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EFFECTIVENESS OF STARFRUIT LEAF EXTRACT AS A NATURAL ANTIBACTERIAL AGENT IN NATIVE CHICKENS

Efektivitas Ekstrak Daun Belimbing Wuluh sebagai Antibakteri Alami pada Ayam Kampung

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Abstract

Bacterial infections such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella pullorum* remain major constraints in native chicken production, reducing productivity and increasing mortality. Excessive use of synthetic antibiotics causes bacterial resistance and residues, thus encouraging the search for safe natural alternatives. This study aimed to evaluate the antibacterial effectiveness of *Averrhoa bilimbi L.* (bilimbi) leaf extract against bacterial isolates from native chickens and to compare its efficacy with oxytetracycline. The leaves were extracted with 96% ethanol and tested at concentrations of 10%, 20%, and 30% using the disk diffusion method against *E. coli* isolates. Data were analyzed using the Kruskal-Wallis and Mann–Whitney tests. Results indicated no significant difference ($p>0.05$) between the inhibition zones of bilimbi leaf extract and oxytetracycline at all concentrations, although descriptively the inhibition zone increased with concentration (average 0.86–1.74 mm). This suggests a concentration-dependent antibacterial activity that remains moderate compared to conventional antibiotics. Bioactive compounds such as flavonoids, tannins, and saponins are presumed to contribute to bacterial growth inhibition. In conclusion, *A. bilimbi* leaf extract exhibits moderate antibacterial activity. It holds potential as a natural phytobiotic alternative to reduce antibiotic dependence in native chicken production, supporting sustainable and eco-friendly poultry farming. For further research, it is recommended to use: concentration >40% or thick extract from fractionation, further testing of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Keywords: Antibacterial, free range chicken, starfruit leaf extract, phytobiotics

Abstrak

Infeksi bakteri seperti *Escherichia coli* masih menjadi masalah utama dalam produksi ayam kampung karena menurunkan produktivitas dan meningkatkan mortalitas. Penggunaan antibiotik sintetis yang berlebihan menimbulkan risiko resistensi dan residu, sehingga diperlukan alternatif alami yang aman. Penelitian ini bertujuan untuk mengevaluasi efektivitas Ekstrak Daun Belimbing Wuluh (EDBW) (*Averrhoa bilimbi L.*) sebagai antibakteri alami

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terhadap isolat bakteri dari ayam kampung serta membandingkannya dengan oksitetrasiklin. Daun belimbing wuluh diekstraksi dengan etanol 96%, kemudian diuji pada konsentrasi 10%, 20%, dan 30% dengan metode difusi cakram terhadap isolat *E. coli*. Analisis data dilakukan menggunakan uji Kruskal-Wallis dan Mann–Whitney. Hasil menunjukkan bahwa tidak terdapat perbedaan signifikan (p>0,05) antara zona hambat EDBW dan oksitetrasiklin pada semua konsentrasi, meskipun secara deskriptif zona hambat meningkat seiring kenaikan konsentrasi (rata-rata 0,86–1,74 mm) menggunakan jangka sorong. Hal ini mengindikasikan adanya potensi aktivitas antibakteri yang meningkat dengan dosis, namun belum mencapai tingkat efektivitas antibiotik konvensional. Senyawa bioaktif seperti flavonoid, tanin, dan saponin diduga berperan dalam mekanisme penghambatan pertumbuhan bakteri. Kesimpulannya, ekstrak daun belimbing wuluh menunjukkan aktivitas antibakteri dan berpotensi dikembangkan sebagai fitobiotik alami untuk mendukung sistem pemeliharaan ayam kampung bebas antibiotik dan ramah lingkungan. Untuk penelitian lanjutan disarankan menggunakan: konsentrasi >40% atau ekstrak kental hasil fraksinasi, Uji lanjut *Minimum Inhibitory Concentration (MIC)* dan *Minimum Bactericidal Concentration (MBC)*

Kata kunci: Antibakteri, ayam kampung, ekstrak daun belimbing wuluh, fitobiotik

INTRODUCTION

Bacterial infections remain one of the major constraints in the production of native chickens in Indonesia. Infections caused by *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella pullorum* frequently lead to reduced productivity, impaired growth, and increased mortality. Synthetic antibiotics such as oxytetracycline and enrofloxacin are widely used both for therapeutic purposes and as growth promoters. However, prolonged antibiotic use poses risks of bacterial resistance and drug residues in animal-derived products (Agarwal et al., 2021). Consequently, the search for natural antibacterial sources that are effective, safe, and environmentally friendly has become a pressing need in sustainable poultry production systems.

One local plant with strong potential as an antibacterial agent is belimbing wuluh (*Averrhoa bilimbi* L.). Its leaves contain various bioactive compounds—including flavonoids, saponins, tannins, and organic acids—known for their antimicrobial and antioxidant properties (Zohdi et al., 2022). These characteristics make *A. bilimbi* leaf extract a promising natural alternative for suppressing pathogenic bacterial growth without promoting resistance or leaving residues in poultry meat. With increasing restrictions on the use of synthetic antibiotics in poultry production at both national and global levels, the development of safe and effective antibacterial alternatives is essential. Phytobiotic research based on locally available plants such as *A. bilimbi* is highly relevant to supporting "antibiotic-free livestock" initiatives. Besides being readily available, the use of local plants may reduce production costs and enhance the added value of local poultry commodities (Alghirani et al., 2021). This study is therefore strategically important, as it provides scientific justification for utilizing Indonesia's natural resources in the development of natural poultry antibacterials.

Recent phytobiotic studies show that various tropical plants possess antibacterial potential for poultry. *A. bilimbi* stands out due to its high phenolic and flavonoid content, which can inhibit bacterial protein synthesis and disrupt cell membranes (Arifin & Jumal, 2020). Several studies have demonstrated the positive effects of leaf and fruit extracts of *A. bilimbi* in reducing pathogenic bacteria and improving intestinal health in broiler chickens (Pratama et al., 2021; Pauzan et al., 2025). Moreover, Alghirani et al. (2021) emphasized that phytobiotics as antibiotic substitutes can enhance mucosal immunity without leaving harmful residues. Taken

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together, these findings highlight *A. bilimbi* as a promising local antibacterial candidate for use in native chicken production systems.

Aziz et al. (2014) reported that leaf and fruit extracts of *A. bilimbi* show antibacterial activity against *S. aureus* and *E. coli*, with the strongest effect observed in 80% ethanol extracts. Sugiharto (2020) further found that a 40% ethanolic leaf extract of *A. bilimbi* exerted a significant inhibitory effect on *Salmonella pullorum*, producing an inhibition zone of 9.4 mm. Rante et al. (2024) added that ethanolic extracts of *A. bilimbi* leaves effectively inhibited test bacteria at a concentration of 1.25% (equivalent to 0.25 mg per paper disc). Likewise, Iwansyah et al. (2021) reported that compared to aqueous extracts, ethanol extracts exhibited greater DPPH scavenging activity (82.82 mg GAE/g DW) and stronger inhibitory effects against *Salmonella* sp., *Escherichia coli*, and *Staphylococcus aureus*.

This study aims to evaluate the antibacterial activity of *A. bilimbi* leaf extract against bacterial isolates obtained from native chickens, compare its effectiveness with oxytetracycline as a positive control, and analyze the statistical relationship between extract concentration and bacterial growth inhibition as a basis for assessing its potential application in antibiotic-free native chicken production systems. Although several previous studies have reported the antibacterial potential of *A. bilimbi* leaves against pathogenic bacteria (Atiyah et al., 2022; Anjasari, 2024; Reshma, 2023), most of these studies focused only on in-vitro assays using laboratory reference strains (e.g., *S. aureus* and *E. coli*) and often employed high extract concentrations or organic solvents—conditions that are less applicable to practical use in poultry systems. Therefore, their findings cannot yet be generalized to natural isolates from native chickens (Aziz et al., 2014; Sugiharto, 2020). Moreover, limited research has compared the antibacterial effects of *A. bilimbi* leaf extract with conventional antibiotics across low-to-moderate concentration ranges. Hence, this study addresses this research gap by conducting an empirical evaluation of *A. bilimbi* leaf extract on field isolates from local poultry and by directly comparing its activity to oxytetracycline.

RESEARCH METHODS

Ethical Clearence

This study did not require ethical approval because it utilized chicken meat and did not involve the use or intervention of live animals or experimental animals.

Research Objects

This study used native chicken meat obtained from areas around Kupang City. *Averrhoa bilimbi* leaves were also collected from Kota Raja District, Kupang City. The leaf samples consisted of leaves taken from the upper three rows, the middle section, and the lower three rows of the plant.

Research Design

Extraction

The plant part of *A. bilimbi* used for antibacterial testing was the leaf. The dried simplicia was air-dried at room temperature, after which 500 g were macerated in 700 mL of 96% ethanol. The mixture was covered with aluminium foil and left for 48 hours. The resulting macerate was filtered using a glass funnel and filter paper until the solid residue was effectively separated. The filtrate was concentrated using a vacuum rotary evaporator. The resulting extract was prepared as a stock solution at a concentration of 100%, then diluted to obtain working solutions of 10%, 20%, and 30%.

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Isolation and Identification of *E. coli* from Chicken Meat

A total of 10 g of chicken meat was added to 100 g of peptone/lactose broth and homogenized. One millilitre of the mixture was taken using a micropipette, and the cover film of the selective MC-Media Pad *E. coli* was opened. The sample was inoculated onto the MC-Media Pad and incubated for 18–24 hours at 37°C. Colonies appearing purple were interpreted as positive for *E. coli*.

Antibacterial Inhibition Zone Test of *A. bilimbi* Leaf Extract

The inhibition zone test was performed using MHA medium with oxytetracycline (10 µg) as the antibiotic control. Bacterial turbidity was adjusted to McFarland Standard No. 5 (1.5×10^8 CFU/mL). One millilitre of *E. coli* from the MC-Media Pad was cultured onto MHA using the spread method and allowed to dry. Antibiotic discs were then placed on the MHA medium and incubated for 24 hours at 37°C. The diameter of the inhibition zone was measured using a calliper (mm) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020). A clear or transparent area surrounding the paper disc indicated bacterial growth inhibition (inhibition zone).

Research Variables

The independent variables consisted of three concentrations of *A. bilimbi* leaf extract (10%, 20%, and 30%), along with a Positive Control (Oxytetracycline 10 µg, Oxoid) and a Negative Control. These variables served as the treatment factors expected to produce different inhibitory effects on the growth of *Escherichia coli*. The dependent variable was the antibacterial activity, measured as the diameter of the inhibition zone (mm). The inhibition zone was assessed as the clear area around the paper disc containing the extract placed on MHA (Mueller Hinton Agar). Control variables included all experimental conditions and non-treatment materials (media, bacterial culture, temperature, incubation time, solvent, extraction method, etc.).

Data Collection Method

Data were collected through laboratory-based experimental procedures, with quantitative measurement of inhibition zone diameters.

Data Analysis

Data were analyzed using the Kruskal Wallis test followed by the Mann–Whitney test.

RESULTS AND DISCUSSION

Results

The findings of this study (Table 1) show that at all tested concentrations, oxytetracycline produced larger inhibition zones compared with the *Averrhoa bilimbi* leaf extract. The extract demonstrated a progressive increase in antibacterial activity with increasing concentrations, particularly at 30%. Considerable variability was observed among replicates, especially in the oxytetracycline group (inhibition zones ranging from 0 mm to 6 mm). Because of this variability, non-parametric statistical tests (Kruskal Wallis and Mann Whitney) were required to determine whether the differences in mean ranks were statistically significant.

The Kruskal Wallis analysis (Table 2) indicated distinct mean rank values across concentrations for both the *A. bilimbi* extract and oxytetracycline. For the extract, the mean rank increased from 10% (5.00), to 20% (7.50), and further to 30% (11.50), indicating a concentration-dependent enhancement of antibacterial activity. A similar pattern was observed in oxytetracycline, where the mean rank at 10% was 7.20, decreased slightly at 20% (5.80),

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and rose again at 30% (11.00). Overall, the 30% concentration yielded the highest mean rank in both treatments, suggesting the largest inhibition zones at this concentration. These differences in mean ranks indicate that concentration influences inhibition zone size, and the Kruskal Wallis test would likely yield significant results if $p < 0.05$.

The Mann Whitney test (Table 3) was performed to compare the inhibition zones between *A. bilimbi* leaf extract and oxytetracycline at equivalent concentrations.

10% Concentration

The Mann–Whitney results showed a mean rank of 4.20 for the extract, lower than oxytetracycline (6.80). This suggests that oxytetracycline produced larger inhibition zones at 10%. If $p < 0.05$, the difference is statistically significant. Biologically, at low concentrations, the *A. bilimbi* leaf extract has not yet demonstrated antibacterial activity comparable to oxytetracycline.

20% Concentration

At 20%, the extract had a mean rank of 5.30, while oxytetracycline had 5.70. The minimal difference between these values suggests that both treatments produced nearly equivalent inhibition zones. If $p > 0.05$, no significant difference exists. Biologically, this implies that at moderate concentrations, the extract begins to show antibacterial potential approaching that of the commercial antibiotic.

30% Concentration

At 30%, the extract recorded a mean rank of 3.70, which was markedly lower than oxytetracycline (7.30). If $p < 0.05$, this indicates a statistically significant difference. Biologically, although the *A. bilimbi* extract exhibits increased antibacterial activity at higher concentrations, its effectiveness still does not match oxytetracycline, which produced the largest inhibition zones overall.

Discussion

The present study focused on evaluating the antibacterial activity of *Averrhoa bilimbi* (belimbing wuluh) leaf extract against bacterial isolates obtained from native chickens and comparing its effectiveness with the commercial antibiotic oxytetracycline. Based on the statistical analyses, the Kruskal Wallis test yielded a significance value of 0.066 (>0.05) (Table 2), indicating no significant differences in the mean inhibition zones among the extract concentrations tested (10%, 20%, and 30%). A similar outcome was observed for the positive control, oxytetracycline (sig = 0.157 > 0.05). This suggests that all concentrations of *A. bilimbi* leaf extract produced relatively comparable inhibitory effects, although the descriptive data showed a gradual increase in inhibition zone diameters with higher concentrations (mean 0.86 mm at 10%, 1.00 mm at 20%, and 1.74 mm at 30%). While this pattern reflects a tendency toward enhanced antibacterial activity with increasing concentration, the change was not statistically significant. The Mann–Whitney test results (Table 3) further support these findings, as comparisons between *A. bilimbi* extract and oxytetracycline at each concentration also did not show statistically meaningful differences ($p > 0.05$).

These findings align with the results of Rante et al. (2024), who reported that ethanolic extracts of *A. bilimbi* leaves inhibited the growth of *S. aureus* and *E. coli*, producing inhibition zones within the moderate range (6–10 mm) and showing increasing antibacterial activity at higher extract concentrations. They emphasized the roles of flavonoids and tannins as the primary active compounds and noted that the effectiveness of the extract is strongly influenced by concentration and solvent type. According to Prastiyanto et al. (2020), ethanolic extracts of *A. bilimbi* produced inhibition zones of 10–14.5 mm, whereas methanolic extracts produced 7–10.5 mm, indicating that

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the antibacterial efficacy is highly dependent on the solvent and concentration. Anindita et al. (2024) also documented inhibition zones ranging from 9.8 to 13.5 mm against *S. aureus* at 50–90% extract concentrations, with higher concentrations consistently yielding stronger antibacterial activity. These studies collectively demonstrate that active compounds such as flavonoids, tannins, and saponins in *A. bilimbi* leaves possess antibacterial potential, although their effectiveness is strongly concentration- and solvent-dependent.

Mareta et al. (2020), Siregar (2023), and Sugiharto (2020) previously reported that *A. bilimbi* leaf extract reduced intestinal *E. coli* counts in broiler chickens, although its effects were weaker than those of conventional antibiotics. These findings support the extract's potential use as a supplementary antibacterial agent rather than a direct replacement for standard antibiotics. According to Aristyantari et al. (2022) and Prastiyanto et al. (2020), *A. bilimbi* extract demonstrated significant antibacterial activity against *Salmonella*, with inhibition zones reaching approximately 9.4 mm, particularly at higher concentrations and when organic solvents were used. Variations among studies may stem from differences in extract concentration, solvent type, and extraction method, as organic solvents often enhance antibacterial activity by improving the solubility of active compounds. The discrepancies between previous findings and the present study may be attributed to the relatively low extract concentrations tested here and the use of ethanol as the extract solvent. Active compounds such as flavonoids and phenolics tend to dissolve more effectively in organic solvents, thus improving antibacterial performance.

Studies by Pramiastuti et al. (2020) and Anindita et al. (2024) reported that *A. bilimbi* leaf extract effectively inhibited *S. aureus* at high concentrations (≥90–100%), producing inhibition zones of approximately 12–13 mm, but activity declined sharply below 30%. At lower concentrations, the extract appears insufficiently potent to exert maximal antibacterial effects. Overall, differences across studies can be explained by variations in solvents, extraction methods, bacterial species tested, and laboratory techniques, including diffusion versus dilution assays.

The very small inhibition zones observed in this study (0.86–1.74 mm) indicate that the antibacterial activity of *A. bilimbi* leaf extract against native chicken isolates is relatively weak. This may be due to the extract concentrations used (10–30%), which likely did not reach the MIC (Minimum Inhibitory Concentration). Previous studies reported that measurable antibacterial activity from *A. bilimbi* typically becomes evident only at higher concentrations (≥50–100%), producing inhibition zones of 9–14 mm (Anindita et al., 2024; Prastiyanto et al., 2020). Lower concentrations tend to yield minimal inhibition, as the quantities of dissolved flavonoids, tannins, and phenolic compounds are insufficient to effectively suppress bacterial growth. Another possible explanation is limited diffusion of the active compounds into the agar, particularly because plant extracts often contain polar and semipolar components with larger molecular sizes. Cowan (1999) noted that phenolic and flavonoid compounds in herbal extracts frequently exhibit poor diffusion in agar-based assays, resulting in smaller inhibition zones despite possessing actual antibacterial activity.

Furthermore, bacterial isolates from native chickens may exhibit higher levels of resistance due to environmental exposure, feed, or prior antibiotic usage in local farming systems. Field isolates of bacteria such as *E. coli* and *Staphylococcus aureus* from poultry are often more resistant than laboratory strains (Manyi-Loh et al., 2018; Nhung et al., 2017), which may contribute to the minimal inhibition produced by plant extracts with mild antibacterial properties. Another contributing factor is the use of the disc diffusion method, which has known limitations for testing plant extracts. Disc diffusion frequently produces smaller inhibition zones because many phytochemical compounds do not diffuse efficiently through agar (Balouiri et al., 2016). Consequently, MIC or MBC assays often provide more representative measurements of antibacterial potency.

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Considering these factors, the small inhibition zones observed in this study remain reasonable and consistent with the characteristics of low-concentration extracts and the diffusion method employed. Although the results were not statistically significant, the upward trend in inhibition zone diameter from 10% to 30% suggests potential for further optimization. With proper adjustment of solvent type and concentration, *A. bilimbi* leaf extract may hold promise as a natural antibacterial alternative, particularly for native chickens, offering advantages such as accessibility, low cost, and minimal antibiotic residues.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study demonstrates that the leaf extract of *Averrhoa bilimbi* exhibits moderate antibacterial activity against bacterial isolates obtained from native chickens. However, no significant differences were observed among the tested concentrations nor when compared with oxytetracycline. Despite this, the upward trend in inhibitory activity at higher extract concentrations suggests its potential for further development as a natural antibacterial agent, particularly in antibiotic-free poultry production systems. In practical terms, *A. bilimbi* leaf extract may be explored as a natural phytobiotic additive in native chicken feed to help reduce reliance on synthetic antibiotics and to support sustainable poultry farming.

Recommendations

Further investigations are needed to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

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Tables

Table 1. Results of inhibition zone measurements

Antibacterial Agent	10% Concentration	20% Concentration	30% Concentration
A. bilimbi Leaf Extract	1.30 mm	0.90 mm	1.00 mm
A. bilimbi Leaf Extract	0.80 mm	0.60 mm	1.50 mm
A. bilimbi Leaf Extract	0.80 mm	1.00 mm	0.90 mm
A. bilimbi Leaf Extract	0.70 mm	0.90 mm	2.80 mm
A. bilimbi Leaf Extract	0.70 mm	1.60 mm	2.50 mm
Oxytetracycline	2.50 mm	4.00 mm	3.80 mm
Oxytetracycline	0.00 mm	1.00 mm	6.00 mm
Oxytetracycline	1.00 mm	0.00 mm	1.00 mm
Oxytetracycline	4.50 mm	4.00 mm	6.00 mm
Oxytetracycline	4.00 mm	0.00 mm	5.00 mm

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Table 2. Kruskal Wallis test results

Group	Concentration	n	Mean Rank
A. bilimbi Extract	10%	5	5.00
	20%	5	7.50
	30%	5	11.50
	Total	15	—
Oxytetracycline	10%	5	7.20
	20%	5	5.80
	30%	5	11.00
	Total	15	—

Table 3. Mann Whitney test results

Concentration	Antibacterial Agent	n	Mean Rank	Sum of Ranks
10%	*A. bilimbi* Leaf Extract	5	4.20	21.00
	Oxytetracycline	5	6.80	34.00
	Total	10	—	—
20%	*A. bilimbi* Leaf Extract	5	5.30	26.50
	Oxytetracycline	5	5.70	28.50
	Total	10	—	—
30%	*A. bilimbi* Leaf Extract	5	3.70	18.50
	Oxytetracycline	5	7.30	36.50
	Total	10	—	—