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In vitro antibacterial screening of bamboo leaves extract against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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ABSTRACT

The study evaluated the *in vitro* antibacterial activity of bamboo *(Bambusa* spp.*)* leaf extract against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using the disc diffusion method. Conducted at the Regional Animal Disease Diagnostic Laboratory in Tuguegarao City from July 19 to August 15, 2024, the experiment utilized a Complete Randomized Design with two treatments: Treatment 1 (bamboo leaf extract) and Treatment 2 (commercial antibiotic control). The antibacterial efficacy was measured based on the diameter of the zone of inhibition (ZOI) in millimeters. Results revealed that the bamboo leaf extract exhibited minimal inhibition against *E. coli* with a mean ZOI of 1.00 mm compared to 3.00 mm from the commercial antibiotic, indicating poor effectiveness. However, the extract showed moderate antibacterial activity against *S. aureus* and *P. aeruginosa*, with mean ZOIs of 3.00 mm and 3.33 mm, respectively, as compared to 5.17 mm from the commercial control. Statistical analysis revealed significant differences in inhibition zones for *S. aureus* and *P. aeruginosa* but not for *E. coli*. These findings indicate that bamboo leaf extract possesses partial antibacterial potential against Gram-positive and certain Gram-negative bacteria, suggesting that it may serve as a potential alternative or supplementary natural antimicrobial agent, particularly for infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Further *in vitro* and *in vivo* studies using other bamboo plant parts and extraction methods are recommended to confirm its antibacterial efficacy and broaden its potential medicinal applications.

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INTRODUCTION

A wide variety of pharmaceutical drugs produced by different manufacturers are available in the market and are commonly used to treat various animal diseases. These drugs are scientifically formulated to ensure effectiveness in curing specific health problems and are distributed in different forms such as tablets, injectables, lotions, and aerosols. They may be administered orally, topically, parenterally, intravenously, or intramuscularly, making them convenient and widely used by veterinarians, farmers, and backyard raisers alike. However, for many individuals living in rural or remote areas, access to such commercial medicines is often limited due to their high cost and unavailability, leading farmers to rely on locally sourced plant-based remedies.

The Philippines is endowed with a rich diversity of medicinal plants traditionally used as alternative treatments for both humans and animals. These plants are commonly prepared in crude forms such as decoctions, poultices, or liniments and have long been utilized to manage common animal health problems including parasitic infections, respiratory diseases, and digestive disorders. Although the pharmacological properties of many of these plants have yet to be scientifically validated, their widespread use and reported efficacy underscore their potential as affordable and sustainable therapeutic options.

One such promising plant is bamboo (*Bambusa* spp.), which has been widely recognized in Asia for its medicinal properties. Bamboo is known for its rapid growth and remarkable natural resistance to bacteria and pests, attributed to its inherent antibacterial compounds. These properties make it a potential source of antimicrobial agents that could help address the growing problem of antibiotic resistance- a major concern in both human and veterinary medicine. In light of this, the present study was conducted to determine the in vitro antibacterial efficacy of bamboo leaf extract against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results of this investigation aim to provide baseline information for the possible development of bamboo leaf extract as a natural and cost-effective alternative to commercially available antibiotics.

MATERIALS AND METHODS

Research design

The study was conducted using a Completely Randomized Design (CRD) with two treatments and two replications to evaluate the antibacterial activity of bamboo leaf extract against selected bacterial species.

Experimental treatments

Treatment 1 (T_1): Bamboo leaf extract
Treatment 2 (T_2): Commercial antibiotic (control)

Experimental materials

The following materials were used in the experiment: Bamboo leaves, autoclave, desiccator, filter paper, test organisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*), nutrient agar, nutrient broth, distilled water, Petri plates, test tubes, incubator, transparent ruler, and antibiotic discs**.**

Collection and preparation of plant material

Fresh bamboo leaves were collected, washed thoroughly, and cut into small pieces. The leaves were pulverized using a mortar and pestle, and the aqueous extract was obtained by squeezing and filtering the homogenized sample.

Preparation of test organisms

Pure cultures of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were obtained from the Regional Animal Disease Diagnostic Laboratory in Carig, Tuguegarao City. A broad-spectrum antibiotic was used as a positive control**.**

Preparation of culture media

Nutrient agar (NA) and nutrient broth were prepared according to the manufacturer's instructions and sterilized in an autoclave at 121°C for 15 minutes. These media were used for maintaining bacterial cultures and performing the antibacterial assay.

Preparation of inoculum

A loopful of bacterial culture was transferred into test tubes containing nutrient broth and incubated at 37°C for 24 hours to produce active bacterial suspensions for the test**.**

Antibacterial susceptibility test

The disc diffusion method was employed to determine the antibacterial activity of the bamboo leaf extract. Sterile filter paper discs (3 mm diameter) were saturated with the extract and placed on solidified nutrient agar plates inoculated with the test bacteria. Plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters using a transparent ruler.

Data collection and statistical analysis

The zone of inhibition (ZOI) was recorded for each treatment and replicate. Data were analyzed using Analysis of Variance (ANOVA) under the Complete Randomized Design to determine significant differences between treatments. Mean comparisons were interpreted using the appropriate statistical test at a 5% level of significance.

RESULTS AND DISCUSSION

General observations

The antibacterial activity of bamboo leaf extract was evaluated against three bacterial pathogens-*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using the disc diffusion method. The presence of a clear zone or inhibition zone (measured in millimeters) around the paper discs indicated the effectiveness of the extract in preventing bacterial growth. After 24 hours of incubation, results revealed variable inhibition effects among the three bacterial species tested.

Antibacterial activity against *Escherichia coli*

Table 1 presents the antibacterial effect of bamboo leaf extract and the commercial antibiotic against *Escherichia coli*. The bamboo leaf extract (Treatment 1) exhibited a mean Zone of Inhibition (ZOI) of 1.00 mm, while the commercial antibiotic (Treatment 2) produced a ZOI of 3.00 mm.

Table 1. Mean zone of inhibition (mm) of bamboo leaf extract against *Escherichia coli*

Treatment	Mean (ZOI) (mm)
T1–Bamboo leaf extract	1.00a
T2–Commercial antibiotic (Control)	3.00a

ZOI= Zone of Inhibition

Means with the same letters are not significantly different at 5% level of significance.

The results showed that the bamboo leaf extract exhibited minimal inhibitory activity against *E. coli* compared to the commercial antibiotic. Statistical analysis indicated no significant difference between treatments, suggesting that bamboo leaf extract was ineffective in suppressing *E. coli* growth. This may be due to the complex structure of gram-negative bacterial cell walls that can restrict the penetration of plant-based compounds.

Antibacterial activity against *Staphylococcus aureus*

Results of the antibacterial test against *Staphylococcus aureus* are summarized in Table 2. The bamboo leaf extract (T1) produced a mean ZOI of 3.00 mm, whereas the commercial antibiotic (T2) produced 5.17 mm.

Table 2. Mean zone of inhibition (mm) of bamboo leaf extract against *Staphylococcus aureus*

Treatment	Mean (ZOI) (mm)
T1–Bamboo leaf extract	3.00
t2–Commercial antibiotic (control)	5.17

ZOI= Zone of Inhibition

Analysis of Variance revealed a significant difference between treatments at 5% level. The bamboo leaf extract demonstrated moderate antibacterial activity against *S. aureus*, as shown by the clear zone around the discs. This finding supports earlier studies indicating that bamboo leaves contain bioactive compounds, such as phenolic acids and flavonoids, that may disrupt bacterial cell wall synthesis or protein function.

Antibacterial activity against *Pseudomonas aeruginosa*

As presented in Table 3, the bamboo leaf extract (T1) exhibited a mean ZOI of 3.33 mm, while the

commercial antibiotic (T_2) achieved 5.17 mm against *Pseudomonas aeruginosa*.

Table 3. Mean zone of inhibition (mm) of bamboo leaf extract against *Pseudomonas aeruginosa*

Treatment	Mean (ZOI) (mm)
T_1–Bamboo leaf extract	3.33
T_2–Commercial antibiotic (Control)	5.17

ZOI= Zone of Inhibition

The results indicated that bamboo leaf extract was effective in inhibiting the growth of *P. aeruginosa*, although less potent than the commercial antibiotic. The observed inhibition suggests that certain phytochemicals in bamboo leaves, possibly flavonoids or phenolic compounds, may interfere with the metabolic processes of gram-negative bacteria like *P. aeruginosa*. Statistical analysis confirmed a significant difference between the two treatments, indicating that while effective, the extract's antibacterial action remains weaker than that of the standard antibiotic.

Comparative antibacterial effectiveness

Comparing all test organisms, the bamboo leaf extract showed the highest inhibitory activity against *Pseudomonas aeruginosa* (3.33 mm), followed by *Staphylococcus aureus* (3.00 mm), and the least effect against *Escherichia coli* (1.00 mm). This pattern suggests that the extract may be more active against certain Gram-negative and Gram-positive bacteria but its potency is still limited compared to commercial antibiotics (Fig. 1).

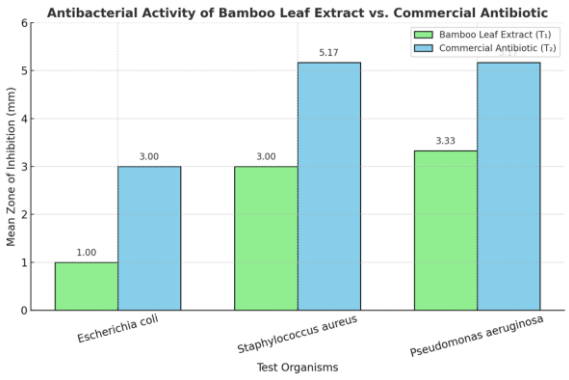

Fig. 1. Antibacterial activity of bamboo leaf extract vs. commercial antibiotic

Overall, the findings demonstrate that bamboo leaf extract possesses partial antibacterial properties, particularly against *S. aureus* and *P. aeruginosa*, validating its traditional use as a herbal remedy. However, its minimal effect on *E. coli* indicates the need for further studies to enhance extraction methods, concentration levels, or explore other plant parts for higher efficacy.

CONCLUSION

Based on the results of the study, the bamboo leaf extract (Treatment 1) demonstrated antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as indicated by the presence of distinct zones of inhibition around the filter paper discs saturated with the extract. This activity was interpreted as partially active, showing moderate effectiveness compared to the commercial antibiotic (Treatment 2), which exhibited a stronger inhibitory effect and was considered very active. However, the bamboo leaf extract showed no significant antibacterial effect against *Escherichia coli*, indicating its limited spectrum of action.

These findings suggest that bamboo leaf extract possesses potential as a natural antibacterial agent, particularly against *Pseudomonas aeruginosa* and, to a lesser extent, *Staphylococcus aureus*. Therefore, it may serve as an alternative or supplementary treatment to commercial antibiotics such as penicillin for certain bacterial infections. Further studies are recommended to improve its efficacy through different extraction methods, higher concentrations, or testing other parts of the bamboo plant. With continued research, bamboo leaves could contribute to the development of affordable and sustainable herbal alternatives for managing bacterial diseases in animals and possibly humans.

RECOMMENDATION(S)

Based from the findings, the researcher recommends the used of bamboo leaves extract (Treatment 1) as an alternative to commercialized drugs (Penicillin)

against the diseases caused by *Pseudomonas aeruginosa*. Likewise, deeper investigation using other method of *in vivo* and *in vitro* testing by using other parts of bamboo plants including the different varieties of bamboo and by the used of other test organisms.

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