can be concluded that ethanol extract of *G. apus* has the potential as a natural antibacterial agent against pathogenic bacteria.

Keywords: Antibacterial Activity, Bamboo Leaves, MIC, MBC, Phytochemical Compound

Introduction

Diarrhea is a disease that can cause disadvantages and mortality, especially for young ruminants. Schinwald *et al.* (2022) stated that calf mortality caused by diarrhea can reach up to 57%. It also agreed with Jessop *et al.* (2024) that diarrhea caused morbidity and mortality in calves. Diarrhea will cause dehydration so the electrolytes in ruminants are unbalanced and in the extreme condition, it will cause mortality (Caffarena *et al.*, 2021). Diarrhea also reduces livestock productivity, such as decreased body weight and milk production, resulting in economic losses for farmers (Abdou *et al.*, 2021). This disease was caused by infection from pathogenic bacteria such as *E. coli* and Salmonella spp. (Caffarena *et al.*, 2021; Gomez *et al.*, 2022). Chigerwe and Heller (2018) stated that another bacteria that can cause diarrhea in ruminants is *S. typhimurium*. El-Seedy *et al.* (2016) also noted that the type of Salmonella most found in feces samples from diarrhea calves was *S. typhimurium*, which reached 30.4%. This pathogenic bacterial infection can come from given feed and water contamination of livestock (El-Seedy *et al.*, 2016). Pathogenic bacterial contamination can also be caused by contact with the mother's udder, bedding material, milk processing tools or feces in the pen (Bekuma and Galmessa, 2018; Deddefo *et al.*, 2023). Salmonella spp. damages the digestive tract epithelium and causes inflammation resulting in malabsorption of water, electrolytes, and other nutrients (Jessop *et al.*, 2024). Diarrheal calves will suffer from dehydration, acidosis, hypovolemia, decreased feed consumption, and difficulty standing (Shehta *et al.*, 2022).

Antibiotics are generally used to manage diseases caused by pathogenic bacteria including diarrhea. The study conducted by Eibl *et al.* (2021) revealed that diarrhea calves, especially newborn livestock, were medicated using antibiotics in several countries. Antibiotics that were often used for diarrhea in livestock were amoxicillin, ceftiofur, ampicillin, nitazoxanide, florfenicol, danofloxacin, marbofloxacin, and so on (Bernal-Cordoba *et al.*, 2023). However, the application of antibiotics was limited because they can cause Antimicrobial Resistance (AMR). In Indonesia, restrictions on the use of antibiotics were contained in Minister of Agriculture Regulation (2017) Number 14, which prohibited the use of antibiotics to increase livestock growth or production. Still, antibiotics used on sick livestock are permitted. However, it must comply with the rules and guidelines for using the correct dose and duration to ensure the safety of livestock products (such as meat, eggs and milk) for consumers. Overuse or misuse of antibiotics will cause bacterial resistance because pathogenic bacteria could mutate the structure of antibiotics DNA target, limiting the uptake of antibiotics through porin channels, inactivating antibiotics, and eliminating antibiotic substances using transporter families and efflux pumping mechanisms (Reygaert, 2018; Devi *et al.*, 2024). This was also in line with Devi *et al.* (2024), who stated that the application of antibiotics in livestock significantly contributed to the spread of resistant bacteria. Therefore, to minimize the use of antibiotics, it was necessary to look for natural ingredients that have antibacterial capabilities to treat livestock with diarrhea.

Bamboo leaves have the potential as an antimicrobial agent. Sola *et al.* (2023) showed that bamboo leaves were able to inhibit the growth of Streptococcus pneumoniae, Staphylococcus aureus, and Klebsiella pneumoniae. The addition of extract bamboo leaves type Bambusa vulgaris can also inhibit the growth of Staphylococcus aureus and Escherichia coli (Musyimi *et al.*, 2023). Bamboo leaves contain secondary metabolite compounds such as flavonoids, saponins, glycosides, tannins, and terpenoids, which can inhibit the growth of pathogenic bacteria (Musyimi *et al.*, 2023). Bamboo leaves also contain 27 other secondary metabolite compounds, including alkaloids, flavonoids, steroids, phenolic acids, fatty acid derivatives, and isoprenoids (Govindan *et al.*, 2018). One of the bamboo leaves that have the potential as a source of metabolite compounds in Indonesia is the Tali bamboo (*Gigantochloa apus*) leaves because tali bamboo is a type of bamboo that grows widely in Indonesia (Rahmawati *et al.*, 2019). Bubonja-Sonje *et al.* (2020) stated that the metabolite compound content of each plant was different, and it would affect their antibacterial activity. Therefore, the metabolite compounds from tali bamboo leaves need to be investigated. Another thing that influences the extraction results of metabolite compounds is the type of solvent used. However, there was limited research conducted to evaluate the antibacterial activity of *G. apus* leaves, so this research aimed to evaluate the antibacterial potential of tali bamboo (*G. apus*) leaves extracted with different solvents to inhibit *E. coli* and *S. typhimurium*.

Materials and Methods

Plant Materials

The bamboo leaves used in this research are old leaves from 4 years old Tali Bamboo (*G. apus*) harvested from Bogor (West Java, Indonesia). The use of 4-year-old bamboo leaves was chosen because, at this stage, the bamboo has reached maturity, resulting in a higher and more stable accumulation of secondary metabolite compounds (Long *et al.*, 2023; Li and Sun, 2024).

Bamboo leaf preparation began with washing the leaves to remove dirt. After that, the bamboo leaves were drained and cut into 5 cm. The bamboo leaves were placed into an oven at 60 ^{0}C for 2 days until the leaves were completely dry. The leaves were then crushed until flour formed.

Extraction Procedure

Bamboo leaf extraction was carried out using 2 solvents (96% methanol and 70% ethanol). The use of ethanol 70% was aimed to extract either water-soluble or non-water-soluble components. The ratio of bamboo leaves and solvent was 1:10, so 50 g of bamboo leaf flour and 500 mL of solvent were added to an Erlenmeyer. After that, the Erlenmeyer was incubated in a shaker water bath for 30 hours at room temperature (Kemit *et al.*, 2016). The maceration results were filtered, and then the solution was concentrated using a rotary vacuum evaporator at 40 ^{0}C for methanol extract and 55 ^{0}C for ethanol extract.

Nutrients and Bioactive Compounds of Bamboo Leaves

In this study, the nutrient content analysis of bamboo leaves was carried out using the proximate method (AOAC, 2005) to measure water content, crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE) and ash. The bioactive compounds analysis was done using the method conducted by Ramli *et al.* (2020) for flavonoids, alkaloids, steroids, triterpenoids, tannins and saponins content.

Antimicrobial Activity: Inhibition Zone Test

The pathogenic bacteria used in this research were *E. coli* and *S. typhimurium* obtained from IPB Culture Collections (IPBCC). The antimicrobial screening was carried out using the agar well diffusion method (Gonelimali *et al.*, 2018). First, 1 mL of fresh bacterial isolate and 20 ml of Mueller Hinton Agar (MHA) media were added into the petri dish. The media was homogenized and kept at room temperature until the agar solidified. After solidifying, the media was perforated using a sterile cork driller with a diameter of 6 mm. Each well of agar was added with 50 µl of bamboo leaves extract with different concentrations (0.00%, 0.02%, 0.04%, 0.06% and 0.08%). In the middle of the agar well, 50 µl of 0.02% cotrimoxazole was added as a positive control. The agar medium added with bamboo leaf extract and antibiotics was placed in the refrigerator for 3 hours to allow the extracted bamboo leaves to be absorbed properly (Magdalena and Kusnadi, 2015). The petri dish was incubated at 37 ^{0}C for 24 hours. The inhibition zone was measured by sizing the area of the clearing zone formed around the well (Bubonja-Sonje *et al.*, 2020).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC value was the minimum concentration of an ingredient that can inhibit the growth of bacteria, while the MBC is the minimum concentration of an ingredient that can kill bacteria (Schrader *et al.*, 2023). The concentrations of bamboo leaf extract used in this test were 0.0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, and 0.9%. Determination of MIC and MBC values was carried out by making 10 test tube series, and each tube contained 11.232 µL of sterile Nutrient Broth (NB) media. The tubes with 0.0% extract were made by adding 11.232 µL of sterile NB + 0µL of extract fluid + 648 µL of sterile aquadest. In comparison, series tubes with the highest concentration used in this study (0.9%) were made by adding 11.232 µL of sterile NB + 648 µL extract fluid + 0 µL sterile aquadest and so on, so each series of test tubes is filled with 11.880 µL of solution. After the entire series was ready, all tubes were added with 120 µL of bacterial suspension, so the total fluid in the tube was 12 ml (12.000 µL). Each tube was homogenized using a vortex for 1-2 minutes, then incubated at 37 °C for 24 hours. The percentage of bacterial growth inhibition is calculated using the formula:

$$\text{Inhibition Percentage} = 100 - \left(N_t \times \frac{100}{N_0}\right)$$

Where:

N_t is the Number of bacteria in the extract addition treatment (CFU/ml)

N_0 is the Number of bacteria in the original inoculum (CFU/ml)

Data Analysis

Antimicrobial activity results were analyzed with a descriptive method, and MBC was calculated using a simple linear regression analysis. The MBC value was obtained based on the regression equation y = 0.403x – 39.286 for *E. coli* and y = 1.06x - 105.04 for *S. typhimurium* with y = extract concentrations and x = % of inhibition.

Results and Discussion

Nutrient Content of Bamboo Leaves

The nutrient content of tali bamboo (*G. apus*) leaves in this research is presented in Table 1. The data showed that bamboo leaves contain a high moisture level (43.39%), a moderate protein level (14.81%), and a low ether extract (4.07%). These findings suggest that tali bamboo leaves have the potential to be a valuable feed resource. Singhal (2023) said that 27 types of bamboo leaves have crude protein in the range from 9-19% and crude fiber range from 18 to 34% and Andriarimalala *et al.* (2019) showed that 9 types of bamboo leaves used in their research had nutrient content DM 44.50-61.90%, crude protein 7.71 – 15.40%, crude fiber 26.30-32.60%, and ash 6.68-18.50%. The results in Table 1 showed that the crude fiber content of tali bamboo leaves (45.26%) was higher than Singhal (2023) and Andriarimalala *et al.* (2019). This was caused by the old bamboo leaves used in this study. The fiber content in plants was influenced by harvested age (Khalil *et al.*, 2015). Other factors that influenced the nutrient content in plants were climate, soil properties, and heavy metal content in the soil (Khalil *et al.*, 2015).

Table 1: Nutrient content of tali bamboo leaf

Nutrient	Value (% DM)
Ether Extract	4.07
Crude Protein	14.81
Crude Fiber	45.26
Ash	15.50
NFE	20.36

Note: All values are expressed on a dry matter basis (100% DM).

DM = Dry Matter; NFE = Nitrogen Free Extract

NFE = 100 – (Crude Protein + Ether Extract + Crude Fiber + Ash)

Phytochemical Compounds

The phytochemical results of extract ethanol tali bamboo leaf were presented in Table 2. The result showed that ethanol extracts of tali bamboo contain a high concentration of tannins, and a medium concentration of flavonoids, saponins and steroids. The result also showed the extract ethanol did not contain alkaloids and triterpenoids. The research conducted by Pujiarti *et al.* (2020) showed that extract ethanol and hot water of Dendrocalamus asper and Gigantochloa verticillata bamboo leaves contained flavonoids and tannins but did not contain saponins and alkaloids. In contrast, the results of Maya *et al.* (2023) showed that extract methanol of Bambusa tulda and Dendrocalamus strictus leaves contained terpenoids, flavonoids, saponins, phenols, alkaloids, glycosides, and steroids. Differences in the type of bamboo and solvent used caused this. The Solvent was a factor that determines the extraction process and influences the amount and types of secondary metabolite compounds extracted from plants (Khan *et al.*, 2022). Kamarudin *et al.* (2021) also said that the quality and quantity of secondary metabolite compounds extracted from plants were influenced by the extraction method, the type and polarity of the solvent, and the chemical structure of the extracted target compounds. Plants phytochemical compounds were also influenced by several factors, such as geographical location and climatic conditions, temperature, humidity and rainfall (Hayat *et al.*, 2020). Table 2 also showed that tannin was the most abundant phytochemical compound extracted using ethanol solvent. This was in line with Hayat *et al.* (2020) that plants extracted using ethanol produce tannins higher than flavonoids and polyphenols. Because ethanol was a polar solvent, tannins with higher polarity than flavonoids can be easily extracted. Tannin was a secondary metabolite compound that had high polarity and a hydroxyl group (Chang *et al.*, 2019; Maharani *et al.*, 2020; Ruiz-Aquino *et al.*, 2023) so it is easy to make hydrogen bonds with ethanol (McRae *et al.*, 2015).

Table 2: Phytochemical compounds of tali bamboo leaf ethanol extract

Chemical Compounds	Result
Flavonoids	++
Alkaloids	-
Tannins	+++
Saponins	++
Steroids	++
Triterpenoids	-

Note: - = Absent; + = Low concentration; ++ = Moderate concentration; +++ = High concentration

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Antimicrobial Activity: Inhibition Zone Test

The ability of tali bamboo leaf extract against the growth of pathogenic bacteria such as *E. coli* and *S. typhimurium* is presented in Table 3. Davis and Stout (1971) stated that the antimicrobial activity is categorized as very strong when the inhibition zone is more than 20 mm, 10-20 mm strong, 5-10 mm medium, and less than 5 mm are low. The result showed that the ethanol extract of tali bamboo had low antimicrobial activity, and methanol extract did not have antimicrobial activity. Meanwhile, cotrimoxazole (antibiotics) had very high antimicrobial activity for *E. coli* and high activity for *S. typhimurium*. This study showed lower results than Indriatie *et al.* (2019), which showed that administration of 5.00 mg/ml *G. apus* leaf extract formed a 3.94 mm clearing zone in inhibiting the growth of *E. coli*. It was caused by the use of 70% ethanol in this study, whereas Indriatie *et al.* (2019) used ethanol 96%. Hikmawanti *et al.* (2021) stated that differences in the concentration of ethanol used will affect the amount of metabolite compounds extracted. The use of 70% ethanol in this study aimed to extract both water-soluble and non water-soluble compounds from bamboo leaves. However, the results showed that 70% ethanol produced a smaller clearing zone compared to 96% ethanol. This may be due to the dominance of non-water-soluble phytochemical compounds in bamboo leaves, making 96% ethanol more effective as an extraction solvent for bamboo leaves.

Table 3: Antibacterial activity of tali bamboo (*G. apus*) leaf extract

Solvent	Concentration (% w/v)	Diameter of inhibition zone (mm)	
		E. coli	*S. typhimurium*
Ethanol	0.00	-	-
	0.02	-	-
	0.04	0.46 ± 0.37	0.92 ± 0.03
	0.06	0.63 ± 0.32	1.13 ± 0.57
	0.08	2.89 ± 1.12	2.13 ± 0.85
Methanol	0.00	-	-
	0.02	-	-
	0.04	-	-
	0.06	-	-
	0.08	-	-
Cotrimoxazole	0.02	25.82 ± 1.05	16.85 ± 2.31

Note: - = No inhibition zone (0.00 mm)

The study conducted by Borges *et al.* (2020) showed that mimosa (Acacia dealbata) leaves extracted using the solid-liquid method with ethanol solvent produced higher antibacterial activity than methanol. Borges *et al.* (2020) also showed that extracting methanol from mimosa leaves does not have an inhibition zone (clearing zone = 0.00 mm) against *E. coli* as obtained in this study. The difference in this antibacterial activity was due to differences in the ability of solvents to extract plants bioactive compounds. Ethanol has a constant dielectric value of 24 and can dissolve polar and some non-polar compounds, so the extracted compounds were more diverse (Pupitasari *et al.*, 2023). The antibacterial activity of plant extracts was the combined effect of various active compounds, not just a single compound (Vaou *et al.*, 2022).

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)

MIC and MBC values of ethanol extract for *E. coli* and *S. typhimurium* were presented in Table 4. The data showed that 0.2% ethanol extract of tali bamboo could decrease 96.12% the population of *E. coli* and 0.3% ethanol extract decreased 91.49% the population of *S. typhimurium*. Fitrial *et al.* (2008) said that a substance had antimicrobial activity if it could reduce the bacterial population by more than 90% and had bactericidal activity if it could inhibit bacterial growth by up to 100%. Table 4 showed that the MIC value of ethanol extract of tali bamboo leaves was 0.2% for *E. coli* and 0.3% for *S. typhimurium*. Regression calculations show that the MBC value of tali bamboo to kill 100% *E. coli* was 1.04% and 0.96% for *S. typhimurium*. The MIC and MBC values also showed that *G. apus* bamboo leaves have antibacterial abilities. This was because bamboo leaves contain phytochemical compounds such as tannins, steroids, saponins, and flavonoids, as presented in Table 2.

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Table 4: MIC and MBC result of extract ethanol tali bamboo leaves

Bacteria	Concentration (% w/v)	% of inhibition	Inhibition Log
E. coli	0.0	0.00	0.00
	0.1	87.99	0.92
	0.2*	96.12	1.97
	0.3	96.42	2.00
	0.4	96.66	2.03
	0.5	97.06	2.09
	0.6	99.09	2.59
	0.7	99.10	2.60
	0.8	99.26	2.69
	0.9	99.70	3.10
S. typhimurium	0.0	0.00	0.00
	0.1	12.02	0.06
	0.2	83.10	0.77
	0.3*	91.49	1.07
	0.4	95.87	1.38
	0.5	98.44	1.81
	0.6	99.65	2.45
	0.7	99.80	2.70
	0.8	99.81	2.72
	0.9	99.93	3.14

*Note: * = MIC ethanol extract of tali bamboo leaf; - = No inhibition; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration.*

Tannin is a metabolite compound that can inhibit the growth of pathogenic bacteria, both gram-positive and gram-negative bacteria (Farha *et al.*, 2020; Villanueva *et al.*, 2022). Tannins can inhibit pathogenic bacteria because they inhibit cell wall formation, disrupt the NorA efflux pump, disrupt cell permeability and disrupt metabolism in cells (Farha *et al.*, 2020; Kaczmarek, 2020; Belhaoues *et al.*, 2020) while the mechanism of action from flavonoids in inhibiting bacterial growth through inhibiting energy formation and electron transfer, inhibiting DNA and protein synthesis, inhibiting the performance of the Sortase A (SorA) enzyme, disrupting cell respiration, disrupting the efflux pump process and disrupting bacterial cell membrane phospholipids (Tan *et al.*, 2020; Yuan *et al.*, 2021; Zhang *et al.*, 2023; Rodriguez *et al.*, 2023). The antibacterial properties of saponins occur through the mechanism of disrupting bacterial cell permeability, inhibiting biofilm formation, interfering with protein formation and enzyme performance, disrupting the bacterial cell respiration system, and lying bacterial cells (Dong *et al.*, 2020; Eylands *et al.*, 2021; Cankaya and Somuncuoglu, 2021). The addition of steroid compounds will also disrupt the permeability of bacterial cells, causing cell lysis, and forming complex compounds with bacterial extracellular proteins (Widowati *et al.*, 2021; Armansyah *et al.*, 2022).

The results in Table 4 showed that the MIC value of ethanol extract tali bamboo leaves to inhibit the growth of *S. typhimurium* (0.3%) was higher than *E. coli* (0.2%). This was caused by that *E. coli* has faster growth than *S. typhimurium* so it reaches the log phase more quickly. Bacteria will be more sensitive to antibacterial compounds in the log phase than in the stationary phase (Tamer and Toprak, 2017; Shree *et al.*, 2023). In the log phase, bacterial cell wall phospholipids were lower compared to the stationary phase (Laydevant *et al.*, 2021). This increases the potential for phytochemical compounds to enter pathogenic bacterial cells and causes cell lysis. The results also showed that the MIC value of *E. coli* was lower than *S. typhimurium*, but on the contrary, it has a higher MBC value than *S. typhimurium*. This showed that the growth of *E. coli* was easily inhibited but more challenging to destroy compared to *S. typhimurium*. *E. coli* has several self-defense systems against antibacterial compounds by forming biofilms and releasing metabolite compounds using an efflux pump (Soto, 2013; Gaurav *et al.*, 2023). Biofilm formation will protect bacterial cells from antimicrobial compounds (Kalia *et al.*, 2023).

Conclusion

In conclusion, ethanol was a more suitable solvent for extracting metabolite compounds from tali bamboo (*G. apus*) leaves than methanol. Tali bamboo (*G. apus*) leaves extracted with ethanol can be used as a natural antibacterial because they have bacteriostatic and bactericidal properties, which inhibit and kill *E. coli* and *S. typhimurium*. The MIC values of *G. apus* extract were 0.2% and 0.3% for *E. coli* and *S. typhimurium*, respectively, with MBC values were 1.04% and 0.96%.

To optimize research outcomes, future studies should explore more effective extraction methods to enhance bioactive compound yield. Additionally, broader antibacterial testing against gram-positive and gram-negative bacteria is necessary to comprehensively evaluate the effectiveness of bamboo leaf extract. Further phytochemical analysis using techniques like HPLC or GC-MS is also recommended to identify active compounds in greater detail.

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Conflict of Interest

The authors have declared no conflict of interest.

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Authors' Contributions

Sinta Agustina: Design of the research conceptualization, collection and interpretation of data and write the original draft.

Komang G Wiryawan: Led the design of the research conceptualization, supervision and evaluation of the research data and content, and reviewed the manuscript.

Sri Suharti: Design of the research conceptualization, supervision and reviewed the manuscript.

Ethics

The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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