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Antimicrobial Activity Test of Alkaloid Rich Extract from *Morinda citrifolia* Leaves Against the Growth of Pathogenic Bacteria *Staphylococcus aureus* and *Escherichia coli*

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Abstract

This study aims to analyze the antimicrobial activity of alkaloid compounds from noni leaf extract (*Morinda citrifolia*) against the growth of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*. The study applied a quantitative experimental laboratory approach. Extraction was carried out using the maceration method with 96% ethanol, followed by qualitative phytochemical analysis using Mayer, Dragendorff, and Wagner reagents to identify the presence of alkaloids. Using the Kirby-Bauer disk diffusion method, the antibacterial efficacy was evaluated at concentrations of 25%, 50%, 75%, and 100%. The primary indicator utilized was the size of the regions where bacteria could not thrive. Basic statistics and ANOVA analyses were utilized for data assessment. About 10.45% of alkaloids were found in the results. As the extract concentration grew, the quantity of bacterial development arrested did as well. The most notable impact was observed at the 100% concentration, with no growth zones of 18.9 mm for S. aureus and 15.8 mm for E. coli. The extract was more effective against Gram-positive bacteria. With p values less than 0.05, the statistical analysis found significant variations among the various treatments. Finally, the alkaloids found in noni leaves have a lot of potential to work as natural antibacterial agents and could be a substitute to fight antibiotic resistance.

Keywords: Antimicrobial activity, Alkaloid chemical compounds, *Morinda citrifolia*, Staphylococcus aureus, Escherichia coli

1. Introduction

Infectious diseases caused by pathogenic microorganisms, particularly bacteria, remain a major global health challenge (Silaen et al., 2025). Bacterial infections not only contribute to increased morbidity but also play a significant role in mortality rates, especially in developing countries (Silaban, Sijabat, et al., 2025). Two bacteria that are commonly associated with human infections are *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is a Gram-positive bacterium commonly found on the skin and in the human respiratory tract; however, it can act as an opportunistic pathogen causing serious infections such as abscesses, pneumonia, and sepsis. Meanwhile, *Escherichia coli* is a Gram-negative bacterium that inhabits the gastrointestinal tract, but certain pathogenic strains can cause diarrhea, urinary tract infections, and other systemic disorders (Ramdani et al., 2025).

In clinical practice, bacterial infections are generally treated with antibiotics (Sinaga et al., 2024). However, the irrational use of antibiotics—such as self-medication, inappropriate dosing, and premature discontinuation of treatment—has led to the increasing emergence of antibiotic resistance (Silaban et al., 2025). This resistance reduces the effectiveness of previously reliable drugs, complicates treatment, and increases healthcare costs (Sigiro et al., 2024). Consequently, there is a growing need to explore alternative sources of antimicrobial agents that are more effective, safe, and sustainable (Handayani et al., 2022).

One promising approach is the exploration of bioactive compounds derived from plants as natural medicinal resources (Sirait, 2025). Indonesia, as a megabiodiversity country, has enormous potential in the utilization of traditional medicinal plants (Sirait et al., 2023). These plants have long been used empirically by local communities and are known to contain various secondary metabolites (Alexander et al., 2025). Among these, noni (*Morinda citrifolia*) has attracted considerable attention (Liu et al., 2024).

Morinda citrifolia is widely recognized in traditional medicine due to its numerous health benefits. Almost all parts of the plant, including the fruit, roots, stems, and leaves, contain diverse bioactive compounds (Putri et al., 2023). Noni (*Morinda citrifolia*) is one such plant widely recognized for its medicinal value. Its leaves, in particular, contain various active compounds, including alkaloids, which play an important role in antimicrobial activity (Maharani et al., 2026). These compounds play a crucial role in providing pharmacological effects, including antimicrobial activity (Geofani et al., 2022).

Among these compounds, alkaloids are a group of secondary metabolites with significant biological activity (Pane et al., 2025). Alkaloids are nitrogen-containing compounds that can disrupt bacterial cell structures, inhibit enzyme activity, and interfere with nucleic acid synthesis. These mechanisms enable them to suppress or eliminate bacterial growth. (Sirait et al., 2026). Through these mechanisms, alkaloids are capable of inhibiting the growth of or even killing bacterial cells (Sirait et al., 2021).

Previous studies have reported that noni leaf extracts exhibit antibacterial activity against various microorganisms (Alexander et al., 2024). However, most of these studies have focused on crude extracts without isolating specific active compounds (Sirait et al., 2024). Identification and testing of specific compounds, such as alkaloids, are essential to determine their precise contribution to antimicrobial activity (Vama et al., 2020). Furthermore, differences in cell wall structures between Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli* are important factors influencing the effectiveness of antimicrobial agents (Siahaan et al., 2026). Therefore, testing against both types of bacteria can provide a more comprehensive understanding of the antimicrobial spectrum of alkaloid compounds (Susanti et al., 2025).

Studies on the antibacterial action of alkaloids obtained from noni leaves are extremely important, not only as a scientific endeavor to produce plant-based medicines but also as a possible answer to the growing issue of antibiotic resistance (Ramdani et al., 2025). Furthermore, the findings of this study are anticipated to enhance scientific knowledge on the potential of native medicinal plants and to facilitate the growth of phytopharmaceuticals in Indonesia (Pasaribu et al., 2024).

However, despite numerous studies reporting the antibacterial activity of *Morinda citrifolia*, most investigations have primarily focused on crude extracts without clearly identifying the specific role of individual bioactive compounds, particularly alkaloids. In addition, limited studies have quantitatively analyzed alkaloid content and systematically evaluated its antibacterial effectiveness against both Gram-positive and Gram-negative bacteria. Therefore, the specific contribution of alkaloid compounds to the antibacterial activity of noni leaves remains insufficiently understood. Based on this gap, this study aims to isolate, quantify, and evaluate the antibacterial activity of alkaloid-rich extract from noni leaves against *Staphylococcus aureus* and *Escherichia coli*.

Given this background, this study intends to isolate and assess the antibacterial activity of alkaloid components from noni leaf extract (*Morinda citrifolia*) against *Staphylococcus aureus* and *Escherichia coli*, two pathogenic bacteria. This study is meant to lay the groundwork for more research on the creation of safe and effective alternative medicines and to offer scientific data on the potential of alkaloids as natural antimicrobial compounds.

2. Methods

Using a quantitative approach, this study is a laboratory experimental investigation meant to examine the antibacterial activity of alkaloid components from noni leaf extract (Morinda citrifolia) against the development of pathogenic bacteria Staphylococcus aureus and Escherichia coli. The study used a Totally Randomized Design (CRD) with various extract concentrations as treatments: 25%, 50%, 75%, and 100%. It also included a positive control (standard antibiotic) and a negative control (solvent). Every therapy was done thrice to guarantee data validity and dependability (Pardede et al., 2024).

The study was carried out in the Chemistry Laboratory and Microbiology Laboratory over a period of approximately three months. The sample used consisted of noni leaves obtained from healthy and uncontaminated plants (Silaen et al., 2025). Sample preparation began with washing the leaves under running water, followed by drying at 40–50°C until dry simplicia were obtained. The dried material was then ground into powder form to facilitate the extraction process (Harita et al., 2025).

Extraction was performed using the maceration method with 96% ethanol at a ratio of 1:10 (w/v) for 3 × 24 hours with periodic stirring. The filtrate obtained was then filtered and concentrated using a rotary evaporator to obtain a thick extract. The extraction efficiency was determined by calculating the yield using the following formula:

$$\text{Yield (\%)} = \frac{\text{mass of extract}}{\text{initial mass of simplicia}} \times 100\%$$

Phytochemical analysis was then conducted qualitatively to identify the presence of alkaloid compounds in the extract (Simatupang et al., 2025). The tests were carried out using Mayer, Dragendorff, and Wagner reagents. A positive result for alkaloids was indicated by the formation of a cream-white precipitate (Mayer), orange precipitate (Dragendorff), or brown precipitate (Wagner). In addition, quantitative analysis of alkaloid content was performed using UV-Visible spectrophotometry to determine the concentration of alkaloid compounds in the extract (Racioppo et al., 2023). Quantitative analysis of alkaloid content was performed using UV-Visible spectrophotometry. Prior to measurement, alkaloid compounds were reacted with Bromocresol Green (BCG) reagent to form a yellow ion-pair complex. The maximum absorption wavelength (λmax) of the complex was determined by scanning in the range of 200–800 nm, and the λmax was found at 417 nm. A calibration curve was constructed using quinine as a standard alkaloid at concentrations of 2, 4, 6, 8, and 10 ppm. The absorbance values were measured at λmax, and a linear regression equation ($y = ax + b$) with correlation coefficient (R^2) ≥ 0.99 was obtained. The total alkaloid content of the sample was then calculated based on this calibration curve and expressed as percentage (% w/w).

The antibacterial test demonstrated that the extract inhibited the growth of both tested bacteria. The inhibition zones increased with higher concentrations, indicating a dose-dependent effect. Sterilized Nutrient Agar was used as the growth medium (Silaban et al., 2024). The positive control used in this study was chloramphenicol at a concentration of 30 µg/disc, while the negative control consisted of the solvent used for extraction. The use of a standardized concentration of chloramphenicol allows for a valid comparison of antibacterial potency between the extract and a conventional antibiotic. The antibacterial activity was interpreted based on inhibition zone diameter according to the classification: <5 mm (inactive), 5–10 mm (weak), 10–20 mm (moderate), and >20 mm (strong activity).

Using the disk diffusion technique (Kirby-Bauer), antimicrobial activity was assessed. The agar surface was evenly covered with the bacterial suspension, and sterile paper discs soaked in extracts at different concentrations were carefully laid on the medium (Sirait et al., 2025). The positive control employed a regular antibiotic; the negative control used the solvent. Plates were incubated at 37°C for 24 hours. (Alexander et al., 2025).

After incubation, antimicrobial activity was observed based on the formation of inhibition zones around the disks (Sinaga et al., 2026). The diameter of the inhibition zones was measured using a caliper in millimeters from multiple directions, and the average was calculated using the following formula:

$$\bar{x} = \frac{\sum x}{n}$$

To determine data variability, the standard deviation was calculated using the formula:

$$SD = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$$

Furthermore, the relative inhibition percentage compared to the positive control was calculated to evaluate extract effectiveness using the following formula (Sinurat et al., 2026):

$$\text{Relative Inhibition (\%)} = \frac{\text{inhibition zone of sample}}{\text{inhibition zone of positive control}} \times 100\%$$

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) to evaluate differences among treatment groups. Prior to analysis, data were tested for normality and homogeneity of variance. When significant differences were detected, a Tukey's HSD post hoc test was conducted to identify pairwise differences between groups. Statistical significance was set at $p < 0.05$. (Silaban, et al., 2020). Furthermore performed was a comparative investigation between Gram-positive and Gram-negative bacterial responses to gauge the extract's antibacterial range (Alexander et al., 2025).

However, the disk diffusion method has several limitations. The size of the inhibition zone is not solely influenced by antibacterial potency but also by the diffusion ability of the compounds in agar media. Compounds with larger molecular size or lower solubility may exhibit smaller inhibition zones despite having strong antibacterial activity. In addition, this method does not provide quantitative parameters such as Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC), which are essential to determine the exact antibacterial potency.

The study is expected to provide comprehensive information regarding the alkaloid content and antimicrobial potential of noni leaf extract as a natural antibacterial agent through this integrated approach.

3. Result and Discussion

Results

This research sought to assess the antibacterial activity of alkaloid components derived from noni leaf extract (*Morinda citrifolia*) against the proliferation of pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*. The results include extraction, phytochemical identification, determination of alkaloid content, and testing for antimicrobial activity.

Macerating noni leaves with 96% ethanol generated a thick, dark green extract with a distinct aroma. The extraction produced around 13.2% of the starting simplicia weight. This yield suggests that ethanol is a good solvent for getting secondary metabolites out of noni leaves, such as alkaloids.

Qualitative phytochemical study revealed that the noni leaf extract includes alkaloid chemicals. Positive reactions to the three reagents utilized point to this. The Mayer test generated a cream-white precipitate, therefore pointing to the development of a complex between alkaloids and mercury ions. The Dragendorff test produced an orange precipitate, implying a reaction between alkaloids and bismuth potassium iodide. The Wagner test showed a brown precipitate caused by the reaction between alkaloids and iodine solution. These outcomes regularly support the extract's alkaloid concentration.

UV-Vis spectrophotometry quantitative analysis showed that the extract had about 10.45% (w/w) total alkaloids. This value suggests that the noni leaf extract has a relatively high concentration of alkaloids, which could play a significant role in its biological activity, especially as an antimicrobial agent. The total alkaloid content was calculated using the following formula:

$$\text{Total Alkaloid Content (\%)} = \frac{C \times V \times DF}{W} \times 100$$

where:
C = concentration obtained from calibration curve (mg/L)
V = volume of extract solution (L)
DF = dilution factor
W = weight of sample (mg)

The total alkaloid content of 10.45% indicates the proportion of alkaloid compounds present in the extract, calculated based on spectrophotometric analysis using a standard calibration curve.

Using the disk diffusion (Kirby-Bauer) technique, the results of the antimicrobial activity test revealed that the alkaloid extract of noni leaves was able to block the development of both Staphylococcus aureus and Escherichia coli at a range of examined doses. The development of distinct inhibition zones around the disks holding the extract suggested this activity.

The inhibition zone data are summarized in Table 1 to clearly illustrate the dose–response relationship of the alkaloid extract against both bacterial strains.

Concentration (%)	*S. aureus* (mm)	*E. coli* (mm)	Activity Category
25%	7.2 ± 0.5	6.1 ± 0.4	Weak–Moderate
50%	10.8 ± 0.6	9.3 ± 0.5	Moderate
75%	14.7 ± 0.7	12.6 ± 0.6	Strong
100%	18.9 ± 0.8	15.8 ± 0.7	Strong
Positive Control (Chloramphenicol)	23.5 ± 0.9	22.1 ± 0.8	Very Strong
Negative Control	0	0	No Activity

Table 1. Inhibition Zone Diameter of Noni Leaf Alkaloid Extract

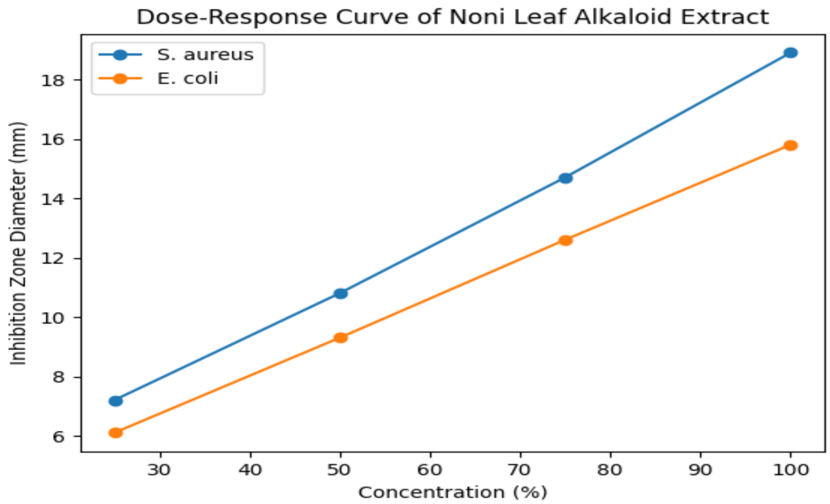

Figure 1. Inhibition Zone Diameter of Noni Leaf Alkaloid Extract

At a concentration of 25%, the average inhibition zone diameter against Staphylococcus aureus was 7.2 ± 0.5 mm, whereas against Escherichia coli it was 6.1 ± 0.4 mm, indicating weak to moderate activity. For Staphylococcus aureus, at 50% concentration the inhibition zones grew to 10.8 ± 0.6 mm, and for Escherichia coli, they increased to 9.3 ± 0.5 mm, therefore falling into the moderate group.

A more significant increase was observed at 75% concentration, with inhibition zones of 14.7 ± 0.7 mm for *Staphylococcus aureus* and 12.6 ± 0.6 mm for *Escherichia coli*, indicating strong activity. At the highest concentration (100%), the inhibition zones reached 18.9 ± 0.8 mm for *Staphylococcus aureus* and 15.8 ± 0.7 mm for *Escherichia coli*, demonstrating strong antibacterial activity approaching that of the positive control.

The positive control, chloramphenicol, showed inhibition zone diameters of 23.5 ± 0.9 mm against *Staphylococcus aureus* and 22.1 ± 0.8 mm against *Escherichia coli*, which are categorized as very strong activity. In contrast, the negative control showed no inhibition zone, confirming that the solvent had no antibacterial effect.

Statistical analysis using ANOVA indicated a significant difference ($p < 0.05$) among the different extract concentrations in terms of inhibition zone diameter for both bacterial strains. This suggests that increasing extract concentration significantly enhances antimicrobial activity. Further post hoc analysis revealed that each concentration differed significantly from the others.

Discussion

The findings of this investigation show that noni leaf extract (Morinda citrifolia) rich in alkaloid components have antibacterial properties against Staphylococcus aureus and Escherichia coli. The development of inhibition zones around the disk's points to this activity since they mirror the capacity of the extract to inhibit bacterial development. The dose-dependency of the antibacterial activity is shown by the rising inhibition zone diameter with increasing extract concentrations.

These findings are consistent with the study conducted by (Geofani et al., 2022) which reported that noni plants exhibit inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. Their literature review highlighted that secondary metabolites such as alkaloids, flavonoids, and tannins play a significant role in antibacterial activity. The present study supports these findings by specifically demonstrating that alkaloids contribute directly to the observed antibacterial effects.

In addition, a study by (Putri et al., 2023), showed that ethanol extract of noni leaves has antibacterial activity against *Pseudomonas aeruginosa*. Although the tested bacterium differs and is known to be more resistant as a Gram-negative bacterium, their findings support the notion that noni leaf extract possesses a broad spectrum of antibacterial activity. This is in line with the present study, which demonstrates inhibitory effects against *Escherichia coli*, albeit with lower effectiveness compared to *Staphylococcus aureus*.

The relatively high alkaloid content (10.45%) suggests that the extraction method was effective in concentrating bioactive compounds, which may contribute to the observed antibacterial activity.

The higher antibacterial effectiveness of the alkaloid-rich extract against *Staphylococcus aureus* compared to *Escherichia coli* can be explained not only by differences in cell wall structure, but also by specific molecular interactions between alkaloid compounds and bacterial membranes. *Morinda citrifolia* is known to contain nitrogen-containing bioactive compounds, including alkaloid-like molecules such as xeronine precursors and other nitrogenous metabolites, as well as phenolic compounds like scopoletin, which may act synergistically (Pardede et al., 2025).

Alkaloids are typically basic compounds containing nitrogen atoms that can become protonated under physiological conditions, forming positively charged species. In Gram-negative bacteria such as *E. coli*, the outer membrane is rich in lipopolysaccharides (LPS), which create a highly selective permeability barrier. Although electrostatic interactions may occur between protonated alkaloids and negatively charged components of LPS, this outer membrane limits penetration due to its tightly packed lipid A and polysaccharide structure. In addition, porin selectivity and efflux pump systems further restrict intracellular accumulation of these compounds (Abubakar et al., 2025).

In contrast, Gram-positive bacteria such as *S. aureus* lack an outer LPS membrane, allowing alkaloid compounds to more readily interact with the cytoplasmic membrane and peptidoglycan layer. The cationic nature of alkaloids facilitates interaction with negatively charged phospholipids in the bacterial membrane, potentially disrupting membrane integrity, altering permeability, and interfering with essential intracellular processes such as enzyme activity and nucleic acid synthesis. Furthermore, compounds like scopoletin, although not an alkaloid, may enhance antibacterial activity through membrane destabilization and oxidative stress induction, thereby potentiating the effect of alkaloids. This synergistic interaction may contribute to the stronger antibacterial activity observed against Gram-positive bacteria.

Therefore, the reduced effectiveness against *E. coli* is likely due to a combination of outer membrane barrier function, limited diffusion, and active resistance mechanisms, rather than solely structural differences, highlighting the importance of considering molecular-level interactions in interpreting antibacterial activity.

The identification of alkaloid compounds in this study lends additional support to earlier research indicating that noni's biological activity is greatly influenced by its secondary metabolites. Alkaloids are believed to have an antibacterial effect by interfering with vital enzymatic processes inside bacterial cells, blocking protein synthesis, and upsetting cell membrane integrity. In the end, these consequences cause decreased cellular metabolism and bacterial cell death.

The data presented in Table 1 demonstrate a clear dose-dependent increase in antibacterial activity for both *Staphylococcus aureus* and *Escherichia coli*. However, the inhibition zones were consistently larger in *S. aureus* compared to *E. coli* at all concentrations (Obeng-boateng et al., 2024).

While this difference is commonly attributed to variations in cell wall structure, additional factors may also contribute. Gram-negative bacteria such as *E. coli* possess an outer membrane containing lipopolysaccharides, which not only acts as a physical barrier but also limits the penetration of hydrophobic compounds such as alkaloids. Furthermore, the presence of efflux pumps and enzymatic degradation mechanisms in Gram-negative bacteria may reduce intracellular accumulation of active compounds, thereby lowering antibacterial effectiveness.

In contrast, Gram-positive bacteria like *S. aureus* lack this outer membrane, allowing easier interaction between alkaloid compounds and cellular targets, leading to stronger antibacterial activity.

The antibacterial action of noni leaf extract against Pseudomonas aeruginosa points to this plant's effectiveness not just against common bacteria like Staphylococcus aureus and Escherichia coli but also against more sophisticated opportunistic pathogens; these findings are in agreement with those of (Putri et al., 2023). This confirms the possibility of utilizing noni leaves as a natural antibacterial source with a wide range of action.

But the noni leaf extract in this study has less antibacterial activity than the conventional antibiotic chloramphenicol (Sitinjak et al., 2024). This is anticipated because crude extracts include a mix of substances and the active components have not yet undergone complete purification. Still, the activity seen, especially at greater concentrations, shows great promise for more development via compound isolation and formulation optimization (Silaban et al., 2021).

The consistent increase in inhibition zones with rising concentrations indicates a strong correlation between extract concentration and antibacterial potency. The results of this study generally support and coincide with earlier studies from experimental studies as well as literature reviews. Noni leaf alkaloid compounds have been found to have strong antibacterial activity, especially against Gram-positive bacteria, and have the potential to be used as natural antibacterial agents to combat the growing problem of antibiotic resistance.

4. Conclusion

Noni leaf extract has been shown in this study to have notable antibacterial properties due to its alkaloid components. With activity rising at larger concentrations, the extract successfully stops the development of Escherichia coli and Staphylococcus aureus. The antibacterial action is more noticeable against Gram-positive bacteria. Though less strong than traditional medicines, the extract shows great promise as a natural substitute for antibacterial compounds. Noni leaf alkaloids show promising antibacterial potential warranting further MIC, MBC, and mechanistic studies.

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