

Antibacterial activity of *Moringa oleifera*, *Solanum nigrum*, and *Sesbania grandiflora* against Bacteria isolated from wound infections

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Abstract

Around the world, most of the medical systems usually employ medicinal herbs to treat both acute and chronic wounds. The current study aims to evaluate the antibacterial properties of *Moringa oleifera*, *Solanum nigrum*, and *Sesbania grandiflora*. These plants were tested for antibacterial activity against wound infections by collecting and extracting the plant's crude material. By using the Hanging drop method and the agar-well diffusion method, three distinct bacterial colonies were identified. Ethanol extract showed the most significant antibacterial activity, attributed to the presence of alkaloids, tannins, saponins, phenols, and steroids. The antibacterial activity varied for different test samples of 500 µg/ml against Gram-negative bacteria, with the positive control serving as a reference of the expected effect. *Solanum nigrum*'s potent antibacterial properties suggest its potential as a valuable natural resource in combating bacterial diseases, potentially aiding in future herbal medicine and pharmaceutical research. The results suggest these plants could be valuable natural resources for combating bacterial diseases.

1. Introduction

A breach in the skin's epithelial integrity or a loss of cellular, anatomical, or functional continuity of live tissue can also be considered a wound. They can damage people's health conditions directly or indirectly and come in a variety of forms, including skin tears, bruises, ulcers, and post-operative. It could eventually result in death if improperly treated. Certain species of bacteria, such as *Streptococci*, *Staphylococci*, *Pseudomonas sp.*, and bactericides, cause persistent wound infections (Bernatchez and Bichel 2023). Antibiotics have significantly improved human health worldwide, especially in developing nations with inadequate public health infrastructure, by lowering the morbidity and mortality rates brought on by bacterial infections. However, the public has abused and misused antibiotics, which has led to a rise in the rates of antibiotic resistance in several microorganisms. Many antibacterial and antifungal products on the market are derived from microbiological sources. However, the emergence of antibiotic resistance poses a problem for the agents that are already in use. Research on evaluating the antimicrobial activity of traditionally used medicinal plants is crucial for locally accessible plant species, particularly for the detection of crude drugs (Salam et al. 2023). Traditional herbal medicines will be safe and reduce pathogen resistance because of pathogens that exist in plant cells as joined forms of multiple molecules. Secondary metabolites in drugs have fewer negative effects; thus, alternative medications have been examined for their pharmacological potential from a variety of plants. Many plant species have substantial medicinal benefits for healing human problems (Keita et al. 2022). *Moringa oleifera*, also known as the "miracle tree." The finding shows that urinary tract infections, caused by both Gram-positive and Gram-negative bacteria can be potentially prevented by the methanol leaf extract of *M. oleifera*. *M. oleifera* leaf extract has many health benefits, which include hepatoprotection, antioxidant, neuroprotective, anticancer, anti-inflammatory, antifungal, and antibacterial properties (Pareek et al. 2023). By using the excision wound model, wound-healing property of a methanol extract of *Sesbania grandiflora* (L) bark in Wistar albino rats was assessed. Comparing methanol extract to the usual 1% framycetin sulfate, a 10% w/w dosage showed considerable wound healing activity. The outcomes verified that the bark of *Sesbania grandiflora* exhibited noteworthy wound-healing properties in the methanol extract form. *Solanum nigrum* is sometimes referred to as black nightshade or maku. People have used *S. nigrum* for a long time to treat various conditions, such as fever, discomfort, and inflammation (Barua et al. 2012; Ofori-Kwakyie et al. 2011).

Recent phytochemical studies have shown that *S. nigrum* crude extract and some chemicals have various activities, including anticancer, antioxidant, anti-inflammatory, hypotensive, immune modulator, antibacterial, and liver-protecting effects. Patients who have wounds or infections face serious health issues because of the loss of epithelial continuity (Chen et al. 2022). Clinically available antibiotics are no longer effective against the pathogenic bacteria that cause wounds. The need to prevent the propagation of infectious bacteria has therefore made it necessary for researchers to search for new sources of antibacterial chemicals made from therapeutic plants (Chinemerem Nwobodo et al. 2022; Muteeb et al. 2023). The current study focuses on evaluating the antibacterial activity of leaf extracts from *Moringa oleifera*, *Sesbania grandiflora*, and *Solanum nigrum* against microbes linked to wound samples.

2. Materials and Method

2.1. Collection of the plant leaves

Lively leaves of *Moringa oleifera*, *Sesbania grandiflora*, and *Solanum nigrum* were gathered for the study, cleaned and allowed to air dry for three weeks (Ademiluyi et al. 2018).

2.2. Collection of the plant leaves

The wound sample was collected from a clinic in Trichy. A cotton swab moistened with regular saline was used to clean the wound. The cotton swab tip was rotated over at least a 1 cm² area of viable wound bed tissue for 5 seconds with enough pressure to extract fluid from the patient's wound tissue without contamination (Ramsay et al. 2016). This was done aseptically using the Levine wound collection method. The material from the wound swab was brought to the lab for bacteriological examination.

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2.3. Enumeration of Microorganisms

2.3.1. Preparation of a sample

Using Spread plate method, 100 µl of the test sample (WP) was taken. For isolation of bacteria 10⁶ dilution was used (Shokryazdan *et al.* 2014).

2.3.2. LB Agar Medium

The medium was prepared by dissolving 1.75 grams of agar powder, 1 gram of tryptone, 1 gram of NaCl, 0.5 grams of yeast extract, and 1 gram of commercial LB Agar medium in 100 millilitres of distilled water. It was dissolved and autoclaved for 15 minutes under 121°C and 15 pounds pressure. After autoclaving, the media was well combined while still molten and then poured into 100mm petri plates (25–30 ml/plate). Using the spread plate method, the 10⁶ dilution was plated on the LB agar medium, and incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated and plated onto a new plate (Parker *et al.* 2025).

2.3.3. Isolating Pure Colonies

Three distinct bacterial colonies were separated and plated onto a brand-new LB agar plate following incubation for 24 hours at 37°C.

2.3.4. CFU (Colony Forming Unit)

Using the spread plate method, the specified sample (WP-100µl) dilution was spread and cultivated overnight under 37°C using a bacteriological incubator (Yadav *et al.* 2016).

$$CFU/ml = \frac{\text{(No. of colonies} \times \text{Total dilution factor)}}{\text{The volume of culture plated (ml)}}$$

2.3.5. Gram Staining

The glass slide contained a loop that was loaded with bacterial culture. In front of the flame, the slide was smeared. After staining the slides for a minute with crystal violet dye, they were cleaned in distilled water. After adding and incubating Gram's iodine for a minute, distilled water was used to rinse. Following the addition of the decolorizing agent and a minute of incubation, the safranin stain was introduced, and a minute later, it was cleaned with distilled water. Gram-positive bacteria were identified by the purple color of the slides under the trinocular microscope, while gram-negative organisms were indicated by the pink tint (Kumar *et al.* 2025).

2.3.6. Motility Test – Hanging Drop Method

The hanging-drop method was used to conduct the motility test. Vaseline was applied to the edge of the coverslip where it was taken. When the coverslip was put over the cavity slide, the test samples were moved to it. The organisms' motile or non-motile properties were noted when the slide was examined at 100X magnification (Cleary *et al.* 2002).

2.4. Preparation of an extract from leaves

The plant components were dissolved in 100 milliliters of distilled water solution and incubated at 4 degrees Celsius for additional research. After submerging the components in distilled water, the extract was filtered using Whatman No. 1 paper, and muslin cloth, and then dried using a rotary evaporator (Raghuvanshi *et al.* 2021).

2.5. Test for Antibacterial Activity

Using Agar well diffusion method, the antibacterial activity of leaf extracts against wound infections was evaluated. At concentrations of 500µg/ml, 250µg/ml, 100µg/ml, and 50µg/ml the diluent was added to the crude extract. On Muller-Hinton agar plates the test organism was swabbed at various concentrations were added to wells of (5 mm) of plate. The zone of inhibition that formed around the well after the leaf extracts were incubated for 24 hours under 37°C was used to measure the extracts antibacterial efficacy. As a positive control, gentamicin was utilized.

3. Results

3.1. Antimicrobial Potential of Medicinal Leaf Extracts

In this study, the antibacterial activity of various commonly used leafy medicinal plants varieties was evaluated against gram-negative bacteria isolated from wound infections. The results demonstrate significant variations in the antimicrobial efficacy among tested leafy medicinal plants samples. Each plant variety exhibited a distinct antibacterial profile, suggesting diverse bioactive compounds are present in these extracts. The inhibitory effects on bacterial growth were observed through various assays, executes the potential of medicinal plants as a natural antimicrobial agent.

3.2. CFU (Colony Forming Unit)

It is the measure of viable colonogenic cell numbers in CFU/mL. An indicated number of cells that remain viable was enough to proliferate and form small colonies.

Table 1. Count of Bacterial colonies adhered (LB Agar)

S. No	Test sample	Growth of Bacterial colonies- 24 hrs	Total volume of test sample	CFU/ml/gm- 24 hrs
1	WP- S6	46	100 µl	4.6×10 ⁸

3.3. Identification of Bacterial organism

Gram staining was used to identify the bacterial organism, which uses crystal violet or methylene blue as the primary colour. Gram-positive organisms retain the primary colour and appear purple-brown under a microscope. Gram-negative organisms do not take up primary stain and appear red under a microscope.

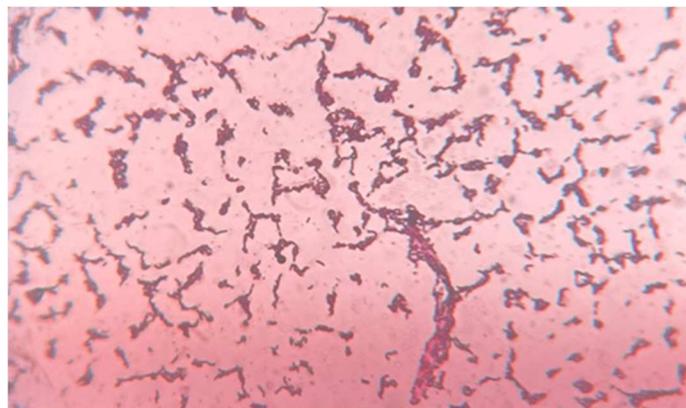


Figure 1. Gram-negative Bacteria (Rod Chain)

3.3. Motility Test

Bacteria exhibit widespread motility, enable them move towards favorable conditions and facilitates host-associated processes such as colonization. Environmental conditions, highly regulate the expression of different bacterial motility types.

Table 2. The result of the Motility test

No	Name of sample	No. of colonies	Result of the Hanging Drop method
1.	WP	1	Motile

Table 3. shows the Zone of inhibition for samples WP1, WP2, and WP3 against Culture 1

No	Test sample	Zone of inhibition (mm) Mean±SD				PC
		500µg/ml	250µg/ml	100µg/ml	50µg/ml	
1	WP1	4.75±0.35	1.9±0.14	0	0	21.5±0.7071
2	WP2	4.75±0.35	1.15±0.212	0.65±0.212	0	20.5±0.7
3	WP3	25.6±0.84	19.75±0.35	14.7±0.4	8.8±1.13	20.5±0.707

SD – Standard Deviation, *Significance - p < 0.05

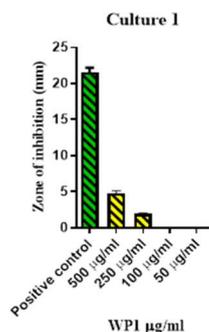
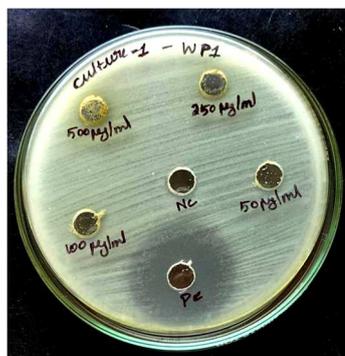


Figure 2. The effect of sample WP1 (*S. grandiflora*) was tested against Culture 1.

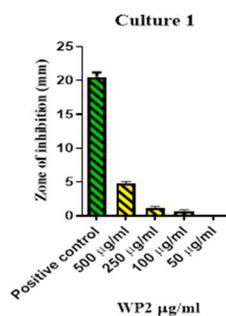


Figure 3. The effect of sample WP2 (*M. oleifera*) was tested against Culture 1.

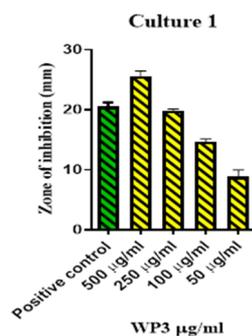
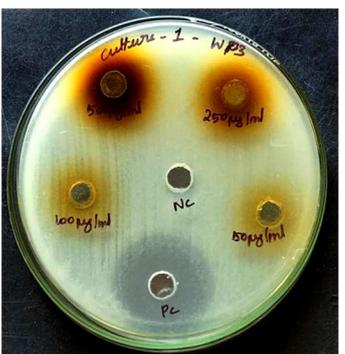


Figure 4. The effect of sample WP3 (*S. nigrum*) was tested against Culture 1

The antibacterial screening of the culture-1 test samples, shows variable inhibition zones at different concentrations, which indicates dose dependent antibacterial activity. At 500µg/ml, WP1 shows exhibit highest activity, later it decreased progressively at lower concentrations. At 100 and 50µg/ml, it disappeared completely. Similarly, measurable inhibition was produced by WP2 at 100µg/ml but none in 50µg/ml. Compared the positive control, the test samples show weaker activity, depend on the concentration the inhibition zone increases, suggests that the antibacterial potential is due to presence of bioactive compounds. The ethanol-based extracts show higher inhibition rate among the test samples, which aligns with previous studies which indicates the ethanol extracts secondary metabolites such as phenolics, tannins and alkaloids (Bibi et al. 2025). These compounds collectively contribute to the antibacterial action, and associated with membrane disruption, enzyme inhibition, and protein precipitation. Previously researchers have reported similar findings, who identified phenolics, flavonoids, terpenoids and alkaloids are the key phytochemicals to potent antibacterial effect in *Moringa oleifera* (El-Sherbiny et al. 2024). Studies on phytochemical rich extracts from *Vernonia amygdalina*, *Azardirachta indica*, and *Acalypha wilkesiana* has potent anti-staphylococcal agents. Previous evidence also shows similar result as current findings, due to better solubility of bioactive metabolites, methanol extracts show stronger antibacterial activity than acetone

extracts (Lee et al. 2024). Similarly, the previous literature work shows that the compounds isolated from *C. patentinervium* leaves, inhibit wound pathogens. Because of the ethanol derived fractions has potent antibacterial constituents like hyperin and cymaroside (Mogana et al. 2020). Also, some evidence of research shows report that *Jatropha tanjorensis* methanol extract has higher inhibitory effects, among diverse phytochemicals these attributes to enhanced activity. For inhibiting multidrug-resistant bacteria, several medicinal plants like *Myrianthusarboresus*, *Psidium guajava*, *Alchornea cordifolia*, *Momordica charantia* and *Justica flava* are shown previously (Viswanathan et al. 2012). In an era of increasing antibiotic resistance, these findings strengthen the hypothesis that plant-derived metabolites act as promising templates for developing novel antimicrobial agents (Abdallah et al. 2023). Previous research reports provide additional supporting evidence, that the *P. scorpi*a extracts activity against burn wound pathogens due to the presence of secondary metabolites and some researchers also demonstrated antibacterial behaviour on concentration dependent extracts rich in tannins and saponins (Roointan et al. 2020). The significant activity of *Aloe megalacantha* was reported to show the antimicrobial efficacy is relevance to phytochemicals such as anthraquinones, polyphenols, flavonoids, alkaloids, saponins, terpenoids and tannins (Asmerom et al. 2020). *S. aureus* was the most sensitive species by their observation which parallel to the sensitivity patterns observed in the current study. Overall, the findings reveal that the moderate antibacterial activity increases with concentration from the Culture 1, suggest that to inhibit the test organism is due to extracts containing bioactive constituents (Nwankwo and Nasiru 2011). Compare to positive control, the inhibition zones were lower. As reported by previous literatures, the patterns observed are consistent with phytochemicals for antimicrobial action. These results provide as a base for further phytochemical profiling and purification of active fractions to specifically identify the compounds which are responsible for antibacterial properties (To et al. 2026). In future, work should include Minimum Inhibitory concentration assays (MIC), isolation of compounds, and *in-vivo* validation of culture 1 derived samples to establish their therapeutic potential.

4. Conclusion

The investigation of the findings leads us to conclude that edible medicinal herbs. nigrum has potent antibacterial qualities in its methanol extracts due to its pharmacological activity, presence of saponins and organic acids makes it as a valuable natural resource in the fight against bacterial illnesses developed from Gram-negative bacteria isolated from wound infections. The extracts of *S. grandiflora* and *M. oleifera* leaves, however, demonstrated a moderate level of efficacy against the tested strain at 500 µg/ml. These results support the conventional wisdom regarding the antibacterial properties of three leafy medicinal plants concerning wound infections. However, before suggesting their use, further research must be undertaken to isolate the potential active compounds from the leaves and need *in-vivo* validation for validate the efficacy and safety.

5. Disclosure Statements

5.1. Author Contribution

BR-Writing-Original draft, MT- Conceptualization, SH-Preparation, ARY-Methodology, AH-Validation, MM-Validation, PV-Writing-Review and Editing, Supervision. All the authors have read and approved the final manuscript.

5.2. Declaration of Generative AI

The authors declare that no generative AI tools were used in the drafting, writing, or editing of the manuscript. All scientific interpretations and conclusions are the author's own.

5.3. Ethics approval (for clinical/animal studies)

Ethical review and approval were waived for this study by the Institutional Review Board of [Holy Cross College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India], as the research involved publicly available data.

5.4. Informed Consent Statement

Not applicable.

5.5. Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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5.8. Conflicts of Interest

The authors declare that they have no known financial, personal, academic, or other relationships that could inappropriately influence, or be perceived to influence, the work reported in this manuscript. All authors confirm that there are no competing interests to declare.

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