African Journal of Sciences and Traditional Medicine

ISSN : 1595-7942

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJSTM.v3i2.10476

# Assessment of the Phytochemicals and Antibacterial Potential of *Azadirachta indica* (Neem) Leaves Extract against Selected Clinical Isolates

**Agbo Anthony Ogbonnia[1], Mbah-Omeje Kelechi Nkechinyere[2], Eze Vincent C.[3]**

[1]Federal Polytechnic, Ohodo, Enugu State, Nigeria; [2]Enugu State University of Science and Technology, Enugu, Nigeria; [3]Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

anthonyagbo2019@gmail.com; kelechi.omejemaba@esut.edu.ng

**Article Info:**

| Submitted: | Revised: | Accepted: | Published: |
|---|---|---|---|
| Apr 22, 2026 | May 20, 2026 | Jun 1, 2026 | Jun 6, 2026 |

## Abstract

The rapid emergence of antimicrobial resistance has intensified the search for alternative therapeutic agents from medicinal plants. *Azadirachta indica* (neem) is widely recognized for its broad-spectrum biological activities; however, comparative evidence on aqueous and ethanolic leaf extracts against clinical isolates, accompanied by phytochemical profiling, remains limited. This study aimed to evaluate the phytochemical constituents and antibacterial potential of aqueous and ethanolic leaf extracts of *A. indica* against selected clinical bacteria. Mature neem leaves were collected, authenticated, air-dried, pulverized, and extracted using distilled water and ethanol. The extracts were concentrated using a rotary evaporator. Qualitative and quantitative phytochemical analyses were conducted using standard procedures, while antibacterial activity was assessed through the agar well diffusion method against *Escherichia coli*, *Shigella* sp., *Lactobacillus* sp., and *Staphylococcus aureus*. Zones of inhibition were measured, and minimum inhibitory concentrations were determined. Phytochemical screening revealed the presence of alkaloids, saponins,

flavonoids, tannins, and glycosides, with glycosides being the most abundant constituent (22.85 ± 0.084) and flavonoids the least abundant (6.55 ± 0.087). At 200 mg/mL, the aqueous extract demonstrated higher antibacterial activity than the ethanolic extract, with inhibition zones of 27 mm against *E. coli*, 26 mm against *Shigella* sp., 23 mm against *Lactobacillus* sp., and 20 mm against *S. aureus*. The minimum inhibitory concentration results indicated resistance of *E. coli* to both extracts and reduced sensitivity of *Lactobacillus* sp. to the ethanolic extract. These findings suggest that aqueous extraction may be more effective for harnessing the antibacterial properties of neem leaves. This study contributes to phytomedicine and antimicrobial research by highlighting the potential of *A. indica* leaf extract as a cost-effective plant-based antimicrobial agent for managing bacterial infections, particularly in resource-limited settings.

**Keywords**: *Azadirachta indica*; Antibacterial Activity; Phytochemical Constituents; Clinical Isolates; Antimicrobial Resistance.

## INTRODUCTION

Humanity is endowed by nature with a wide variety of plants, herbs, and shrubs, each of which has a distinct function. In Africa and other continents, thousands of plant species are known to provide health benefits. Since ancient times, people have used different parts of several medicinal plants to treat specific ailments (WHO, 2023). The most important dietary components for optimum health are thought to be plant-based foods. Antioxidants can be found in abundance in fruits and vegetables (Shirajum *et al.*, 2015).

These medicinal plants according to Atanasov *et al.* (2021) are the major sources of bioactive compounds that have contributed significantly to the discovery and development of modern therapeutic agents. The essential roles these plant-derived phytochemicals played in the drug discovery are not unconnected with their diverse chemical structures and biological activities. Their various plant parts—including fruits, leaves, vegetables, nuts, oils, and whole grains—contain numerous bioactive constituents that exhibit beneficial metabolic and immunological effects (Atanasov *et al.*, 2021). These compounds can help meet the growing human desire for healthier lifestyles and improved disease prevention strategies.

Reports have shown that such phytochemicals have helped maximally at reducing the danger of several chronic and life-threatening diseases, including cancer, cardiovascular

diseases (CVD), obesity, and neurological disorders. These beneficial effects are largely attributed to their antioxidant properties, which enable them to detoxify free radicals and prevent oxidative cellular damage (Shirajum *et al.*, 2015). As highlighted by Shrinet *et al.* (2021), plant-derived bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds possess significant pharmacological activities that support their application in disease prevention and management.

Medicinal plants have long been recognized as valuable sources of therapeutic agents and continue to play a significant role in primary healthcare across the world. According to the WHO, (2023), nearly 80% of the global population depends on plant-based remedies for their basic healthcare needs. Extracts obtained from these plants have demonstrated a wide spectrum of biological activities, including antibacterial, antifungal, and antiviral properties (Agbo *et al.*, 2025; Kebede *et al.*, 2021). These properties are largely attributed to the presence of bioactive phytochemicals that exert diverse pharmacological effects and contribute to disease prevention and management.

However, the rapid and continuous emergence of multidrug-resistant pathogens resulting from the indiscriminate use and misuse of antibiotics in the treatment of infectious diseases has become a major global clinical concern (*Tsaku et al.*, 2017). Antimicrobial resistance has significantly reduced the effectiveness of many conventional antibiotics, thereby complicating the treatment of infectious diseases and increasing morbidity and mortality rates worldwide. In response to this challenge, researchers have intensified efforts toward the screening and evaluation of medicinal plants as potential sources of new antimicrobial agents. Such investigations have generated increasing scientific interest and global acceptance of plant-based therapeutic alternatives (Abdallah *et al.*, 2023).

Available records indicated that more than 20,000 plant species possess medicinal properties and have been traditionally used for the treatment of various ailments (Kunle and Egharevba, 2013). Among these are plants such as neem, guava, and lemon, which are widely recognized for their therapeutic benefits. These plants have attracted considerable research attention because of their availability, cost-effectiveness, specificity of action, low toxicity, and minimal residual effects.

One such medicinal plant that has attracted considerable scientific interest is *Azadirachta indica*, commonly known as neem. Neem leaves are rich in several bioactive

phytochemicals that exhibit antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties. These characteristics make neem a promising natural resource for the development of alternative antimicrobial agents, particularly in the face of increasing antibiotic resistance among clinical pathogens. In Nigeria, neem is popularly referred to as "Dogonyaro" and has been widely used in traditional medicine for the treatment of various ailments including malaria, diabetes, eczema, and cardiovascular disorders. Beyond its medicinal applications, neem has also been utilized in the production of toothbrushes, lubricants, insect repellents, and pesticides (Virshette *et al.*, 2020).

Scientific investigations have revealed that different parts of the neem plant—including the leaves, stem, bark, and roots—contain numerous bioactive compounds with antibacterial, antifungal, and antiviral properties (Khanal, 2021). The pharmacological activities of neem have been attributed to a wide range of phytochemicals such as alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones. These compounds are associated with diverse biological activities including anti-inflammatory, anti-mutagenic, anti-carcinogenic, antioxidant, antihyperglycemic, anti-ulcer, and anti-diabetic effects (Khanal, 2021). Among the most notable active constituents of neem is azadirachtin, a complex compound consisting of several isomeric molecules identified as azadirachtin A–G, with azadirachtin E reported to exhibit greater biological effectiveness (Zillich *et al.*, 2015). Other biologically active constituents include salannin, meliantriol, volatile oils, and nimbin (Khanal, 2021).

Historically, neem has been utilized to address a wide range of global agricultural, environmental, and health challenges due to its versatile biological properties (Santhosh and Navartnam, 2013). Neem oil, for instance, has been employed in the treatment of certain diseases such as diabetes and tuberculosis, while also serving industrial and agricultural purposes as a lubricant, pesticide, and medicinal preparation (Virshette *et al.*, 2020). These multifunctional applications further highlight the therapeutic and economic importance of the plant.

Although antibiotics remain the primary agents used in the treatment of bacterial infections, their misuse for both preventive and therapeutic purposes has contributed significantly to the emergence of antibiotic-resistant bacteria (Banerjee *et al.*, 2013). The WHO has recognized antimicrobial resistance as a major global health security threat requiring coordinated action across healthcare systems, governments, and society (Mehta

and Diwakar, 2021). Consequently, there is an increasing need to explore alternative antimicrobial agents, particularly those derived from medicinal plants. These may serve as complementary or alternative therapeutic options in the management of resistant infections. Herbal preparations derived from crude plant extracts contain multiple bioactive compounds, making it more difficult for pathogens to develop resistance compared with single-compound synthetic antibiotics (Mehta *et al*., 2016; Radji *et al*., 2013).

Several studies have investigated the antimicrobial properties of medicinal plants, and among the numerous species evaluated, neem has consistently been identified as a promising and time-tested source of antimicrobial agents (Mamman *et al*., 2013). The continued search for novel antibacterial agents from plant sources remains important due to their relatively low toxicity to humans and animals compared with many synthetic drugs (Kunle and Egharevba, 2013). Furthermore, the use of conventional antibiotics is sometimes associated with adverse side effects, which further justifies the exploration of safer natural alternatives (Alekshun and Levy, 2007). Previous studies have demonstrated that neem leaf extracts exhibit significant antimicrobial activity against a wide range of microorganisms;including *Bacillus* spp., *E.coli*, *Staphylococcus* spp., *Pseudomonas* spp., *Clostridium* spp., *Shigella* spp., and *Salmonella* spp. (Mamman *et al*., 2013).

Despite the numerous studies conducted on neem and its antimicrobial properties, certain gaps remain regarding the phytochemical composition and antibacterial efficacy of its extracts against specific clinical bacterial isolates. Therefore, in light of these considerations, this study was undertaken to assess the phytochemical constituents and evaluate the antibacterial activity of aqueous and ethanolic extracts of neem leaves against selected clinical bacterial isolates.

## MATERIALS AND METHODS

### Collection and identification of the plant samples

Fresh and healthy leaves of *A. indica* were collected from Carmelite farm in Enugu State, Nigeria. This sample was identified and authenticated by Prof. Garuba Omosun, Department of Plant Science and Biotechnology, Micheal Okpara University of Agriculture, Umudike, Nigeria.

Preparation of plant material

The leaves were washed in potable water to remove debris and air-dried at room temperature for one week. The dried leaves were then coarsely pulverized with blending machine, and extracted using aqueous and ethanol (Soxhlet) methods. For aqueous, five hundred (500) gram of the sample was weighed and soaked in water (1,000 ml) for 24 hours and filtered. In Soxhlet method, five hundred (500) gram of the sample was weighed out, wrapped in filter paper and then put in the thimble of the Soxhlet apparatus compartment. Thereafter, the condenser was carefully and efficiently connected. An initial 1,000 ml volume of the solvent (ethanol) were added with the aid of a funnel by passing it through the thimble containing the sample to the round bottom flask system of the Soxhlet. The inlet and outlet of the condenser were connected to a hose respectively, for the recycling of the cold water during the extraction. Thereafter, the heat source was switched on about 5cm from the flask. Finally, the crude extracts (aqueous and ethanol) were concentrated at 50 $^{o}$C using a rotary evaporator, and the resultant extracts were stored separately in sterile screw-capped bottles and kept at 4 $^{o}$C in a refrigerator for further use.

Qualitative phytochemical analysis

Preliminary qualitative phytochemical screenings were determined using the standard procedures. A modified method of Ankita and Sapan (2018) was used in these analyses. Essentially, phytochemical screenings were carried out to identify the presence of secondary plant metabolites. The saponin, alkaloids, tannin, flavonoids, glycosides, phenol and steroid were determined following these protocols.

Quantitative phytochemical evaluation

Quantitative tests of these phytochemicals were done by the gravimetric method. The standard procedures described by Harborne (1973) with slight modifications by Muhammad and Abubakar (2016) were employed to quantify the phytochemical constituents.

Isolation of organisms

The bacteria organisms used include; *Escherichia coli*, *Shigella sp*, *Lactobacillus sp* and *Staphylococcus aureus*. These organisms were isolated from urine samples at the Microbiology Laboratory, Department of Science Laboratory Technology, Federal Polytechnic, Ohodo, Enugu State, Nigeria.

Purification of the isolates.

The isolates were first identified, then a sterile wire loop was used to streak them on nutrient agar slants and incubated aerobically at 37 °C for 24 h. The colonies were stored on nutrient agar slants at 4 °C.

Inoculum preparation.

A loopful of each bacterial isolate was inoculated into 5 ml of sterile nutrient broth and incubated at 37 °C for 24 h. Following incubation, the turbidity of each bacterial suspension was standardized to match the 0.5 ml McFarland standard to ensure uniform inoculum density (CLSI, 2012; Kebede *et al*., 2021).

Reconstitution of the concentrated extracts.

To prepare 200 mg/ml (stock sample), weigh 8 g of the extract and dissolve in 4 ml of dimethyl sulfoxide (DMSO) (diluent). Test tubes (1, 2, 3, 4 and 5) were set up, and then 2 ml of the diluent was added into each tube. Then, 2ml from the stock sample was added to two milliliters (2 ml) of dimethyl sulfoxide (DMSO), yielding a concentration of 100 mg/ml. Subsequently, 50, 25 and 12.5 mg/ml were prepared following the above steps, to provide two-fold dilutions.

Antibacterial assay of the plant extracts.

The antibacterial activity of the plant extracts against the isolates was evaluated using the agar well diffusion technique as described by Gonelimali *et al.* (2018). Each of the four bacterial isolates already standardized to 0.5 McFarland turbidity was then inoculated onto Mueller-Hinton agar plates using 0.1 ml of the bacterial suspension. Wells of 6 mm diameter were aseptically created in the agar plates using a sterile cork borer. Different concentrations of the plant extracts (ranging from 200 to 12.5 mg/ml) were introduced into the wells in 0.1 ml volumes. The extracts were allowed to diffuse into the agar for 30 minutes at room temperature before incubation. The plates were then incubated at 37°C for 24 hours.

Following incubation, antibacterial activity was assessed by observing the clear zones of inhibition around each well. The diameters of these zones were measured in millimeters from the underside of the plates using a ruler. The antimicrobial spectrum of the extracts against the bacterial isolates was determined based on the size of the inhibition zones, in accordance with Kebede *et al.* (2021).

Determination of minimum inhibition concentration (M.I.C.)

The minimum inhibitory concentration (MIC) of the plant extracts was determined using the agar well diffusion method. Serial two-fold dilutions of the extracts were prepared to obtain concentrations of 200, 100, 50, 25, and 12.5 mg/ml for antimicrobial susceptibility testing. Then, each bacterial isolate was standardized to 0.5 McFarland turbidity, and 0.1 ml of the suspension was inoculated onto Mueller-Hinton agar plates. Wells of 6 mm diameter were aseptically bored into the agar, and 0.1 ml of each extract concentration was introduced into the respective wells. The inoculated plates were incubated at 28°C for 24 hours, after which the zones of inhibition were observed. The MIC was defined as the lowest concentration of the extract that produced a visible inhibition of bacterial growth (Obodo et al., 2025)

Statistical analysis

The data obtained were analyzed using IBM Statistical package for service solution (SPSS) version 21.0. Descriptive statistical methods were applied, and differences between mean values were considered statistically significant at $p < 0.05$. All experiments were conducted in triplicate ($n = 3$), and the results were presented as mean ± standard error of the mean (± SEM).

## RESULTS

The results of the qualitative and quantitative phytochemical compositions of the phytoextracts of *A. indica* leaves were depicted in Table 1. The qualitative screenings of the extracts, confirmed the presence of several pharmacologically bioactive compounds including alkaloids, flavonoids, tannins, glycosides, phenols, steroids, and saponins. All the detected bioactive compounds analyzed (mg/100g) occurred in moderate concentrations, while glycosides was the most abundance.

The quantitative analysis further revealed varying concentrations of these constituents (mg/100 g). Glycosides were the most abundant compound with a concentration of 22.85 ± 0.084, followed by tannins (13.62 ± 0.012). However, steroids (7.04 ± 0.012) and flavonoids (6.55 ± 0.087) were present in comparatively lower amounts (Table 1).

**Table 1: Results of the qualitative and quantitative (mg/100g) analysis of the phytoextracts of *A. indica* leaves.**

| Parameters | Qualitative | Quantitative (mg/100g) |
|---|---|---|
| Alkaloids | + + | 12.35 ± 0.324 |
| Saponins | + + | 10.12 ± 0.006 |
| Flavonoids | + + | 6.55 ± 0.087 |
| Glycosides | + + + | 22.85 ± 0.084 |
| Tannins | + + | 13.62 ± 0.012 |
| Phenols | + + | 11.61 ± 0.009 |
| Steroids | + + | 7.04 ± 0.012 |

Key: + + = indicates moderate; + + + = indicates high; mg/100g = milligram per 100 grams of the sample. Values are means of triplicate results ± SEM

The antibacterial activities of the aqueous and ethanolic extracts of *A. indica* leaves against selected clinical bacterial isolates are presented in Tables 2 and 3. These antibacterial activities were evaluated against the four bacterial species comprising Gram-negative organisms (*Escherichia coli* and *Shigella* sp.) and Gram-positive organisms (*Lactobacillus* sp. and *Staphylococcus aureus*).

The aqueous and ethanolic extracts exhibited varying degrees of antibacterial activity against the tested organisms. No inhibitory effect was observed against *E. coli* at concentrations ranging from 200 to 12.5 mg/ml in both extracts, while similar observation was exhibited by *Lactobacillus* species in ethanolic extract, thus, indicating complete resistance by these organisms. In contrast, *Shigella* sp. in aqueous extract showed significant susceptibility, with zones of inhibition measuring 22, 11, 9, 7, and 2 mm at concentrations of 200, 100, 50, 25, and 12.5 mg/ml, respectively. Similarly, in aqueous extract, *Lactobacillus* sp. exhibited moderate susceptibility at higher concentrations, producing inhibition zones of 9 and 5 mm at 200 and 100 mg/ml, respectively. *Staphylococcus aureus* demonstrated notable susceptibility to the aqueous extract, with inhibition zones of 21, 19, 11, 9, and 3 mm at concentrations of 200, 100, 50, 25, and 12.5 mg/ml, respectively.

Comparatively, ethanolic extract exhibited lower antibacterial activity as shown the available results in Tables 2 and 3. However, moderate activities were recorded against *Shigella* sp. and *S. aureus*. At concentrations of 200, 100, and 50 mg/ml, inhibition zones of 9, 6, and 2 mm were observed against *Shigella* sp., while 12, 8, and 3 mm inhibition zones were recorded against *S. aureus* at the same concentrations (Table 3).

Table 2: Antibacterial activity of aqueous extract of *A. indica* leaves against clinical bacteria isolates

| Bacterial Isolates | Diameter zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Conc. (mg/ml) | | | | |
| | 200 | 100 | 50 | 25 | 12.5 |
| *E. coli* | 0 | 0 | 0 | 0 | 0 |
| *Shigella sp* | 22 | 11 | 9 | 7 | 2 |
| *Lactobacillus sp* | 9 | 5 | 0 | 0 | 0 |
| *Staphylococcus aureus* | 21 | 19 | 11 | 9 | 3 |

Key: mm = millimeter; mg/ml = milligram per milliliter; Conc. = Concentration; 0 = no growth

Table 3: Antibacterial activity of ethanolic extract of *A. indica* leaves against clinical bacteria isolates

| Bacterial Isolates | Diameter zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Conc. (mg/ml) | | | | |
| | 200 | 100 | 50 | 25 | 12.5 |
| *E. coli* | 0 | 0 | 0 | 0 | 0 |
| *Shigella sp* | 9 | 6 | 2 | 0 | 0 |
| *Lactobacillus sp* | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* | 12 | 8 | 3 | 0 | 0 |

Key: mm = millimeter; mg/ml = milligram per milliliter; Conc. = Concentration; 0 = no growth

The minimum inhibitory concentration (MIC) values of the aqueous and ethanolic extracts were presented in Tables 4 and 5. The aqueous extract demonstrated strong antibacterial activity against *Shigella* sp. and *S. aureus*. The inhibitory activity decreased progressively with decreasing concentrations, and both organisms exhibited MIC values of 12.5 mg/ml.

Moderate inhibition was observed against *Lactobacillus* sp., with MIC value of 100 mg/ml, while *E. coli* remained completely resistant to the aqueous extract. The high activity of the aqueous extract suggests that the antibacterial compounds present in neem leaves—such as glycosides, tannins, flavonoids, and saponins—are more readily extracted in water.

In contrast, the ethanolic extract showed a narrower antibacterial spectrum, inhibiting only *Shigella* sp. and *S. aureus*, with MIC values of 50 mg/ml. No activity was observed against *E. coli* and *Lactobacillus* species.

Table 4: Minimum Inhibitory Concentration (MIC) of Aqueous Extract of *A. indica* Leaves

| | Concentration (mg/ml) | | | | | MIC |
|---|---|---|---|---|---|---|
| Isolates | 200 | 100 | 50 | 25 | 12.5 | |
| E. coli | + | + | + | + | + | 200 |
| Shigella sp | - | - | - | - | - | 12.5 |
| Lactobacillus sp | - | - | + | + | + | 100 |
| Staphylococcus aureus | - | - | - | - | - | 12.5 |

Key: (-) = No bacterial growth; (+) = Bacterial growth

Table 5: Minimum Inhibitory Concentration (MIC) of Ethanolic Extract -of *A. indica* Leaves

| | Concentration (mg/ml) | | | | | MIC |
|---|---|---|---|---|---|---|
| Isolates | 200 | 100 | 50 | 25 | 12.5 | |
| E. coli | + | + | + | + | + | 200 |
| Shigella sp | - | - | - | + | + | 50 |
| Lactobacillus sp | + | + | + | + | + | 200 |
| Staphylococcus aureus | - | - | - | + | + | 50 |

Key: (-) = No bacterial growth; (+) = Bacterial growth

## DISCUSSION

Phytochemical composition of *Azadirachta indica* leaves

The phytochemical screening of leaf extracts revealed the presence of several important secondary metabolites that are known to contribute to the therapeutic potential of medicinal plants. Phytochemical investigations are commonly conducted to identify active chemical constituents such as glycosides, alkaloids, tannins, flavonoids, terpenoids, saponins, reducing sugars, and volatile oils. These bioactive compounds are widely associated with antimicrobial, antioxidant, and pharmacological activities in plants. (Basnayake and Gunatilake, 2024). The efficiency of phytochemical extraction from medicinal plants largely depends on the solvent used, particularly its dielectric constant and polarity. According to Nawaz *et al.* (2020), extraction yield and purity of phytochemicals may also be influenced by several factors including extraction time, temperature, solvent concentration, and solvent polarity. These factors determine the degree to which bioactive compounds are dissolved and recovered from plant materials.

The qualitative and quantitative phytochemical compositions of neem leaves as presented in Table 1 confirmed the presence of several pharmacologically active compounds including alkaloids, flavonoids, tannins, glycosides, phenols, steroids, and saponins. Most of the detected phytochemicals occurred in moderate concentrations, except the glycosides which was the most abundance.

Similar phytochemical constituents have been reported in other medicinal plants such as guava (*Psidium guajava*) (Agbo *et al*., 2025; Kumar *et al*., 2021), and lemon grass (*Cymbopogon citatus*) (Wifek *et al*., 2016), and these plants were reported to have bioactive compounds known for their protective and therapeutic properties. These phytochemicals—including alkaloids, flavonoids, glycosides, and saponins—serve as natural defense mechanisms in plants against microbial invasion and environmental stress (Herrera-Calderon *et al*., 2017). In addition to these compounds, Amadi *et al*. (2017) reported the presence of other phytoconstituents such as carbohydrates, reducing sugars, triterpenoids, and oxalates in medicinal plants.

Quantitative analysis further revealed varying concentrations of the phytochemicals (mg/100 g). Glycosides were the most abundant compound with a concentration of 22.85 ± 0.084 mg/100 g, followed by tannins (13.62 ± 0.012 mg/100 g). Steroids (7.04 ± 0.012 mg/100 g) and flavonoids (6.55 ± 0.087 mg/100 g) were present in comparatively lower amounts as depicted in Table 1. These findings are consistent with earlier observations by Bamishaiye *et al*. (2011), who reported that the distribution and concentration of phytochemicals in plants vary depending on the plant species, extraction method, and geographical location.

The presence of these bioactive compounds supports the medicinal relevance of neem leaves. Several therapeutic activities of neem—including antioxidant, anti-inflammatory, anti-diabetic, anticancer, and antimalarial properties—have been attributed to its rich phytochemical composition (Khanal, 2021).

The bitter taste of neem leaves is largely associated with the presence of tannins. Tannins are naturally occurring polyphenolic compounds widely recognized for their medicinal applications. They function as astringents capable of precipitating proteins in exposed tissues, thereby forming protective coverings over wounds and burns. Tannins have also been used as mild antiseptics for treating diarrhea, controlling minor hemorrhages, and managing conditions such as leucorrhoea, gonorrhea, and piles.

Furthermore, tannins possess anti-inflammatory, antibacterial, antiviral, anti-parasitic, anti-ulcer, and antioxidant activities (Achikanu *et al*., 2022).

The presence of flavonoids and phenolic compounds in *A. indica* leaves further suggest it's significant antioxidant potential. These compounds are known to protect biological systems against free radicals and oxidative stress. Flavonoids have been reported to possess diverse biological activities including anti-inflammatory, antimicrobial, antiviral, anti-cancer, anti-aging, and vasodilatory effects. They also contribute to protection against allergies, ulcers, hepatotoxicity, and platelet aggregation, and may help improve cerebral blood circulation in patients suffering from neurodegenerative conditions such as Alzheimer's disease (Achikanu *et al*., 2022).

Flavonoids are hydroxylated polyphenolic compounds typically produced by plants in response to microbial infection. Previous studies have demonstrated that these compounds exhibit antimicrobial activity against a wide range of microorganisms in vitro, largely due to their ability to form complexes with extracellular proteins and bacterial cell walls (Cowan, 1999).

Saponins detected in the neem extracts may also contribute to the medicinal properties of the plant. These compounds possess surface-active properties and may function as natural cleansing agents. Saponins are known to inhibit inflammation and have been reported to precipitate and coagulate red blood cells (Waziri and Saleh, 2015). Their inhibitory effects against Gram-positive bacteria such as *Staphylococcus aureus* have also been documented. Similar findings have been reported in leaves, where saponins were shown to possess beneficial cholesterol-lowering properties (Bamishaiye *et al*., 2011).

Alkaloids, another important class of phytochemicals identified in this study, are well known for their physiological effects on humans and animals. These compounds are associated with numerous pharmacological activities including anticancer, antimalarial, analgesic, antispasmodic, bactericidal, antioxidant, and stimulant properties (Achikanu *et al*., 2022).

Antibacterial activity of aqueous and ethanolic extracts

The antibacterial activities of the aqueous and ethanolic extracts of *A. indica* leaves against selected clinical bacterial isolates are presented in Tables 2 and 3. The antibacterial activity was evaluated against four bacterial species comprising Gram-negative organisms

(*Escherichia coli* and *Shigella* sp.) and Gram-positive organisms (*Lactobacillus* sp. and *Staphylococcus aureus*).

The aqueous extract exhibited varying degrees of antibacterial activity against the tested organisms. The no inhibitory effect observed against *E. coli* at concentrations ranging from 200 to 12.5 mg/ml, depicting complete resistance of this organism to the aqueous extract. In contrast, *Shigella* sp. showed significant susceptibility, with zones of inhibition measuring 22, 11, 9, 7, and 2 mm at concentrations of 200, 100, 50, 25, and 12.5 mg/ml, respectively.

Similarly, *Lactobacillus* sp. exhibited moderate susceptibility at higher concentrations, thus, producing inhibition zones of 9 mm and 5 mm at 200 mg/ml and 100 mg/ml, respectively, while no inhibition was observed at lower concentrations. *Staphylococcus aureus* demonstrated notable susceptibility to the aqueous extract, with inhibition zones of 21, 19, 11, 9, and 3 mm at concentrations of 200, 100, 50, 25, and 12.5 mg/ml, respectively.

The ethanolic extract exhibited comparatively lower antibacterial activity. No inhibitory effects were observed against *E. coli* and *Lactobacillus* species. However, moderate activity was recorded against *Shigella* sp. and *S. aureus*. At concentrations of 200, 100, and 50 mg/ml, inhibition zones of 9, 6, and 2 mm were observed against *Shigella* sp., while 12, 8, and 3 mm inhibition zones were recorded against *S. aureus* at the same concentrations.

Previous studies have reported similar antibacterial activities of neem leaf extracts against various pathogenic microorganisms (Adewuyi *et al*., 2024). The differences observed in antibacterial activities in both extracts may be attributed to variations in the solubility of phytochemicals and structural differences between bacterial cell walls. Interestingly, the aqueous extract demonstrated stronger antibacterial activity than the ethanolic extract. Similar findings have been reported by Dhanami *et al.* (2017), who suggested that water extraction yielded higher concentrations of certain phytochemicals due to the high polarity of water and the elevated temperature often used during extraction.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values of the aqueous and ethanolic extracts are presented in Tables 4 and 5.

The aqueous extract demonstrated strong antibacterial activity against *Shigella* sp. and *S. aureus*. The inhibitory activity decreased progressively with decreasing concentrations, and both organisms exhibited MIC values of 12.5 mg/ml.

Moderate inhibition was observed against *Lactobacillus* sp., while *E. coli* remained completely resistant to the aqueous extract. The high activity of the aqueous extract suggests that the antibacterial compounds present in neem leaves—such as glycosides, tannins, flavonoids, and saponins—are more readily extracted in water.

In contrast, the ethanolic extract showed a narrower antibacterial spectrum, inhibiting only *Shigella* sp. and *S. aureus*, with MIC values of 50 mg/ml. No activity was observed against *E. coli* and *Lactobacillus* species.

The differences in antibacterial susceptibility between Gram-positive and Gram-negative bacteria observed in this study align with previous reports indicating that Gram-negative bacteria possess an outer lipopolysaccharide membrane that acts as a permeability barrier against many antimicrobial agents (Stefanello *et al*., 2008). In contrast, the relatively permeable peptidoglycan layer of Gram-positive bacteria allows easier penetration of antimicrobial compounds. This observation is consistent with the findings of Yildirim *et al.* (2013), who reported greater susceptibility of Gram-positive bacteria to plant extracts. However, it contrasts with the findings of Mickmaray (2019), while supporting the earlier report by Tajkarimi *et al.* (2010) that Gram-negative bacteria tend to exhibit greater resistance to plant-derived antimicrobials.

The absence of antibacterial activity against *E. coli* in both extracts may be attributed to intrinsic resistance mechanisms such as efflux pumps or limited penetration of bioactive compounds through the bacterial outer membrane. These defense mechanisms may reduce the effectiveness of plant-derived antimicrobial compounds (Herrera-Calderon *et al*., 2017). Overall, the strong inhibitory effects observed against *S. aureus* and *Shigella* sp. indicate that neem leaf extracts possess promising antibacterial potential.

## CONCLUSION

The findings of this study demonstrate that leaf extracts of contain diverse phytochemical constituents with significant antibacterial properties. The qualitative and quantitative analyses confirmed the presence of important bioactive compounds including

glycosides, tannins, flavonoids, alkaloids, phenols, steroids, and saponins, which are known to contribute to the medicinal value of the plant.

Among the bacterial organisms tested, the aqueous extract exhibited stronger and broader antibacterial activity than the ethanolic extract. *Staphylococcus aureus* and *Shigella* sp. were the most susceptible organisms, showing low MIC values of 12.5 mg/ml, whereas *Escherichia coli* demonstrated complete resistance to both extracts.

The superior activity of the aqueous extract suggests that the major antibacterial compounds present in neem leaves are predominantly polar and therefore more efficiently extracted with water. The results also indicate that Gram-positive bacteria were generally more susceptible to the extracts than Gram-negative bacteria, likely due to structural differences in their cell walls.

Overall, these findings support the traditional medicinal use of neem leaves in the treatment of bacterial infections and highlight their potential as a natural source of antimicrobial agents. However, further investigations involving purification and characterization of the active compounds, as well as evaluation against a wider range of pathogenic microorganisms, are recommended to better understand their mechanisms of action and possible pharmaceutical applications.

Acknowledgments

Authors are grateful to Prof. Garuba Omosun, Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria for generously helping to identify the plant leaves used in this study.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. Further inquiries can be directed to the corresponding author(s).

Funding

The authors received no funding from any individuals or organization(s).

## REFERENCES

Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., & Martins, C. H. G. (2023). Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants, 12*(17), Article 3077. https://doi.org/10.3390/plants12173077

Achikanu, C. E., Ujah, I. I., & Ezenwali, M. O. (2022). Proximate and phytochemical composition of *Phyllantus amarus*. *World Journal of Advanced Research and Reviews, 15*(1), 041–047. https://doi.org/10.30574/wjarr.2022.15.1.0517

Adewuyi, G. M., Iyevhobu, B. T., Momoh, A. M., & Samuel, S. O. (2024). Evaluation of African neem plant (Dogonyaro) antibacterial activity against certain pathogenic organisms. *World Journal of Advanced Research and Reviews, 24*(1), 2208–2214.

Agbo, A. O., Mbah-Omeje, K. N., Ikegwu, T. M., & Ameh, O. A. (2025). Proximate, phytochemical and gas chromatography flame ionization detection (GC-FID) profiling of ethanolic extract of guava (*Psidium guajava*) leaves and its antibacterial activities on some bacterial isolates. *International Journal of Research and Innovation in Applied Sciences, 10*(8), 1254–1298. https://doi.org/10.51584/IJRIAS.2025.100800111

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell, 128*(6), 1037–1050. https://doi.org/10.1016/j.cell.2007.03.004

Altemimi, A., Watson, D. G., Choudhary, R., Dasari, M. R., & Lightfoot, D. A. (2016). Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins. *PLoS ONE, 11*(2), Article e0148758. https://doi.org/10.1371/journal.pone.0148758

Amadi, B., Emelieze, M., Agomuo, E., Ogunka-Nnoka, C., & Amadi, P. (2017). Proximate, GC-FID, and micronutrient analysis of extracts of *Azadirachta indica*. *International Journal of Advanced Chemistry, 5*(2), 73–79. https://doi.org/10.14419/ijac.v5i2.8124

Ankita, S., & Sapan, P. (2018). Preliminary phytochemical screening and quantitative analysis of secondary metabolites of *Mentha arvensis* and *Azadirachta indica*. *International Journal of Advanced Research and Development, 3*(1), 114–118.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery, 20*(3), 200–216. https://doi.org/10.1038/s41573-020-00114-z

Bamishaiye, E. I., Olayemi, F. F., Awagu, E. F., & Bamishaiye, O. M. (2011). Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. *Advance Journal of Food Science and Technology, 3*(4), 233–237.

Banerjee, S., Kim, L. M., Shariff, M., Khatoon, H., & Yusoff, F. M. (2013). Antibacterial activity of neem (*Azadirachta indica*) leaves on *Vibrio* spp. isolated from cultured shrimp. *Asian Journal of Animal and Veterinary Advances, 8*(2), 355–361. https://doi.org/10.3923/ajava.2013.355.361

Basnayake, P. I., & Gunatilake, M. (2024). Pharmacological effects of *Pterocarpus marsupium*. *BLDE University Journal of Health Sciences, 9*(1), 3–15. https://doi.org/10.4103/bjhs.bjhs_43_23

Clinical and Laboratory Standards Institute. (2012). *Performance standards for antimicrobial susceptibility testing: Twenty-second informational supplement* (CLSI document M100-S22). Clinical and Laboratory Standards Institute.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582. https://doi.org/10.1128/cmr.12.4.564

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry, 10*, S1193–S1199. https://doi.org/10.1016/j.arabjc.2013.02.015

Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antibacterial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology, 9*, Article 1639. https://doi.org/10.3389/fmicb.2018.01639

Harborne, J. B. (1973). Phenolic compounds. In *Phytochemical Methods* (pp. 33–88). Springer. https://doi.org/10.1007/978-94-009-5921-7_2

Herrera-Calderon, O., Arroyo-Acevedo, J. L., Rojas-Armas, J., Chumpitaz-Cerrate, V., Figueroa-Salvador, L., Enciso-Roca, E., & Tinco-Jayo, J. (2017). Phytochemical screening, total phenolic content, antioxidant and cytotoxic activity of *Chromolaena laevigata* on human tumor cell lines. *Annual Research & Review in Biology, 21*(1), 1–9.

Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS ONE, 16*(3), Article e0249253. https://doi.org/10.1371/journal.pone.0249253

Khanal, S. (2021). Qualitative and quantitative phytochemical screening of *Azadirachta indica* Juss. plant parts. *International Journal of Applied Sciences and Biotechnology, 9*(2), 122–127. https://doi.org/10.3126/ijasbt.v9i2.38050

Kumar, M., Tomar, M., Amarowicz, R., Saurabh, V., Nair, M. S., Maheshwari, C., Sasi, M., Prajapati, U., Hasan, M., Singh, S., Changan, S., Kumar, R., Prajapat, R. K., Berwal, M. K., & Satankar, V. (2021). Guava (*Psidium guajava* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. *Foods, 10*(4), Article 752. https://doi.org/10.3390/foods10040752

Kunle, O. F., & Egharevba, H. O. (2013). Chemical constituents and biological activity of medicinal plants used for the management of sickle cell disease: A review. *Journal of Medicinal Plants Research, 7*(48), 3452–3476. https://doi.org/10.5897/JMPR2013.5333

Mamman, P. H., Mshelia, W. P., Susbatrus, S. C., & Sambo, K. W. (2013). Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. *International Journal of Medicine and Medical Sciences, 5*(1), 14–18. https://doi.org/10.5897/IJMMS12.017

Mehta, A., & Diwakar, M. (2021). Characterization and antimicrobial susceptibility profile of non-lactose fermenting gram-negative bacterial isolates in a tertiary care teaching

hospital of central India. *Asian Journal of Pharmaceutical and Clinical Research, 14*(10), 1–7. https://doi.org/10.22159/ajpcr.2021.v14i10.42822

Mehta, A., Saxena, G., & Mani, A. (2016). Comparative analysis of antibacterial activity of aqueous, ethanolic, methanolic and acetone extracts of commercial green tea and black tea against standard bacterial strains. *International Journal of Current Microbiology and Applied Sciences, 5*(11), 145–152. https://doi.org/10.20546/ijcmas.2016.511.017

Mickymaray, S. (2019). Efficacy and mechanism of traditional medicinal plants and bioactive compounds against clinically important pathogens. *Antibiotics, 8*(4), Article 257. https://doi.org/10.3390/antibiotics8040257

Muhammad, S. A., & Abubakar, S. M. (2016). Qualitative and quantitative determination of phytochemicals in aqueous extract of *Chrysophyllum albidum* seed kernel. *Biosciences Biotechnology Research Asia, 13*(2), 1201–1206. https://doi.org/10.13005/bbra/2153

Obodo, N. L., Tasie, F. O., & Mbah-Omeje, K. N. (2025). Antimicrobial activities of some medicinal plants used in folklore remedies in Enugu State, Nigeria. *GSC Biological and Pharmaceutical Sciences*. https://doi.org/10.30574/gscbps.2025.30.3.0020

Prabagar, S., Nandakumar, Thuraisingam, S., & Prabagar, J. (2020). Antimicrobial activity of leaf and bark extracts of *Cassia fistula*. *World Journal of Agricultural Sciences, 16*(4), 227–232. https://doi.org/10.5829/idosi.wjas.2020.227.232

Radji, M., Agustama, R. A., Elya, B., & Tjampakasari, C. R. (2013). Antimicrobial activity of green tea extract against isolates of methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Pseudomonas aeruginosa*. *Asian Pacific Journal of Tropical Biomedicine, 3*(8), 663–667. https://doi.org/10.1016/S2221-1691(13)60133-1

Santosh, V. K., & Varadhan, V. (2013). Neem: Prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine, 3*(7), 505–514. https://doi.org/10.1016/S2221-1691(13)60105-7

Shirajum, M., Luthfun, N., Manirul, I., Sharmin, S., Sarwar, H., Habiba, K., Humayun, K., Rubel, A., & Mammunar, R. (2015). Analgesic, anti-inflammatory and CNS activities of the methanolic extract of *Artocarpus heterophyllus* seed. *World Journal of Pharmacy and Pharmaceutical Sciences, 4*(5), 91–103.

Shrinet, K., Singh, K., Chaurasia, R. K., Tripathi, A. K., & Kumar, A. (2021). Bioactive compounds and their future therapeutic applications. In *Natural Bioactive Compounds: Technological Advancements* (pp. 337–355). Elsevier. https://doi.org/10.1016/B978-0-12-820655-3.00017-3

Stefanello, M. É. A., Cervi, A. C., Ito, I. Y., Salvador, M. J., Wisniewski, A., Jr., & Simionatto, E. L. (2008). Chemical composition and antimicrobial activity of essential oils of *Eugenia chlorophylla* (Myrtaceae). *Journal of Essential Oil Research, 20*(1), 75–78. https://doi.org/10.1080/10412905.2008.9699427

Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spices compounds in food. *Food Control, 21*(9), 1199–1218. https://doi.org/10.1016/j.foodcont.2010.02.003

Tsaku, P. A., Ehinmidu, J. O., & Mohammed, S. A. (2017). Antibiotic susceptibility and plasmid profile of *Escherichia coli* from door handles in two tertiary institutions in Nasarawa State, Nigeria. *Journal of Advances in Microbiology, 3*(3), 1–13. https://doi.org/10.9734/JAMB/2017/33612

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms, 9*(10), Article 2041. https://doi.org/10.3390/microorganisms9102041

Virshette, S. J., Patil, M. K., Deshmukh, A. A., Shaikh, J. R., & Dhas, M. S. (2020). Phytochemical constituents of different extracts of *Azadirachta indica* leaves in urine solvent of a non-pregnant cow. *Journal of Pharmacognosy and Phytochemistry, 9*(2), 1324–1328.

Waziri, M., & Saleh, I. A. (2015). Proximate analysis and phytochemical screening of *Psidium guajava* (guava) and *Cucumis sativus* (cucumber) grown in Gashua Fadama Area of Yobe State, Nigeria. *International Research Journal of Pure and Applied Chemistry, 6*(2), 77–83. https://doi.org/10.9734/IRJPAC/2015/13775

WHO. (2023). *Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise*. https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise

Wifek, M., Saeed, A., Rehman, R., & Nisar, S. (2016). Lemon grass: A review on its botany, properties, applications and active components. *International Journal of Chemical and Biochemical Sciences, 9*, 79–84.

Wylie, M. R., & Merrell, D. S. (2022). The antimicrobial potential of the neem tree *Azadirachta indica*. *Frontiers in Pharmacology, 13*, Article 891535. https://doi.org/10.3389/fphar.2022.891535

Yildirim, A. B., Karakas, F. P., & Turker, A. U. (2013). In vitro antibacterial and antitumor activities of some medicinal plant extracts growing in Turkey. *Asian Pacific Journal of Tropical Medicine, 6*(8), 616–624. https://doi.org/10.1016/S1995-7645(13)60106-6

Zillich, O. V., Schweiggert-Weisz, U., Eisner, P., & Kerscher, M. (2015). Polyphenols as active ingredients for cosmetic products. *International Journal of Cosmetic Science, 37*(5), 455–464. https://doi.org/10.1111/ics.12218