

Antioxidant and Antibacterial Activities of Noni Fruit (*Morinda citrifolia*) Extract

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Abstract

Noni fruit (*Morinda citrifolia*), also known as noni, is widely used in Indonesia for its antioxidant and antibacterial benefits. This study evaluated the antioxidant and antibacterial activities of ethanol-extracted Noni fruit. Antioxidant activity was assessed using the DPPH method, showing an IC₅₀ value of 28.82 µg/mL. Antibacterial activity was tested using the Resazurin Microtiter Assay (REMA), revealing MIC values against *Bacillus subtilis* (2.5 mg/mL), *Staphylococcus aureus* (10 mg/mL), *Salmonella typhi* (10 mg/mL), *Pseudomonas aeruginosa* (>10 mg/mL), and *Propionibacterium acnes* (0.63 mg/mL). The results indicate that Noni fruit extract has potential as a natural source of antioxidants and antibacterials.

Keywords: antibacterial activity, antioxidant activity, *Morinda citrifolia*, noni fruit

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Highlights

1. The ethanol extract of noni fruit demonstrated notable antioxidant activity, with an IC₅₀ value of 28.82 µg/mL, indicating strong free radical scavenging potential using the DPPH method.
2. Noni extract exhibited effective antibacterial activity against *Propionibacterium acnes* (MIC = 0.63 mg/mL) and *Bacillus subtilis* (MIC = 2.5 mg/mL), but was less effective against Gram-negative bacteria such as *Salmonella typhi* and *Pseudomonas aeruginosa* (MIC = 10 mg/mL and >10 mg/mL, respectively).
3. Noni fruit contains various bioactive compounds—such as alkaloids, flavonoids, and phenolic acids—that contribute to its antioxidant and antibacterial properties, supporting its traditional medicinal use.
4. Due to its natural origin and low potential for inducing resistance, noni fruit extract offers a safer alternative to synthetic antioxidants and antibiotics, especially in functional foods or natural health products.
5. The study supports the development of noni fruit extract as a natural therapeutic agent for disease prevention and as a natural preservative in the food industry, though further research is needed to enhance its effectiveness.



Introduction

Free radicals are molecules containing one or more unpaired electrons, making them highly reactive and unstable. These molecules can form as byproducts of normal metabolic processes in the body, primarily through cellular respiration, or as a result of external factors such as pollution, ultraviolet radiation, and toxic chemicals (Mudjiran & Karneli, 2024). When free radicals react with biological molecules in cells, including lipids, proteins, and DNA, they cause cellular damage that can potentially lead to various degenerative diseases, including cancer, cardiovascular disease, diabetes, cataracts, and neurodegenerative diseases such as Alzheimer's and Parkinson's. This damage is known as oxidative stress, a condition of imbalance between free radical production and the body's ability to neutralize them through antioxidant defense mechanisms (Fitriana et al., 2016).

Antioxidants are compounds that can neutralize free radicals by donating electrons to these reactive molecules without themselves becoming reactive (Mudjiran & Karneli, 2024). This action helps prevent or minimize oxidative damage to cellular components. In the context of health, antioxidants play an essential role in reducing the risk of diseases associated with oxidative stress (Fitriana et al., 2015). Several types of natural antioxidants include vitamin C, vitamin E, flavonoids, carotenoids, and polyphenols, commonly found in fruits, vegetables, and medicinal plants. These natural antioxidants are considered safer than synthetic antioxidants used in the pharmaceutical and food industries because they have fewer side effects (Yuniar Pristiana et al., 2017). Therefore, research into new sources of natural antioxidants, particularly from herbs and fruits, is crucial to developing safer and more effective therapies.

In addition to damage from free radicals, infections caused by pathogenic

bacteria are also a significant health issue (Fitriana et al., 2021). Pathogenic bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, and *Salmonella typhi* can cause various infections and diseases, ranging from skin infections to systemic diseases such as typhoid and sepsis. Antibiotic use in recent decades has reduced the impact of bacterial infections; however, misuse and overuse have led to antibiotic resistance, where bacteria become resistant to conventional antibiotic treatments. This phenomenon drives the search for new antibacterial agents that are both effective and safe, particularly from natural sources. Plant-derived natural compounds often demonstrate good antibacterial activity without inducing resistance, making them an appealing alternative for developing new antibacterial agents (Fitriana et al., 2024).

Indonesia is a country rich in biodiversity, including medicinal plants used in traditional medicine. One plant with considerable potential is the noni fruit (*Morinda citrifolia*), also known as "Noni" in Indonesia. The noni fruit has long been used in traditional medicine across various countries, including Indonesia, Polynesia, and India, to treat a range of conditions such as fever, skin infections, and digestive issues. This fruit contains a variety of bioactive compounds, including alkaloids, flavonoids, iridoids, terpenoids, and phenolic acids, known for their antioxidant, anti-inflammatory, and antibacterial properties. These compounds aid the body in combating oxidative stress and microbial infections while strengthening the immune system (Fatonah et al., 2020).

Research conducted by Sogandi (2019) demonstrated that noni fruit extract has potential as an antioxidant and antibacterial agent. The antioxidant activity of noni fruit is associated with its ability to neutralize free radicals, while its

antibacterial activity is effective against various pathogenic bacteria. Based on these findings, noni fruit could be further developed as a natural resource beneficial for treating and preventing various diseases. Additionally, noni fruit extract could be utilized in the food industry as a natural preservative to prevent food spoilage caused by pathogenic microorganisms (Pakpahan et al., 2015). Further research is needed to fully evaluate the potential of noni fruit and to identify the molecular mechanisms underlying its biological activities.

This study aims to evaluate the antioxidant and antibacterial potential of ethanol-extracted noni fruit. Antioxidant testing is conducted using the DPPH method to measure the extract's ability to neutralize free radicals, while antibacterial testing is conducted using the Resazurin Microtiter Assay (REMA) against relevant Gram-positive and Gram-negative bacteria. The results of this study are expected to provide further insight into the potential of noni fruit as a natural therapeutic agent in health and food preservation. Additionally, this research distinguishes itself from previous studies, such as those by Sogandi (Sogandi & Rabima, 2019), by employing an advanced extraction technique and conducting a comprehensive evaluation of both antioxidant and antibacterial activities. It also targets clinically significant bacterial strains and incorporates a detailed phytochemical analysis to establish correlations between observed activities and active compounds. This approach not only highlights the therapeutic potential of *Morinda citrifolia* but also explores its practical applications in various industries.

Research Methods

Chemicals

The chemicals used in this study include ethanol was used as an organic solvent for extraction. DPPH (Aldrich, 1898-66-4), ethanol (Merck,

1.06009.2500) and gallic acid (Aldrich, 149-91-7) were used for antioxidant assay. DMSO (Merck, 1.02952.1000), Mueller Hinton Agar (MHA) (HIMEDIA, M173-500G), Mueller Hinton Broth (MHB) (HIMEDIA, M391-500G), aquadest, McFarland 0.5 (HIMEDIA, R092-1NO), resazurin (Aldrich, 199303-1G), ampicillin, and gram-positive and negative of isolate bacteria included *B. subtilis* (ATCC-19659), *S. aureus* (ATCC-29213), *S. typhimurium* (FNCC- 0050), *P. aeruginosa* (ATCC-27853), and *P. acnes* (ATCC-6919) were used as materials for antibacterial assay system. All chemicals were analytical grade and obtained from reputable suppliers.

Plant material and sample preparation

The plant material used was noni fruit (*Morinda citrifolia*), collected from Jombang, East Java, Indonesia (7° 33' 0" S, 112° 14' 0" E) on December 2021. Fresh, non-spoiled fruits were selected, washed with clean water to remove impurities, and then dried in the shade at room temperature (25-30°C) for 20 days to reduce moisture content before further processing.

Extraction was performed using the maceration method, and the process was repeated three times. Dried noni fruit powder, weighing 100 grams, was combined with 1 liter of ethanol and left to soak for 48 hours with intermittent stirring. After soaking, the mixture was filtered through Whatman No. 1 filter paper to separate the extract solution from the solid residue. The extract was then concentrated using a rotary evaporator at 40°C to remove the solvent, yielding a thick extract containing active compounds.

In vitro antioxidant activity assay using the DPPH method

Antioxidant activity of the extract was tested using the DPPH method. A DPPH solution was prepared at a concentration of 0.1 mM in ethanol. Noni fruit extract was tested at various concentrations (25, 50, 75, 100, and 125 µg/mL) dissolved in

ethanol. 2 mL of the extract solution was added to 2 mL of the DPPH solution, and the mixture was incubated in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm using a UV-Vis spectrophotometer. Gallic acid was used as a standard or positive control. The percentage inhibition was calculated based on changes in absorbance, and the IC₅₀ value was determined from a plot of percentage inhibition against concentration (Fitriana et al., 2018).

Antibacterial activity assay using Resazurin Microtiter Assay (REMA)

The antibacterial activity of noni fruit extract was tested against five pathogenic bacteria: *Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), *Propionibacterium acnes* (Gram-positive), and *Salmonella typhi* (Gram-negative). The detailed procedure for the antibacterial activity assay using the Resazurin Microtiter Assay (REMA) follows (Sarker et al., 2007):

1) Bacterial preparation

The pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, and *Salmonella typhi*) were incubated on agar media at 37°C until an appropriate density was reached. Bacterial suspensions were then prepared and inoculated into 96-well plates containing nutrient broth, allowing bacterial growth during antibacterial testing.

2) Extract preparation

Noni fruit extract was prepared in ethanol at a concentration of 20 mg/mL, creating a homogeneous solution ready for testing.

3) Testing procedure

From the extract stock solution, 50 µL of extract was added to each well in a 96-well plate containing 100 µL of nutrient medium with bacterial

inoculum. The plate was incubated at 37°C for 24 hours, allowing interaction between the extract and bacteria to observe antibacterial effects.

4) Addition of Resazurin

After incubation, 10 µL of 0.02% resazurin solution was added to each well, and the plate was incubated again for 4 hours at 37°C. Resazurin is an indicator that changes color from blue to pink in the presence of bacterial growth, indicating bacterial metabolic activity.

5) Evaluation of antibacterial activity

A color change from blue to pink indicates bacterial growth, while no color change signifies bacterial inhibition. The Minimum Inhibitory Concentration (MIC) is the lowest concentration of the extract where no color change occurs, indicating that the extract effectively inhibits bacterial growth at that concentration. Ampicillin was used as a positive control.

Data analysis

Antioxidant test results were evaluated based on IC₅₀ values, which represent the extract concentration needed to inhibit 50% of DPPH free radicals. Lower IC₅₀ values indicate stronger antioxidant activity. Antibacterial test results were determined by observing MIC values for each bacterium (Fitriana et al., 2021).

Results and Discussion

Extraction

The ethanol extract of noni fruit (*Morinda citrifolia*) has been successfully obtained through maceration. From a total sample of 800 grams of dried noni fruit powder, 55 grams of ethanol extract were obtained, resulting in a yield of approximately 6.87%.

Antioxidant activity

Antioxidant activity results for the various extracts are shown in Figure 1. Based on the data, ethanol extract of noni fruit demonstrated an antioxidant activity

of 80.7%, while gallic acid (positive control) exhibited an antioxidant activity of around 91.8%. Antioxidant activity testing was conducted using the DPPH

method (2,2-diphenyl-1-picrylhydrazyl) to assess noni fruit extract's ability to scavenge free radicals.

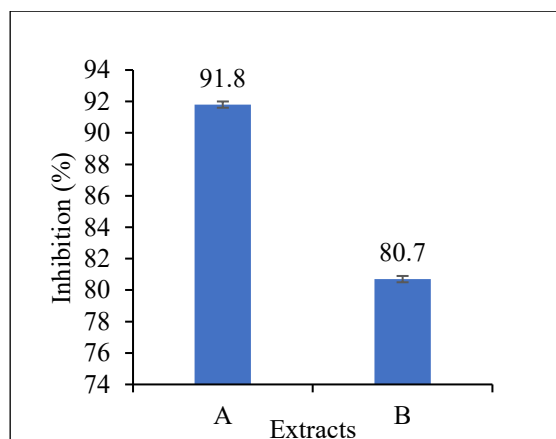


Figure 1. DPPH Antioxidant Activity Test Results: (A) Gallic Acid, (B) Ethanol Extract of Noni Fruit (*Morinda citrifolia*). Each bar represents the mean \pm SD, $n=3$ at a concentration of 319.46 $\mu\text{g/mL}$.

As a reference, Table 1 presents the literature review findings on the antioxidant activity of noni fruit (*Morinda citrifolia*) based on previous studies. In this study, the antioxidant activity of ethanol extract from noni fruit (*Morinda citrifolia*) was tested using the DPPH method, resulting in an IC_{50} value of 28.82 $\mu\text{g/mL}$. This result indicates that the noni extract possesses significant antioxidant activity, although it is higher compared to some other plant extracts that have been extensively studied, such as the extracts of

Curcuma aeruginosa (Black Ginger) and *Curcuma zedoaria* (White Ginger), with IC_{50} values of 0.67 $\mu\text{g/mL}$ and 15.11 $\mu\text{g/mL}$, respectively (Fitriana et al., 2024). A study by Meilawati et al. (Meilawati et al., 2021) also tested the antioxidant activity of noni juice, which resulted in an even higher IC_{50} value of 113.35 $\mu\text{g/mL}$, while the ethanol extract of noni fruit in this study showed a lower IC_{50} value, suggesting promising antioxidant potential.

Table 1. Literature review results on the antioxidant activity of noni fruit (*Morinda citrifolia*)

No	Sample	IC_{50} ($\mu\text{g/mL}$)	Reference
1.	Noni juice extract	113.35 \pm 0.7	(Meilawati et al., 2021)
2.	Ethanol extract of ripened noni juice	24.92 \pm 0.9	
3.	Water extract of ripened noni juice	113.13 \pm 1.4	
4.	Noni fruit extract	61.74	(Puteri et al., 2024)
5.	Ethanol extract of noni fruit	442.40 \pm 5.78	(Qulub et al., 2018)
6.	Ethanol extract of noni leaves	374.29 \pm 4.14	
7.	Noni fruit extract	153.85	(Sukeksi et al., 2018)
8.	Noni fruit extract	25.70	(Rahmawati et al., 2016)
9.	Noni fruit extract	9.84	
10.	Ethanol extract of noni fruit	28,82	This study

The differences in results might be attributed to several factors, including variations in extraction methods, solvent types, or other testing conditions such as extraction time and material concentration. For instance, the use of ethanol as a solvent in this study might optimize the extraction of bioactive compounds soluble in ethanol, which could influence the final antioxidant activity. Additionally, variations in fruit maturity and the source of the material might also affect the chemical composition and antioxidant activity of the extract.

The innovation highlighted in this study lies in the use of ethanol extract from noni fruit, which has not been widely explored, especially in terms of its potential as a natural antioxidant source. This study contributes by demonstrating that, although the IC₅₀ value of noni fruit extract is higher compared to some other plants, it still holds considerable potential as a natural ingredient for applications in the pharmaceutical and food industries.

Another innovation is the direct comparison with well-known plant extracts, providing a clearer perspective on the positioning of noni fruit as a natural resource for health and nutrition applications (Hossain et al., 2015).

Antibacterial Activity

The antibacterial activity of noni fruit extract was tested using the Resazurin Microtiter Assay (REMA) against five pathogenic bacteria: *Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), *Propionibacterium acnes* (Gram-positive), and *Salmonella typhi* (Gram-negative). Noni fruit extract was prepared in a stock solution at a concentration of 20 mg/mL. 50 µL of the extract solution was added to each well containing 100 µL of nutrient medium and bacterial inoculum. After 24 hours of incubation at 37°C, 10 µL of 0.02% resazurin solution was added and incubated for an additional 4 hours. For comparison, Results of noni fruit antibacterial activity are shown in Table 2.

Table 2. Antibacterial activity results of noni fruit (*Morinda citrifolia*) extract

Bacteria	MIC* (mg/mL)	
	Noni Fruit (<i>Morinda citrifolia</i>)	Ampicillin
<i>Bacillus subtilis</i>	2.50 ± 4.93	0.31 ± 2.15
<i>Staphylococcus aureus</i>	10 ± 4.93	<0.08 ± 2.15
<i>Salmonella typhimurium</i>	10 ± 4.93	2.50 ± 2.15
<i>Pseudomonas aeruginosa</i>	>10 ± 4.93	5.00 ± 2.15
<i>Propionibacterium acnes</i>	0.63 ± 4.93	0.63 ± 2.15

Table 3 shows the literature search results on the antibacterial activity of noni fruit (*Morinda citrifolia*) from previous studies. The ethanol extract of noni fruit (*Morinda citrifolia*) tested in this study demonstrated significant antibacterial activity against several pathogenic bacteria, both Gram-positive and Gram-negative. The MIC for *Bacillus subtilis* was 2.5 mg/mL and for

Propionibacterium acnes was 0.63 mg/mL, indicating that the noni extract has considerable antibacterial potential, especially against Gram-positive bacteria (Anggraeni et al., 2019). This finding is consistent with previous studies that also reported antibacterial activity from noni fruit extract, although the MIC values varied depending on the plant part used and the testing conditions.

Table 3. Literature search results on the antibacterial activity of noni fruit (*Morinda citrifolia*)

Plant Part	Test Bacteria	MIC	Reference
Seeds	<i>Staphylococcus aureus</i>	5 mg/mL	(Oktaviana et al., 2019)
		10 mg/mL	
		15 mg/mL	
Leaves	<i>Pseudomonas aeruginosa</i>	300 mg/mL	(Putri et al., 2023)
		400 mg/mL	
		500 mg/mL	
	<i>Propionibacterium acnes</i>	12.5 mg/mL	(Sugiarti & Shofa, 2021)
		25 mg/mL	
		50 mg/mL	
	<i>Salmonella typhimurium</i>	25 mg/mL	(Halimah et al., 2019)
		50 mg/mL	
		75 mg/mL	
	<i>Bacillus subtilis</i>	100 mg/mL	(Ayu et al., 2010)
80 mg/mL			
90 mg/mL			
Fruit	<i>Bacillus subtilis</i>	100 mg/mL	This Study
	<i>Bacillus subtilis</i>	2.50 ± 4.93 mg/mL	
	<i>Staphylococcus aureus</i>	10 ± 4.93 mg/mL	
	<i>Salmonella typhimurium</i>	10 ± 4.93 mg/mL	
	<i>Pseudomonas aeruginosa</i>	>10 ± 4.93 mg/mL	
	<i>Propionibacterium acnes</i>	0.63 ± 4.93 mg/mL	

However, the noni extract exhibited lower antibacterial activity against Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Salmonella typhimurium*, with MIC values above 10 mg/mL. This may be due to the chemical composition of the noni extract, which might be less effective in penetrating the outer membrane of Gram-negative bacteria. Gram-negative bacteria have more complex cell walls with an outer lipopolysaccharide (LPS) layer that can hinder the penetration of active compounds from the plant extract (Setiawan et al., 2017). This explains why the noni extract is more effective against Gram-positive bacteria, which have simpler cell walls.

Compared to previous studies, such as Oktaviana et al. (Oktaviana et al., 2019), who reported a MIC of 5 mg/mL for noni seed extract against *Staphylococcus*

aureus, this study shows that noni fruit extract has better antibacterial potential against Gram-positive bacteria. However, compared to standard antibiotics such as ampicillin, the MIC values for noni extract are still higher. This suggests that although noni extract shows antibacterial activity, its effectiveness may need to be enhanced for broader clinical applications.

The main innovation in this study is the use of noni fruit extract as a natural antibacterial source with promising results, especially for Gram-positive bacteria. Additionally, this study highlights the importance of factors such as extract concentration, contact time, and extraction methods in determining the antibacterial effectiveness of this plant. Further research is needed to identify the bioactive compounds responsible for its antibacterial activity and to develop more

effective formulations to enhance the antibacterial potential of noni fruit extract.

Conclusions

The ethanol extract of noni fruit (*Morinda citrifolia*) demonstrated significant antioxidant activity with an IC₅₀ value of 28.82 µg/mL. While this value reflects good antioxidant potential, it is not as strong as some other plant extracts. In terms of antibacterial activity, the noni fruit extract effectively inhibited several pathogenic bacteria, with an MIC of 2.5 mg/mL for *Bacillus subtilis* and 0.63 mg/mL for *Propionibacterium acnes*. However, the noni extract showed a higher MIC against *Salmonella typhi* and *Pseudomonas aeruginosa* (10 mg/mL each), indicating lower effectiveness against these bacteria compared to standard antibiotics.

The novelty of this study lies in its focus on the ethanol extract of noni fruit, which shows stronger antibacterial potential against *Bacillus subtilis* and *Propionibacterium acnes*, as well as promising antioxidant activity. This study contributes new insights into the development of noni fruit as a natural source for health and pharmaceutical applications, an area that has been less explored compared to other plants with similar potential. Although the findings are promising, further development is needed to enhance the effectiveness of noni fruit extract. Therefore, noni fruit has the potential to be developed as a natural antioxidant and antibacterial source with broader applications in the health and pharmaceutical industries.

Author Contributions

WDF designed the research study, data analysis, writing–original draft, writing–review & editing, supervision, project administration, and funding acquisition. **MFI** data collection and data visualization.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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