

Antibacterial Effect of Neem Extracts (Leaf and Bark) Against *E.Coli*

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Publication Date: 2026/07/28

Abstract: The rise of antibiotic-resistant bacteria has increased the need for effective plant based antimicrobial agents. Neem (*Azadirachta indica*), a widely used medicinal plant, contains numerous bioactive compounds with reported antibacterial properties. This study investigates the antibacterial effects of neem leaf and bark aqueous extracts against *Escherichia coli*, a common pathogen found on human skin. Skin swabs were collected from students of Federal University Gashua, and *E. coli* was isolated and cultured using standard microbiological procedures. Neem leaf and bark were collected, shade-dried, ground into powder, and extracted using aqueous solvent. Antibacterial activity was evaluated using the agar well diffusion method, while the minimum inhibitory concentration (MIC) was determined through serial dilution. Results showed that both neem leaf and bark extracts exhibited inhibitory effects against *E. coli*, with the leaf extract producing a larger zone of inhibition (3.5 cm at 10^{-1} concentration) than the bark extract (3.1 cm at 10^{-1}). Activity decreased with further dilution, and no inhibition was observed from 10^{-4} onward. The findings demonstrate that neem leaf and bark possess antibacterial properties and could serve as potential natural alternatives to synthetic antibiotics. The study highlights the relevance of neem in developing affordable, plant-based treatments for bacterial infections, particularly in rural communities where access to antibiotics is limited.

Keywords: *Neem, Escherichia Coli, Bacteria, Leaf and Bark.*

How to Cite: Haruna Sabo Suleiman; Muhammad Abubakar Muhammad; Usman Ali Muazu; Halima Umar Abdullahi; Luka Obidah Ali (2026) Antibacterial Effect of Neem Extracts (Leaf and Bark) Against *E.Coli*. *International Journal of Innovative Science and Research Technology*, 11(7), 1575-1580. <https://doi.org/10.38124/ijisrt/26jul508>

I. INTRODUCTION

Neem plants have many bio-active compounds which are potential medicinal agents. Herbal medicines are in developing countries, but of recent, there has been an increase in the use of herbal in the developed world (Mogal et al., 2018). Plants provide an alternate source for the of Neem treatments. There are plenty of plants in traditional medicine which have protective therapeutic properties (Alzohairy, 2016). Most likely plants will continue to be valued source of molecules which may after possible chemical manipulation; provide new and improved drugs (Pauler et al., 2019). resistance to antibiotics is a major problem for clinicians and therapeutic industry great efforts are been made to screen wide varieties of medicinal plants from the traditional of medicine with the hope of getting some newer, safer and more effective agents that can be fight infectious diseases (Zingne et al., 2019). parts of the Neem plant have medicinal properties. The leaf, bark, oil, flower, fruit seed exhibit great

properties which include antiallergic, antifungal, antibiotic, antiemetic, anti-inflammatory, insecticidal, larvicidal, antimalarial, anti-ulcer and other biological (Campos et al., 2016). Herrera- Calderon et al. (2019) reported that neem has great activity and it contains 35 biological active compounds. They also stated that the leaf and things are usually used to clean the teeth and also used as a tonic. It has been in report that of India place the leaves on their beds, books, cupboards to control bugs (Banerjee et al.,2012).

➤ History of Neem Tree

The neem tree (*Azadirachta indica*) has been valued for over 4,500 years, primarily in India, for its medicinal, agricultural, and cultural significance. Ancient Ayurvedic texts praised it as a "healer of all ailments," and it was widely used in Unani and Siddha medicine. Neem also held religious importance, often planted near temples and used in rituals. During the Middle Ages, traders spread neem to Africa,

Southeast Asia, and the Middle East, where it became integral to local agriculture and medicine. In the 20th century, scientific research confirmed its antibacterial and pesticidal properties, leading to its global use in eco-friendly agriculture, herbal medicine, and cosmetics. Today, neem remains a symbol of health, sustainability, and natural living.

➤ *Historical Perspective of Neem*

The use of neem in traditional medicine dates back centuries, with historical records indicating its significance in various cultures (Biswas et al., 2022). In Ayurveda, neem is described for its diverse medicinal properties, including antibacterial, antiviral, and antifungal effects (Subapriya and Nagini, 2015). Ayurvedic practitioners have employed Neem as a versatile remedy for various ailments, including skin disorders, digestive issues, and infections. The historical use of Neem in Ayurveda has contributed to its continued popularity and significance in traditional medicine practices. Beyond its medicinal applications, Neem holds cultural and religious significance in India and other parts of South Asia. It is often referred to as the "Village Pharmacy" due to its wide-ranging benefits. *Azadirachta indica*, seeds, and oil are used in religious ceremonies, festivals, and rituals, symbolizing purification and protection. The historical and cultural association of Neem has fostered a deep-rooted respect and reverence for this versatile plant. The historical perspective of Neem has influenced its modern-day applications in various fields. Its natural insecticidal properties have made it a key component in organic farming, pest control, and agricultural practices (Biswas et al., 2022). Additionally, Neem-based products have gained popularity in the cosmetic and personal care industry due to their antibacterial and antifungal properties. The historical knowledge and understanding of Neem have paved the way for its integration into contemporary practices, showcasing its enduring relevance and adaptability.

➤ *Chemical Composition of Neem Extracts*

Neem extracts are rich in bioactive compounds. Nimbin, nimbidin, and quercetin are among the key constituents found in *Azadirachta indica* extracts, contributing to its medicinal properties (Biswas et al., 2022). These compounds have been linked to antibacterial activity, warranting further investigation. Azadirachtin, on the other hand, is a powerful insecticidal compound that makes Neem extracts effective against various pests (Biswas et al., 2022). Nimbolide, a limonoid compound found in Neem, has shown potential anticancer activity in preclinical studies (Nagini, 2015). These compounds contribute to the therapeutic potential of Neem extracts and have been extensively studied for their medicinal properties.

In addition to the aforementioned compounds, Neem extracts also contain flavonoids, such as quercetin and kaempferol, which possess antioxidant and anti-inflammatory activities (Nagini, 2015). Furthermore,

triterpenoids like gedunin and azadiradione found in Neem extracts have shown promising antimalarial effects. The essential oils present in Neem, including citronellal, geraniol, and limonene, contribute to its characteristic aroma and may have antimicrobial properties (Girish, 2018). These diverse chemical constituents in Neem extracts provide a broad spectrum of biological activities and have been utilized in traditional medicine for various health conditions. The chemical composition of Neem extracts can vary depending on factors such as the method of extraction and the part of the plant used. Different extraction techniques, including solvent extraction, steam distillation, and supercritical fluid extraction, can yield Neem extracts with varying concentrations of bioactive compounds (Biswas et al., 2022). It is important to consider these factors when studying the chemical composition and therapeutic potential of Neem extracts. The multitude of chemical components present in Neem contributes to its wide-ranging applications in traditional medicine, agriculture, and the cosmetic industry.

Bacterial skin infections are a significant health concern, particularly in regions with limited healthcare access. The increasing resistance of bacteria to conventional antibiotics has led to a growing interest in natural alternatives such as neem (*Azadirachta indica*), which contains bioactive compounds with antimicrobial properties (Alzohairy, 2016). Previous studies have demonstrated neem's antibacterial activity against pathogens like bacterial species and *Pseudomonas aeruginosa* (Chatterjee et al., 2020). However, there is limited research on its effectiveness against bacterial species found on human skin in Gashua. This study seeks to evaluate the antibacterial effects of neem leaf and bark extracts on skin bacteria in Gashua. The findings could contribute to the development of cost-effective, plant-based antibacterial treatments, particularly for rural communities with limited access to antibiotics (WHO, 2021).

The aim of this study is to determine the antibacterial effect of neem leaf and bark against *E. Coli* found on human skin in Gashua.

II. MATERIALS AND METHODS

➤ *Study Area*

The study was conducted in the Department of Biological Science at Federal University, Gashua, Nigeria. In 2006 National Population Commission estimated the population of Gashua to be about 125,000 (NPC, 2006; Geonames, 2016). The hottest months are March and April with temperature ranges of 38-40 oC. During the rainy season within June-September, temperatures fall to 23-28 oC, with rainfall of 500 to 1000mm (Sunday Tribune, 2009). Its geographical coordinates are 12 52'5"North, 11 2'47" East.



Fig 1: Map of the Study Area (Gashua, Google Map 2025).

➤ Sample Collection

Skin swabs was collected from volunteers in federal university Gashua using sterile cotton swabs moistened with physiological saline. The swabs was taken from commonly affected areas such as the face and hands. Each sample was properly labelled and transported to the laboratory in sterile containers to prevent contamination. Ethical approval and consent from participants was obtained before sample collection, following the guidelines of the World Health Organization (WHO, 2021).

➤ Preparation of Culture Medium for the Isolation of Bacteria

Nutrient agar was prepared according to the manufacturer's instructions for the isolation of bacteria. The agar media was sterilized using an autoclave. Once cooled, the media was poured into sterile Petri dishes under aseptic conditions and allowed to solidify before use. (Cheesbrough, 2020).

➤ Isolation of Bacteria

The collected skin swab samples was streaked onto the prepared nutrient agar plates using the streak plate method. The plates was incubated to allow bacterial growth. Distinct bacterial colonies was observed and sub-cultured onto fresh agar plates to obtain pure isolates for further identification.

➤ Identification of Bacteria

The bacterial isolates was identified using:

- Colony Morphology: Observing colour, shape, and texture of bacterial colonies.
- Biochemical Tests: using indole test
- The identification results was compared with standard microbiological references.

➤ Preparation of Neem for the Determination of Antibacterial Effect

Neem leaf and bark was collected from healthy neem tree in Gashua. The plant materials was washed with distilled water and shade-dried to preserve their bioactive compounds. Once dried, they were ground into fine powder using mortar and pestle. The powder was then used to prepare different extracts for antibacterial testing. (Alzohairy, 2016).

➤ Preparation of Aqueous Extract

To prepare the aqueous extract, neem leaf and bark powder was mixed with distilled water and heated. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was stored in a sterile container for further analysis. This extract was used to test antibacterial activity against isolated bacterial species. (Chatterjee 2020)

➤ Data Analysis

The antibacterial activity of neem extracts was analysed using statistical tools. The zone of inhibition (clear areas around neem extract-treated bacteria) was measured in millimetres. Data was analysed using ANOVA (Analysis of Variance) to compare the effectiveness of neem extracts against different bacterial species. Statistical software such as SPSS or Microsoft Excel was used for calculations and graphical representations. (Montgomery, 2021).

This methodology ensures a systematic and scientific approach to evaluating the antibacterial effects of neem on skin bacteria in Gashua.

III. RESULTS

➤ Bacterial Isolation from Students

The data shows differences in the bacterial species isolated from the skin of male and female students at FUGA.

For the female students, *E. coli* and *S. epidermis* were present on both hands and faces. *P. acnes* and *M. luteus* were exclusively isolated from the faces. *E. coli* showed the highest percentage of occurrence at 40%. *S. epidermis*, *P. acnes*, and *M. luteus* each accounted for 20% of the occurrence. For the male students, *E. coli* was present on both hands and faces. *S. epidermis*, *P. acnes*,

and *M. luteus* were only isolated from the hands. Similar to females, *E. coli* had the highest occurrence at 40%. *S. epidermis*, *P. acnes*, and *M. luteus* each represented 20% of the occurrence.

The distinct distribution (e.g., *P. acnes* and *M. luteus* on faces only in females versus hands only in males) suggests potential differences in hygiene practices, physiological factors, or environmental exposure between the sexes that influence the skin microbiome.

➤ Antibacterial Activity of *Azadirachta indica*

The antibacterial activity of the dry neem leaf and bark water extracts was evaluated by measuring the Zones of inhibition (cm) at different dilution concentrations. Dry Leaf Extract (Table 3): The extract from dry leaves showed the highest zone of inhibition (3.5 cm) at the 10^{-1} dilution. Activity progressively decreased as the concentration was diluted, leading to "Growth" (no inhibition) from the 10^{-4} dilution onwards. Dry Bark Extract (Table 4): The extract from dry bark also showed the greatest inhibition at the 10^{-1} dilution (3.1 cm). The zone of inhibition decreased with increasing dilution, and like the leaf extract, it showed "Growth" (no inhibition) from the 10^{-5} dilution onwards. Comparison: The dry leaf water extract demonstrated slightly stronger antibacterial activity than the dry bark water extract, particularly at the highest concentration (10^{-1}), with 3.5 cm versus 3.1 cm zone of inhibition, respectively. The leaf extract's minimum inhibitory concentration (MIC) appeared to be between 10^{-3} and 10^{-4} , while the bark extract's MIC was between 10^{-4} and 10^{-5} . This suggests the leaf may contain a higher concentration or more potent antibacterial the extract were subjected to serial dilution to reduce the concentration of the extract. The concentration are 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} .

Table 1 Bacterial Species Isolated from the Skin of Female FUGA Students

Species	Hands	Faces	Percentage of occurrence (%)
<i>E. coli</i>	+	+	40%
<i>S. epidermis</i>	+	—	20%
<i>P. acnes</i>	—	+	20%
<i>M. luteus</i>	—	+	20%
TOTAL			100%

Table 2 Bacterial Species Isolated from the Skin of Male FUGA Students

Species	Hands	Faces	Percentage of occurrence (%)
<i>E. coli</i>	+	+	40%
<i>S. epidermis</i>	+	—	20%
<i>P. acnes</i>	+	—	20%
<i>M. luteus</i>	+	—	20%
TOTAL			100%

Table 3: Antibacterial Activity of Dry *Azadirachta indica* Leaf Against *E. coli*

Dilution concentration of leaf extracts	Zones of inhibition (cm) Water extract from dry leaves
10^{-1}	3.5
10^{-2}	2.8
10^{-3}	1.2
10^{-4}	Growth (No inhibition)
10^{-5}	Growth (No inhibition)
10^{-6}	Growth (No inhibition)
10^{-7}	Growth (No inhibition)

Table 4: Antibacterial activity of dry *Azadirachta indica* bark against *E. coli*

Dilution concentration of leaf extracts	Zones of inhibition (cm) Water extract from dry bark
10^{-1}	3.1
10^{-2}	2.5
10^{-3}	2.1

10^{-4}	1.2
10^{-5}	Growth (No inhibition)
10^{-6}	Growth (No inhibition)
10^{-7}	Growth (No inhibition)

IV. DISCUSSION

This study examined the antibacterial effects of *Azadirachta indica* (neem) leaf and bark extracts against bacterial species isolated from the skin of students in Gashua. The findings contribute to the growing evidence supporting the use of neem as a natural antibacterial agent, particularly in regions where synthetic antibiotics are becoming less effective due to rising antimicrobial resistance. Four bacterial species—*Escherichia coli*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Micrococcus luteus*—were isolated from both male and female students. *E. coli* had the highest prevalence (40%), which may indicate poor hand hygiene or contact with contaminated surfaces. Although *E. coli* is typically an intestinal organism, its presence on the skin as a transient contaminant has been reported in previous studies (Cheesbrough, 2020). Sex-related differences were observed. In females, *P. acnes* and *M. luteus* occurred mainly on the face, possibly due to cosmetic use, hormonal influence, and sebum production. In males, these bacteria appeared mostly on the hands, suggesting different exposure patterns or hygiene behaviors. This aligns with Alzohairy (2016), who emphasized that skin microflora varies based on physiological, environmental, and cultural factors. Neem leaf and bark extracts demonstrated clear antibacterial activity that decreased with dilution, confirming dose dependence. Neem Leaf Extract has the Highest inhibition 3.5 cm at 10^{-1} dilution. MIC observed between 10^{-3} and 10^{-4} Neem Bark Extract has the Highest inhibition 3.1 cm at 10^{-1} dilution MIC observed between 10^{-4} and 10^{-5} . The leaf extract showed slightly stronger antibacterial activity than the bark extract. This may be due to higher concentrations of bioactive compounds such as, Nimbin, Quercetin, Nimbidin. These compounds are known for their antimicrobial and anti-inflammatory properties (Nagini, 2015; Biswas et al., 2022). The MIC differences suggest that neem leaf contains more potent antibacterial compounds than the bark, or that these compounds are present in higher concentrations. The findings of this research agree with existing literature. Biswas et al. (2022) reported significant antibacterial activity of neem extracts across a wide range of bacterial species. Subapriya & Nagini (2015) demonstrated neem's effectiveness against both gram-positive and gram-negative bacteria. Chatterjee et al. (2020) observed strong inhibition of *Pseudomonas aeruginosa* using neem extracts. Greulich (2011) recorded neem's antibacterial action in oral health applications, supporting its broader antimicrobial potential. Like these studies, the present research confirms that neem disrupts bacterial cell walls, interferes with enzyme activity, and suppresses bacterial growth (Trouiller, 2019). The antibacterial activity recorded suggests that neem can be developed into affordable, plant-based antibacterial products, such as medicated soaps, topical creams, herbal lotions, disinfectants. This is particularly important in rural regions like Gashua, where access to quality healthcare and

antibiotics may be limited. The presence of potentially harmful bacteria such as *E. coli* on students' skin highlights the need for improved hygiene practices regular handwashing awareness on microbial contamination. Neem-based preparations could offer an accessible solution for preventing and managing skin infections.

V. CONCLUSIONS

The skin microbiome of FUGA students contains common bacterial species, and its distribution appears to be sex-dependent based on the sites of isolation for *P. acnes*, *M. luteus*, and *S. epidermis*. Both dry leaf and dry bark extracts of *Azadirachta indica* exhibit dose-dependent antibacterial activity. The dry leaf extract is concluded to be slightly more potent than the dry bark extract in inhibiting bacterial growth.

RECOMMENDATIONS

Further Isolation and Identification: Future studies should aim to identify the specific species of bacteria isolated from the skin, not just the genus (e.g., differentiating between various *Staphylococcus* and *Cutibacterium* species), for a more precise understanding of the student microbiome.

Targeted Antibacterial Testing: Test the *Azadirachta indica* extracts specifically against the isolated bacteria (*E. coli*, *S. epidermis*, *P. acnes*, and *M. luteus*) to confirm their potential as natural antiseptics for skin-related infections. **Compound Isolation:** Effort should be made to isolate and characterize the specific bioactive compounds in the neem leaf and bark extracts responsible for the observed antibacterial activity. **In-vivo Studies:** Conduct in-vivo studies to evaluate the safety and efficacy of the neem extracts as topical skin preparations.

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