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In Vitro Evaluation of the Antimicrobial Activity of *Azadirachta Indica* (Neem) Leaf Extracts against Microbial Isolates from the Midwest in the United States

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Abstract
The increasing resistance of microorganisms to standard antimicrobial drugs has created a growing need for alternative therapeutic agents from natural sources. This study evaluated the antimicrobial activity of aqueous, methanolic, and ethanolic extracts of *Azadirachta indica* (neem) leaves against selected bacterial and fungi. Neem leaves were extracted using water, methanol, and ethanol, and the antimicrobial activity of the extracts was assessed using the disc diffusion method. Test organisms included *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella typhi*, *Pseudomonas aeruginosa (*ATCC 27853), *Candida albicans*, *Aspergillus fumigatus*, and *Penicillium camemberti*. Minimum inhibitory concentration (MIC) and phytochemical screening were also carried out, with the results showed that all extracts exhibited antimicrobial activity against the test organisms, with the ethanolic extract demonstrating the highest inhibitory activity, while the aqueous extract showed the lowest activity. Zones of inhibition increased with extract concentration. Moreover, antifungal activity was also observed, particularly with methanolic and ethanolic extracts. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, phlobatannins, terpenoids, glycosides, steroids, phenol, oil and reducing sugar which may contribute to the observed antimicrobial activity. The *Azadirachta indica* leaves possess bioactive compounds with potential antimicrobial and antifungal properties and may serve as a promising source of plant-derived therapeutic agents for the control of pathogenic microorganisms.

Keywords: *Azadirachta indica*, antimicrobial activity, antibacterial activity, antifungal activity, phytochemicals, medicinal plants, antibiotic resistance

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1. Introduction
Infectious diseases caused by bacteria and fungi remain a major public health concern worldwide, particularly with the increasing emergence of antimicrobial resistant microorganisms. The reduced effectiveness of standard antibiotics has created an urgent need for alternative antimicrobial agents from natural sources. The *Azadirachta indica* plant parts have long been explored for their therapeutic properties because they contain bioactive compounds capable of inhibiting microbial growth and as well cultivated for foods (Olson, 2002; Bukar *et al.*, 2010). Even before the discovery of microorganisms, plants were widely used in the treatment of infectious diseases across different cultures, reflecting a long-standing belief in their healing potential (Rios and Recio, 2005; Priyadharsini *et al.*, 2014). *Azadirachta indica* is a globally recognised plant that has been extensively studied for its remarkable nutritional value and medicinal properties. Its leaves, bark, seeds, seed oil, fruits, flowers, roots, twigs, and stem are widely utilised in many African countries, including Ghana, Ethiopia, Nigeria, and regions of East Africa and Malawi, as well as in Europe and America (Nwosu & Okafor; Anyanwueze, 2026). Today, herbal remedies remain widely utilized because they are accessible, cost effective, and often associated with fewer side effects compared to synthetic or standard drugs. The medicinal value of this plant is largely attributed to the presence of bioactive compounds that exert physiological and antimicrobial effects in humans and animals (Edeoga *et al.*, 2005, Chuang *et al* 2007).

The growing scientific interest in medicinal plants has led to increased investigation into their antimicrobial properties, particularly in response to the rising challenge of antimicrobial resistance (Dahot, 1998; Hafia, R.E. 2000; Ebo *et al* 2026). The increasing resistance of microorganisms to many commonly used antimicrobial drugs has reduced the effectiveness of several affordable therapeutic agents, creating a major public health concern worldwide (Montefore *et al*., 1989; Rahman *et al* 2009).). Several plants have proven to consist of antibacterial properties (Oluma *et al* 2004). Consequently, there is an urgent need to explore alternative therapeutic strategies, especially plant-based compounds that are readily available and associated with fewer adverse effects (Khulbe & Sati, 2009). Medicinal plants are regarded as promising sources of antimicrobial agents because they contain secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds that can inhibit microbial growth or interfere with cellular metabolism and integrity (Ratnasooriya *et al*., 2005). These compounds have demonstrated inhibitory activity against a wide range of pathogenic microorganisms and continue to attract attention in antimicrobial research.

Among the numerous medicinal plants investigated, *Azadirachta indica*, commonly known as neem, is one of the most widely recognized species because of its broad therapeutic importance. Neem is predominantly distributed in tropical and subtropical regions, especially within the Indian subcontinent, where it has been used for centuries in traditional medicine Tewari (1992). Various parts of the plant including the leaves, bark, seeds, roots, flowers, and fruits have been utilized in the treatment and management of infections, particularly those affecting the skin and oral cavity (Subapriya & Nagini, 2005). Neem twigs have also traditionally been used as chewing sticks for maintaining oral hygiene and dental health (Almas & Ansallafi, 1995; Yerima, *et al* 2012).

The neem tree has gained considerable scientific attention because of its diverse pharmacological and therapeutic properties. Studies have shown that extracts obtained from neem leaves and other plant parts possess antimicrobial, antifungal, anti-inflammatory, antimalarial, antihyperglycemic, antioxidant, antimutagenic, and anticancer activities (Subapriya and Nagini, 2005; Talwar *et al*., 1997). These biological activities are largely associated with the presence of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds (Anyanwu and Nwosu, 2014). Previous studies have also demonstrated that aqueous neem leaf extracts exhibit antihyperglycemic effects in both insulin dependent and non-insulin dependent diabetes mellitus (Bajaj & Srinivasan, 1999). In traditional medicine, neem leaves are additionally used for the treatment of skin allergies and for enhancing the healing of wounds associated with diseases such as smallpox and chickenpox (Almas and Ansallafi, 1995; Hla *et al*., 2011).

Microorganisms can invade host tissues through natural body openings, injuries, or latent infections, often resulting in internal deterioration that may not be immediately visible externally. This further emphasizes the importance of discovering effective antimicrobial agents capable of controlling microbial growth and infection. Plant derived extracts from *Azadirachta indica* have therefore continued to receive scientific attention because of their potential antimicrobial activities against pathogenic microorganisms Anyanwueze *et al*., (2025). Despite numerous studies on neem, variations in extraction solvents and microbial susceptibility continue to produce differences in antimicrobial effectiveness.

Therefore, this study evaluated the antimicrobial activity and phytochemical composition of aqueous, methanolic, and ethanolic neem leaf extracts against selected bacterial and fungal isolates to identify effective plant derived alternatives to standard antibiotics for the control of microbial infections. The study also compared the antimicrobial effectiveness of the neem leaf extracts with standard antibiotics against selected pathogenic microorganisms.

2. Materials and Methods

2.1 Collection of plant material

Fresh leaves of *A. indica* were purchased from a local supplier in St. Louis, Missouri, USA. The collected samples were packed in clean plastic containers and transported to the Department of Biology, Botany Unit at Southeast Missouri State University, Cape Girardeau Missouri, USA where the leaves were authenticated and washed with distilled water to remove dirt and debris prior to preparation of the neem leaf extract.

2.2 Plant extraction

A total of 250 g of fresh *A. indica* leaves were shade-dried at room temperature (30–37 °C) for five days until completely dry. The dried leaves were ground into a fine powder using a mortar and pestle (Farberware Professional Marble Mortar and Pestle). A 250 g portion of the powdered sample was separately extracted in 850 mL of distilled water (aqueous extraction), 60% methanol (methanolic extraction), and 90% ethanol (ethanolic extraction) using 1 L conical flasks. The flasks were sealed with rubber corks, shaken at 120 rpm for 25 min, and left at room temperature for five days with intermittent manual agitation every 24 h using a sterile glass rod. The extracts were filtered using sterile Whatman No. 1 filter paper to obtain clear filtrations. The filtrates were subsequently concentrated using an evaporator and lyophilized to obtain dry extracts Anyanwueze (2026).

2.3 Bacterial Media (Agar Media)

A total of 28 g of nutrient agar was dissolved in 1 L of distilled water and sterilized by autoclaving at 121 °C and 15 psi for 20 min. The sterile medium was aseptically poured into sterile Petri dishes and allowed them to solidify. Wells of 6 mm diameter were created in the agar using a sterile cork borer, and the plates were subsequently used for antibacterial analysis. Two Gram-positive bacterial species, *Staphylococcus aureus* and *Enterococcus faecalis*, and two Gram-negative bacterial species, *Salmonella typhi* and *Pseudomonas aeruginosa* (ATCC 27853), were used as test organisms. The bacterial isolates were maintained on nutrient agar slants as stock cultures at 4 °C. Biochemical characterization was carried out to confirm the identity of each test organism.

2.4 Fungal Media (Potato dextrose sugar)

Three hundred grams of peeled and sliced potatoes were boiled in 1.5 L of distilled water for 30 min, and the resulting potato infusion was filtered using sterile cheese cloth. 30 g of dextrose and 20 g of agar were added to the filtration as the carbon source and solidifying agent, respectively. The medium was thoroughly mixed, and the volume was adjusted to 1.5 L with distilled water before sterilization by autoclaving at 121 °C and 15 psi for 20 min. The sterile medium was aseptically poured into sterile Petri dishes and allowed them to solidify. Wells of 6 mm diameter were created in the agar using a sterile cork borer, and the plates were subsequently used for antifungal analysis against *Candida albicans*, *Aspergillus fumigatus*, and *Penicillium camemberti.*

2.5 Antimicrobial activity assay (*in vitro*)

Antimicrobial activity of the aqueous, ethanolic and methanolic extracts of *A. indica* leaves. The concentrated leaf extracts were dissolved in 5% dimethyl sulfoxide (DMSO) and sterile discs (6mm, Hi-media, india) were each impregnated. The extract was dissolved in sterile distilled water to form dilution such as 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml for aqueous, ethanolic and methanolic extracts. Each concentration of the plant extract was tested against *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella typhi* and *Pseudomonas aeruginosa* (ATCC 27853) bacteria. The antifungal activity was observed at the concentration of 30 mg/ml for aqueous, ethanolic and methanolic extract (Maragathavalli *et al.,* 2012) and modified by (Anyanwueze 2026). The discs were carefully and firmly placed on the Mueller-Hinton Agar (MHA) plates earlier seeded with standardized bacterial suspensions (0.5 McFarland (~10^8 CFU/ml) Anyanwueze (2026). The plates were incubated at 37 °C for 24 h. The zones of inhibition were achieved by measuring the diameter of the inhibition zone around the well (in mm) from end to end using metric ruler.

2.6 Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was evaluated using the broth dilution technique according to the procedure by (Wiegand *et al* 2008). For bacterial isolates, concentrations of 200 mg/mL, 100 mg/ml, 50 mg/ml, and 25 mg/ml were used, while for fungal isolates, extract concentrations of 30mg/ml were prepared. Thereafter, 0.1 mL of each standardized microbial suspension was inoculated into test tubes containing the various extract concentrations. Control tubes containing only the solvent and test organisms without extracts were also included. All tubes were incubated at 37°C for 24 hours. Following incubation, the concentration of the extracts that showed no visible microbial growth was measured (mm) and recorded.

2.7 Phytochemical screening

Qualitative phytochemical screening was carried out to detect the presence of alkaloids, tannins, saponins, flavonoids, phlobatannins, steroids, terpenoids, glycosides, phenol, oil, and reducing sugars in the extracts using standard chemical tests.

Test for the presence of alkaloids

Two millilitres of neem leaf extract were acidified with a few drops of diluted hydrochloric acid in a clean test tube. Subsequently, 2–3 drops of Wagner's reagent were added. The formation of a reddish-brown precipitate indicated the presence of alkaloids in the extract.

Test for the presence of Tannins

Two millilitres of filtered neem leaf extract were added into a clean test tube followed by 2–3 drops of 5% ferric chloride ($FeCl_3$)solution. The mixture was gently shaken, and the formation of a dark green coloration indicated the presence of tannins in the extract.

Test for presence of Saponins

A 2 ml aliquot of neem leaf extract was mixed with 2 mL of distilled water in a clean test tube and shaken vigorously for 15–30 seconds. The mixture was allowed to stand undisturbed for 10–15 minutes. The formation of a stable foam layer measuring at least 1 cm in height indicated the presence of saponins in the extract.

Test for presence of Flavonoids

Equal volumes (2 mL each) of neem leaf extract were mixed with a few drops of 10% sodium hydroxide (NaOH) solution in a clean test tube. The development of an intense yellow coloration was observed. Subsequently, a few drops of dilute hydrochloric acid (HCl) were added, and the disappearance of the yellow color indicated the presence of flavonoids in the extract.

Test for presence Phlobatannins

Exactly 2ml of neem leaf extract were added into a clean test tube followed by 2 mL of 1–2% aqueous hydrochloric acid (HCl). The mixture was gently mixed and heated in a boiling water bath for 5–10 minutes. The formation of a brick-red precipitate indicated the presence of phlobatannins in the extract.

Test for steroids

Two millilitres of concentrated neem leaf extract were dissolved in 2 mL of chloroform in a clean test tube. One to two milliliters of acetic anhydride were added and mixed gently. Subsequently, 1–2 mL of concentrated sulfuric acid (H_2SO_4)were carefully introduced down the side of the test tube without shaking. The formation of a blue-green coloration in the upper layer indicated the presence of steroids in the extract.

Test for terpenoids

Exactly 2ml of neem leaf extract were dissolved in 2 mL of chloroform in a clean test tube. One to two milliliters of acetic anhydride were added and mixed gently. Subsequently, 1–2 mL of concentrated sulfuric acid was carefully added down the side of the test tube. The appearance of a deep red or violet coloration in the lower layer indicated the presence of terpenoids, while a blue-green coloration in the upper layer indicated the presence of steroids.

Test for glycosides

Into a clean test tube, 2 ml of neem leaf extract was added, followed by 2–3 drops of dilute hydrochloric acid (HCl). The mixture was heated in a boiling water bath for 5 minutes and allowed to cool. A few drops of sodium hydroxide (NaOH) solution were then added to neutralize the mixture. Equal volumes (1 mL each) of Fehling's solution A and Fehling's solution B were mixed in a separate test tube, after which 1 mL of the neutralized extract was added. The mixture was heated in a boiling water bath for 5–10 minutes. The formation of a brick-red precipitate indicated the presence of glycosides in the extract.

Test for oils

One gram of neem leaf extract was introduced into a clean test tube and mixed with 2 mL of sodium hydroxide (NaOH) solution. The mixture was gently heated in a hot water bath for 5–10 minutes with occasional shaking. The formation of a translucent appearance indicated the presence of oils in the extract.

Test for reducing sugars

A 1 mL of the neem leaf extract was added into a clean test tube followed by 2 mL of Benedict's reagent. The mixture was heated in a boiling water bath for 3–5 minutes. The formation of a brick-red precipitate indicated the presence of reducing sugars in the extract.

3. Results

The aqueous neem leaf extract demonstrated antibacterial activity against all the tested bacterial isolates, although the degree of inhibition varied across concentrations and organisms (Table 1). At 200 mg/ml, the highest inhibition zone was observed against *Enterococcus faecalis* (22 mm), followed by *Staphylococcus aureus* (20 mm), while *Pseudomonas aeruginosa* ATCC 27853 showed the lowest inhibition zone (15 mm).

Table 1: Inhibition zone diameter (mm) of aqueous extract of neem leaf at different concentrations

Test Bacteria	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
Staphylococcus aureus	20	17	15	12
Enterococcus faecalis	22	20	14	11
Salmonella typhi	17	14	12	10
Pseudomonas aeruginosa ATCC 27853	15	12	9	8

Table 2: Inhibition zone diameter (mm) of methanolic extract of neem leaf at different concentrations

Test Bacteria	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
Staphylococcus aureus	23	20	17	14
Enterococcus faecalis	25	21	19	12
Salmonella typhi	19	17	16	10
Pseudomonas aeruginosa ATCC 27853	17	15	10	5

Table 3: Inhibition zone diameter (mm) of ethanolic extract of neem leaf at different concentrations

Test Bacteria	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
Staphylococcus aureus	28	25	20	18
Enterococcus faecalis	26	23	19	17
Salmonella typhi	20	19	16	11
Pseudomonas aeruginosa ATCC 27853	18	14	11	10

Table 4: Minimum inhibitory concentration (mg/ml) of neem leaf extract on test bacteria

Test Bacteria	Aqueous extract	Methanol Extract	Ethanol Extract
Staphylococcus aureus	3.125	1.562	1.562
Enterococcus faecalis	3.125	1.562	0.781
Salmonella typhi	1.562	0.781	0.781
Pseudomonas aeruginosa ATCC 27853	6.250	3.125	1.562

Table 5: Antibacterial zone of inhibition (mm) on reference antibiotics

Test Bacteria					
Gram Positive Bacteria	APX	AM	ERY	CPX	GEN
Staphylococcus aureus	17	12	18	37	15
Enterococcus faecalis	19	14	R	21	25
Gram Negative Bacteria	APX	AM	ERY	CPX	GEN
Salmonella typhi	20	14	10	28	12
Pseudomonas aeruginosa ATCC 27853	15	13	5	10	17

Key: *APX = Ampiclox; R = Resistance; AM = Ampicillin; ERY = Erythromycin; CPX = Ciprofloxacin; GEN = Gentamicin*

Table 6: Phytochemical (phytonutrients) screening of neem leaf extracts

Components	Aqueous	Ethanol	Methanol
Alkaloids	-	+	++
Tannins	+++	++	+++
Saponins	+	+++	++
Flavonoids	+	++	+++
Phlobatannins	-	+	++
Steroids	++	+	++
Terpenoids	+	+++	+++
Glycosides	+++	+++	+++
Phenol	+	+++	+++
Oil	+	++	++
Reducing Sugar	+++	+++	++

Table 7: Inhibition zone diameter (mm) of aqueous extract, Methanol, Ethanol of neem leaf (30 mg/ml) for fungi

Test Fungi	Ketoconazole (Control)	Aqueous	Methanol	Ethanol
Candida albicans	4.0	12.0	18.0	20.0
Aspergillus fumigatus	2.0	15.0	21.0	14.0
Penicillium camemberti	1.0	13.0	11.0	16.0

Table 8. Minimum inhibitory concentration (MIC) of neem leaf extracts against fungi (mg/ml)

Test Fungi	Aqueous Extract	Methanol Extract	Ethanol Extract
Candida albicans	4.25	3.12	1.56
Aspergillus fumigatus	3.12	1.56	2.12
Penicillium camemberti	6.25	2.25	3.12

A progressive reduction in inhibition zone diameter was observed with decreasing extract concentration from 200 mg/ml to 25 mg/ml across all organisms tested. The methanolic neem leaf extract also exhibited antibacterial activity against all test bacteria (Table 2). The largest inhibition zone at 200 mg/ml was recorded against *Enterococcus faecalis* (25 mm), while the lowest inhibition was observed against *Pseudomonas aeruginosa* ATCC 27853 (17 mm). This is like the aqueous extract, where the inhibition zone diameters decreased as the extract concentration decreased. At the lowest concentration (25 mg/ml), *Pseudomonas aeruginosa* ATCC 27853 showed the smallest inhibition zone (5 mm). The ethanolic neem leaf extract produced the highest antibacterial activity among the solvent extracts tested (Table 3). At 200 mg/ml, *Staphylococcus aureus* showed the highest inhibition zone diameter (28 mm), followed by *Enterococcus faecalis* (26 mm). *Pseudomonas aeruginosa* ATCC 27853 exhibited the lowest susceptibility with an inhibition zone diameter of 18 mm at 200 mg/ml. Reduction in antibacterial activity was also observed with decreasing extract concentration.

The minimum inhibitory concentration (MIC) values of the neem leaf extracts against the test bacteria are presented in Table 4. The ethanol and methanol extracts generally showed lower MIC values than the aqueous extract. The lowest MIC value recorded was 0.781 mg/ml against *Enterococcus faecalis* and *Salmonella typhi* using the ethanol extract, and against *Salmonella typhi* using the methanol extract. The aqueous extract produced the highest MIC value of 6.250 mg/ml against *Pseudomonas aeruginosa* ATCC 27853.

The reference antibiotics demonstrated varying antibacterial activity against the test organisms (Table 5). Ciprofloxacin produced the highest inhibition zone against *Staphylococcus aureus* (37 mm), while gentamicin showed the highest activity against *Enterococcus faecalis* (25 mm). Among the Gram-negative bacteria, ciprofloxacin produced the highest inhibition zone against *Salmonella typhi* (28 mm), whereas gentamicin showed the highest activity against *Pseudomonas aeruginosa* ATCC 27853 (17 mm). *Enterococcus faecalis* showed resistance to erythromycin.

Phytochemical screening of the neem leaf extracts revealed the presence of several bioactive compounds in varying concentrations (Table 6). Glycosides and reducing sugars were highly present (+++) in both aqueous and ethanol extracts. Terpenoids, saponins, and phenols were more abundant in the ethanol extract, while tannins and steroids were more concentrated in the aqueous extract. Alkaloids and phlobatannins were absent in the aqueous extract but present in the ethanol extract.

The antifungal activity of the neem leaf extracts against the tested fungi is presented in Table 7. The ethanol extract showed the highest inhibition zone against *Candida albicans* (20 mm) and *Penicillium camemberti* (16 mm), while the methanol extract exhibited the highest activity against *Aspergillus fumigatus* (21 mm). The aqueous extract demonstrated lower inhibition zones compared to the methanol and ethanol extracts across the fungal isolates tested. The MIC values of the neem leaf extracts against the fungi are presented in Table 8. The methanol and ethanol extract generally produced lower MIC values than the aqueous extract. The lowest MIC value recorded was 1.56 mg/ml against *Candida albicans* using the ethanol extract and against *Aspergillus fumigatus* using the methanol extract. The aqueous extract showed the highest MIC value against *Penicillium camemberti* (6.25 mg/ml).

The minimum inhibitory concentration results presented in Table 4 demonstrated that the neem leaf extracts possessed antibacterial activity against all tested organisms, although the degree of effectiveness varied among the solvents and bacterial species. Lower MIC values were generally observed for the methanol and ethanol extracts compared to the aqueous extract, indicating stronger antibacterial activity of the organic solvent extracts. *Salmonella typhi* and *Enterococcus faecalis* showed higher susceptibility to the extracts, particularly the ethanol extract, with MIC values as low as 1.561 mg/ml. On the other hand, *Pseudomonas aeruginosa* ATCC 27853 exhibited the highest MIC values, especially with the aqueous extract (6.250 mg/mL), indicating greater resistance to the neem leaf extracts. These findings suggest that solvent type influenced the extraction of bioactive compounds and that ethanol and methanol were more effective in extracting antibacterial constituents from neem leaves.
The standard antibiotics used as positive controls produced varying zones of inhibition against the test bacteria (Table 5), confirming the susceptibility and resistance patterns of the organisms. Comparison between the antibiotic controls and neem leaf extracts demonstrated that the extracts possessed measurable antibacterial activity. The MIC results further showed that the ethanol extract exhibited greater antibacterial effectiveness against most of the test organisms when compared with the aqueous and methanol extracts, depicting that ethanol was more efficient in extracting bioactive antibacterial compounds from the plant material. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, glycosides, and reducing sugars in *A. indica* extracts (Table 6). These phytochemical constituents are known to contribute to the natural defense mechanisms of plants and may be responsible for the observed antimicrobial activity against the test organisms.

The *A. indica* leaf extracts demonstrated antifungal activity against all the tested fungi as presented in (Table 7). Generally, the methanol and ethanol extracts exhibited greater zones of inhibition compared to the aqueous extract, suggesting that the active anti-fungal constituents were more effectively extracted using organic solvents. The ethanol extract showed particularly strong inhibitory activity against *Candida albicans* and *Penicillium camemberti* while the methanol extract exhibited the highest activity against *Aspergillus fumigatus*. The aqueous extract also demonstrated antifungal activity but was comparatively less effective. The MIC results in (Table 8) further supported these findings, with lower MIC values observed for the methanol and ethanol extracts, indicating stronger anti-fungal potency. These findings suggest that organic solvents were more efficient in extracting bioactive antifungal compounds from neem leaves.

4. Discussion

The study demonstrated that *A. indica* leaf extracts possessed considerable antimicrobial activity against the tested microorganisms, with the methanolic and ethanolic extracts showing greater activity than the aqueous extract. This variation may be associated with the ability of organic solvents to extract higher amounts of bioactive phytochemical compounds from the plant material. Many standard antibiotics are often associated with undesirable side effects, highlighting the need to investigate plant derived alternatives that are both effective and safer for therapeutic use (Srivastava *et al*., 2000, Ugwu, 2019). In the present study, the ethanolic extract demonstrated the highest antimicrobial activity, whereas the aqueous extract showed the lowest activity against the tested organisms. Similar variations in antimicrobial effectiveness based on extraction solvent have been reported in previous studies. For instance, Anyanwueze (2026) reported that methanolic extracts exhibited the strongest antibacterial activity, suggesting that methanol was more efficient in extracting bioactive antimicrobial compounds from plant leaves, while the ethanolic extract displayed the weakest activity. On the other hand, Doughari *et al*. (2007), who evaluated the antibacterial activity of aqueous, acetone, and ethanolic extracts of *A. indica* leaves, observed that the ethanolic extract produced the greatest antibacterial effect, whereas the aqueous extract exhibited the least antibacterial activity. Different solvents vary in their ability to extract compounds, as they dissolve different types of phytochemicals depending on their chemical properties (Majorie, 1999; Srinvasan *et al.*, 2001; Oluduro 2012), this means the choice of solvent directly influences the composition, concentration, and effectiveness of the extracted bioactive compounds Anyanwueze (2026), aligning with previous reports showing that leaf extracts possess inhibitory effects against pathogenic bacteria (Hala & Alfadhil, 2015).

It has also been widely observed that ethanolic extracts of plant materials tend to exhibit stronger antimicrobial activity than aqueous extracts, likely due to the higher efficiency of ethanol in extracting bioactive compounds (Keqiang & Bruggen 2001). Similar trends have been reported in other studies (Orhue *et al*., 2014; Dhayenithi *et al*., 2010; Autade *et al*., 2015; Ugwu, 2019). The methanolic extract in this current study demonstrated antimicrobial activity against the test organisms, supporting earlier findings by Maragathavalli *et al*. (2012). However, the aqueous extract of neem leaves showed comparatively lower inhibitory effects, suggesting limited extraction of active compounds in water. Previous studies have shown that constituents of some flowering plants possess antimicrobial activity; however, a key limitation of this line of research is the lack of standardized methods used to assess such activity. This inconsistency has led to conflicting results across different studies, and even within the same study when different methods are applied to the same sample (Alade and Irobi, 1993; Iwu, 2000). In this study they compared the antimicrobial efficacy of aqueous extracts of leaf of *A. indica* against human pathogenic bacteria (Raja *et al* 2013). They found out that leaf extract exhibited strong antimicrobial activity against these bacteria at all the concentrations tested. This study is also in agreement with the result that the leaf of this plant has inhibitory effect on *P.aeruginosa*, Ugwu (2019).

In the present study, neem leaf extracts possessed antifungal activity against all the test fungi, although the degree of inhibition varied depending on the extraction solvent and fungal species. In general, the methanolic and ethanolic extracts exhibited greater antifungal activity than the aqueous extract, establishing that organic solvents were more effective in extracting the active antifungal compounds present in neem leaves. The minimum inhibitory concentration results further supported these findings, as the methanolic and ethanolic extracts showed stronger inhibitory effects at lower concentrations compared to the aqueous extract.

These reports indicate that neem leaf extracts contain bioactive compounds with considerable antifungal properties and may have potential applications in the development of alternative antifungal agents against fungal pathogens. In agreement with the findings of Oluduro (2012). In that study, notable antifungal activity of organic solvent extracts of plant leaves. The ethanolic extract demonstrated significant antifungal activity against *Aspergillus flavus*, *Penicillium* species, *Pullarium* species, and *Trichophyton mentagrophytes*, whereas the aqueous and methanolic extracts showed little or no anti-fungal effect, except for moderate activity observed against *A. flavus* with the methanolic extract. These results further support the idea that organic solvents are more effective in extracting antifungal bioactive compounds from plant materials than aqueous solvents. The antimicrobial activity demonstrated by *A. indica* may be due to the presence of several bioactive phytochemical compounds naturally found in the leaves (Odebiyi & Sofowora 1978). Compounds such as azadirachtins, quercetin, β-sitosterol (Subapriya & Nagini 2005), as well as flavonoids, tannins, alkaloids, saponins, even several others which are known to possess antimicrobial properties and contribute to the strong antibacterial and antifungal activity observed in this study. These phytochemicals may inhibit microbial growth through different mechanisms, including disruption of the microbial cell membrane, interference with enzyme activity, inhibition of protein synthesis, and alteration of metabolic processes essential for microbial survival. The *A. indica* leaf extract has potential as a natural therapeutic agent and could serve as an alternative source for the development of antimicrobial drugs, especially in the face of increasing antimicrobial resistance among pathogenic microorganisms.

The present study demonstrated that *A. indica* leaf extracts possessed considerable antimicrobial activity against the tested microorganisms, with the methanolic and ethanolic extracts exhibiting greater activity than the aqueous extract. This variation may be associated with the ability of organic solvents to extract higher amounts of bioactive phytochemical compounds from plant materials. Many conventional antibiotics are associated with undesirable side effects, highlighting the need to investigate plant derived alternatives that are effective and safer for therapeutic use (Srivastava *et al*., 2000; Ugwu, 2019).In the present study, the ethanolic extract demonstrated the highest antimicrobial activity, whereas the aqueous extract showed the lowest activity against the tested organisms. Similar variations in antimicrobial effectiveness based on extraction solvent have been reported in previous studies. Anyanwueze (2026) reported that methanolic extracts exhibited the strongest antibacterial activity, suggesting that methanol was more efficient in extracting bioactive antimicrobial compounds from plant leaves, while ethanolic extracts displayed weaker activity. In contrast, Doughari *et al*. (2007), who evaluated the antibacterial activity of aqueous, acetone, and ethanolic extracts of *A. indica* leaves, observed that ethanolic extracts produced the greatest antibacterial effect, whereas aqueous extracts showed the least activity.

Different solvents vary in their ability to extract phytochemical compounds because they dissolve different classes of bioactive constituents depending on their chemical properties (Majorie, 1999; Srinivasan *et al*., 2001; Oluduro, 2012). Therefore, the choice of solvent directly influences the composition, concentration, and effectiveness of extracted antimicrobial compounds. The findings of the present study align with previous reports demonstrating that leaf extracts possess inhibitory effects against pathogenic bacteria (Hala & Alfadhil, 2015). Previous studies have also reported that ethanolic extracts of plant materials often exhibit stronger antimicrobial activity than aqueous extracts because ethanol is more efficient in extracting bioactive compounds (Keqiang & Bruggen, 2001). Similar observations were reported by (Orhue *et al*. 2014; Dhayenithi *et al*. 2010; Autade *et al*. 2015; and Ugwu 2019).

The methanolic extract in the present study also demonstrated considerable antimicrobial activity against the test organisms, supporting earlier findings reported by Maragathavalli *et al*. (2012). However, the aqueous extract showed comparatively lower inhibitory effects, indicating limited extraction of active compounds in water. Several studies have demonstrated that extracts from flowering plants possess antimicrobial activity; however, differences in extraction procedures and antimicrobial testing methods often contribute to variations in reported results (Alade & Irobi, 1993; Iwu, 2000). Raja *et al*. (2013) evaluated the antimicrobial efficacy of aqueous *A. indica* leaf extracts against human pathogenic bacteria and reported strong antimicrobial activity at different concentrations tested. The findings of the present study are also consistent with the report of Ugwu (2019), who observed inhibitory effects of neem leaf extracts against *Pseudomonas aeruginosa*.

The present study further demonstrated that neem leaf extracts possessed antifungal activity against all the tested fungal isolates, although the degree of inhibition varied depending on the extraction solvent and fungal species. Generally, the methanolic and ethanolic extracts exhibited greater antifungal activity than the aqueous extract, suggesting that organic solvents were more effective in extracting antifungal bioactive compounds from neem leaves. The minimum inhibitory concentration results further supported these findings, as the methanolic and ethanolic extracts produced stronger inhibitory effects at lower concentrations compared to the aqueous extract.These findings indicate that neem leaf extracts contain bioactive compounds with considerable antifungal properties and may have potential applications in the development of alternative antifungal agents against fungal pathogens. Similar findings have been reported for the antifungal activity of organic solvent extracts of plant leaves. Ethanolic extracts demonstrated significant antifungal activity against *Aspergillus flavus*, *Penicillium* species, *Pullarium* species, and *Trichophyton mentagrophytes*, whereas aqueous extracts showed comparatively lower activity. These observations further support the idea that organic solvents are generally more effective than aqueous solvents in extracting antifungal bioactive compounds from plant materials (Oluduro, 2012).

The antimicrobial activity demonstrated by *A. indica* in the present study may be attributed to the presence of several phytochemical compounds naturally found in the leaves (Odebiyi & Sofowora, 1978). Bioactive constituents such as azadirachtins, quercetin, β-sitosterol (Subapriya & Nagini, 2005), flavonoids, tannins, alkaloids, and saponins are known to possess antimicrobial properties and may have contributed to the antibacterial and antifungal activities observed in this study. These phytochemicals may inhibit microbial growth through several mechanisms, including disruption of microbial cell membranes, interference with enzyme activity, inhibition of protein synthesis, and alteration of essential metabolic processes.

The findings of the present study infer that *A. indica* leaf extracts possess significant antimicrobial potential and may serve as a natural source for the development of alternative antimicrobial agents, particularly in response to the increasing global challenge of antimicrobial resistance.

5. Conclusion

These findings point to a promising avenue for identifying potent antimicrobial agents from *Azadirachta indica* (neem) *leaves*. The results indicate that the plant contains bioactive compounds with potential for development into phytomedicines for the treatment of infections. Systematic screening of natural compounds and identification of active constituents remain essential, as early recognition of lead molecules can significantly improve the efficiency of drug development. The extracts demonstrated inhibitory activity against Gram-positive and Gram-negative bacteria, as well as fungal species.

The present study demonstrated that *Azadirachta indica* (neem) leaf extracts possess considerable antibacterial and antifungal activity against the tested microorganisms. The methanolic and ethanolic extracts exhibited greater antimicrobial activity than the aqueous extract, suggesting that organic solvents were more effective in extracting bioactive antimicrobial compounds from the plant material. The extracts demonstrated inhibitory activity against Gram positive bacteria, Gram negative bacteria, and fungal isolates, indicating that neem leaves contain phytochemical constituents with potential therapeutic value. These findings point to the potential of *A. indica* as a promising natural source for the development of alternative antimicrobial agents and phytomedicines, particularly in the face of increasing antimicrobial resistance.

Despite these promising findings, the present study had some limitations. Antimicrobial activity was evaluated only under in vitro conditions, and therefore the effectiveness of the extracts under in vivo conditions remains unknown. The study was also limited to a small number of test organisms and did not include a wide diversity of clinical multidrug resistant isolates. In addition, the specific bioactive compounds responsible for the observed antimicrobial activity were not characterized, and the toxicity, pharmacokinetics, and bioavailability of the extracts were not investigated.

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