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Comparative Phytochemical Profiling and Antimicrobial Potency of Ethanolic Extracts from *Azadirachta Indica* Leaves and Stem Bark

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Abstract* - This study investigated the phytochemical composition and antimicrobial efficacy of ethanolic extracts from neem (Azadirachta indica) leaves and stem bark against Staphylococcus aureus (bacterium) and Aspergillus niger (fungus). Phytochemical analysis revealed distinct profiles: leaves contained alkaloids, tannins, saponins, and steroids, while stem bark contained alkaloids, flavonoids, and saponins. Antimicrobial assays demonstrated that both extracts were effective, but stem bark extract was significantly more potent. Notably, the stem bark extract at 100 mg/mL exhibited superior activity against S. aureus, producing a larger zone of inhibition (30.00 mm) than the Control control (27.00 mm). Minimum Inhibitory Concentration (MIC) results confirmed the stem bark's greater potency, showing lower MIC values for both test organisms. The study concludes that neem stem bark is a highly effective antimicrobial source, with its efficacy being dependent on the specific plant part and extract concentration, highlighting its potential as a natural alternative to conventional antibiotics. Clinical Applications: Given its superior efficacy against S. aureus, neem stem bark extract at 100 mg/ml should be further investigated for developing topical antimicrobial formulations or complementary therapies for bacterial infections. Agricultural and Preservation Uses: Given its antifungal properties, neem stem bark extract could be explored as a natural preservative in food and agricultural products to combat fungal contaminants like A. niger.

***Keywords:* Azadirachta Indica; Antimicrobial Resistance; Phytochemicals; Minimum Inhibitory Concentration; Staphylococcus Aureus; Aspergillus Niger.**

I. INTRODUCTION

The Neem tree (*Azadirachta indica* A. Juss.) is a cornerstone of traditional medicinal systems across Asia and Africa, reputed for its wide spectrum of biological activities (Biswas et al., 2002). Its usage in treating infections, skin disorders, and fevers is well-documented in Ayurveda and other ethnomedical practices (Subapriya & Nagini, 2005). This historical application is supported by modern scientific evidence demonstrating its antibacterial, antifungal, antiviral, and anti-inflammatory properties (Kumar & Navaratnam, 2013; Patel & Kumar, 2008).

The global antimicrobial resistance (AMR) crisis, driven by the overuse of conventional antibiotics and a stagnating drug development pipeline, has created an urgent need for novel therapeutic agents (Ventola, 2015; Murray et al., 2022). Plant-derived compounds offer a promising alternative due to their structural diversity and multi-target mechanisms of action, which can reduce the propensity for resistance development (Cowan, 1999).

While various parts of Neem have been studied, comparative analyses of the antimicrobial efficacy and phytochemical basis of different anatomical parts, such as leaves and stem bark, remain underexplored. Existing literature presents conflicting findings on which part is more potent, likely due to variations in extraction methods, test organisms, and phytochemical variability (Omar et al., 2018; Shrivastava & Swarnkar, 2014). This study therefore aimed to conduct a direct comparative analysis of the phytochemical composition and *in vitro* antimicrobial efficacy of ethanolic extracts from *A. indica* leaves and stem bark against a Gram-positive bacterium (*Staphylococcus aureus*) and a filamentous fungus (*Aspergillus niger*), to identify the most potent source of antimicrobial activity.

II. MATERIALS AND METHODS

2.1. Collection and Preparation of Plant Material

Fresh leaves and stem bark of *A. indica* were collected from Mubi, Adamawa State, Nigeria, in March 2025. The plant material was authenticated (voucher specimen deposited at the Department of Botany Herbarium, Adamawa State University, Mubi). Samples were washed, shade-dried at room

temperature (25–30°C) for two weeks, and pulverized into fine powder.

2.2. Extraction Procedure

Powdered leaf and stem bark materials (100 g each) were separately macerated in 500 mL of 95% ethanol for 72 hours with occasional shaking. The mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator. The resulting crude extracts were stored at 4°C until use.

2.3. Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extracts was performed using standard protocols (Harborne, 1998) to detect the presence of alkaloids (Wagner's test), flavonoids (Aluminum chloride test), tannins (Ferric chloride test), saponins (Froth test), and steroids (Salkowski test).

2.4. Microbial Strains and Inoculum Preparation

Reference strains of *Staphylococcus aureus* (ATCC 25923) and *Aspergillus niger* (ATCC 16888) were used. Bacterial inoculum was standardized to 0.5 McFarland turbidity (~1.5×10^8 CFU/mL) in sterile saline. Fungal spore suspension was prepared from a 5-day-old culture and adjusted to approximately 1×10^6 spores/mL in saline with 0.05% Tween 80.

2.5. Antimicrobial Susceptibility Testing

The disc diffusion assay was performed as per the Kirby-Bauer method (Bauer et al., 1966). Sterile filter paper discs (6 mm) were impregnated with 20 µL of each extract at concentrations of 50, 100, and 200 mg/mL (dissolved in 10% DMSO). Discs were placed on Mueller-Hinton Agar (MHA) inoculated with *S. aureus* and Potato Dextrose Agar (PDA) inoculated with *A. niger*. Control discs and 10% DMSO (negative control) were included. Plates were incubated at 37°C for 24 h (bacteria) and 28°C for 48 h (fungi). Zones of inhibition (ZOI) were measured in mm. All tests were performed in triplicate.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using a broth microdilution method in 96-well plates (CLSI, 2018). Two-fold serial dilutions of each extract (200 – 6.25 mg/mL) were prepared in Mueller-Hinton Broth (MHB) for *S. aureus* and Potato Dextrose Broth (PDB) for *A. niger*. Each well was inoculated with the standardized microbial suspension. After incubation, the MIC was recorded as the lowest concentration showing no visible growth.

2.7. Statistical Analysis

Data from disc diffusion assays were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post-hoc test was performed using SPSS software (Version 25). A p-value < 0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

3.1. Phytochemical Composition

The qualitative phytochemical profiles of the two extracts were distinct (Table 1). The leaf extract tested positive for alkaloids, tannins, saponins, and steroids but was negative for flavonoids. Conversely, the stem bark extract contained alkaloids, flavonoids, and saponins, but tannins and steroids were absent.

Table 1: Qualitative Phytochemical Analysis of *A. indica* Ethanolic Extracts

Phytochemicals	Leaves Status	Stem bark Status
Alkaloids	+	+
Flavonoids	-	+
Tannins	+	-
Saponins	+	+
Steroids	+	-

Key: + = Present; - = Not detected

This tissue-specific variation aligns with previous reports suggesting differential biosynthesis and accumulation of secondary metabolites in plant organs (Patel & Kumar, 2008). The presence of flavonoids exclusively in the stem bark is significant, as this class of compounds is strongly associated with antimicrobial activity through membrane disruption and enzyme inhibition (Cushnie & Lamb, 2005). The abundance of tannins in the leaves correlates with

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their traditional use for wound healing and astringency (Subapriya & Nagini, 2005).

3.2. Antimicrobial Activity: Disc Diffusion Assay

Both extracts exhibited concentration-dependent antimicrobial activity, but with differing patterns of efficacy (Tables 2 & 3, and integrated analysis in Table 4).

Table 2: Antimicrobial Activity (ZOI in mm) of Neem Leaf Extract

	Zone of Inhibition (mm)	
Concentration (mg/ml)	*Aspergillus niger*	*Staphylococcus aureus*
50	13.00±0.21[d]	26.00±0.13[b]
100	18.00±0.34[b]	20.00±0.11[c]
200	15.00±0.14[c]	18.00±0.10[d]
Control	29.00±0.21[a]	28.33±0.41[a]

Means along the column with similar superscript alphabet are not significantly different at p>0.05.

For the leaf extract, the highest activity against *S. aureus* was paradoxically at the lowest concentration (50 mg/mL), with efficacy decreasing at higher concentrations. This non-linear response may be due to compound aggregation or reduced diffusion efficiency in agar at high concentrations (Ahmad & Beg, 2001). Activity against *A. niger* peaked at 100 mg/mL.

Table 3: Antimicrobial Activity (ZOI in mm) of Neem Stem Bark Extract

Table 4.3: Antimicrobial Effect of Neem Stem Bark Extract

	Zone of Inhibition (mm)	
Concentration (mg/ml)	*Aspergillus niger*	*Staphylococcus aureus*
50	16.00±0.72[d]	24.00±0.20[d]
100	20.00±0.38[c]	30.00±0.14[a]
200	22.00±0.09[b]	25.00±0.11[c]
Control	29.00±0.14[a]	27.00±0.26[b]

Means along the column with similar superscript alphabet are not significantly different at p>0.05.

In stark contrast, the stem bark extract showed a more classic dose-response, particularly against *S. aureus*, where its activity at 100 mg/mL (30.00 mm) significantly ($p < 0.05$) exceeded that of the Control control (27.00 mm). This superior and consistent performance highlights the stem bark as a more potent antimicrobial source.

Table 4: Interaction Effect of Plant Part and Concentration on Antimicrobial Activity

Table 4.4: Antimicrobial Effect of Neem Leaf and Stem Bark Extract

	Zone of Inhibition (mm)	
Treatment	*Aspergillus niger*	*Staphylococcus aureus*
Concentration (mg/ml) – C		
50	14.50[c]	25.00[b]
100	19.00[b]	25.00[b]
200	18.50[b]	21.50[c]
Control	29.00[a]	27.67[a]
S±	0.24	0.15
Plant part – PP		
Leaf	18.75[b]	23.08[b]
Stem bark	21.75[a]	26.50[a]
S±	0.17	0.10
Interaction		
C x PP	S	S

Means along the column of each treatment group with similar superscript alphabet are not significantly different at p>0.05.

The significant interaction ($p < 0.05$) between plant part and concentration (Table 4) underscores that optimal efficacy is not a function of concentration alone but is critically dependent on the source tissue. For *S. aureus*, mid-concentration stem bark extract (100 mg/mL) was optimal, while for *A. niger*, higher concentrations (200 mg/mL) of stem bark were most effective.

3.3. Minimum Inhibitory Concentration (MIC)

The MIC results provided quantitative confirmation of the stem bark's superior potency (Table 5). The stem bark extract had a four-fold lower MIC against *S. aureus* (12.5 mg/mL) and a two-fold lower MIC against *A. niger* (50 mg/mL) compared to the leaf extract.

Table 5: Minimum Inhibitory Concentration (MIC) of *A. indica* Extracts

Test Organism	MIC – Leaf Extract (mg/mL)	MIC – Stem Bark Extract (mg/mL)	Standard Drug	MIC of Standard
Staphylococcus aureus	50	12.5	Control	0.5–1 µg/mL
Aspergillus niger	100	50	Control	0.5–1 µg/mL

While these MIC values are orders of magnitude higher than those of pure antibiotics (typically in µg/mL range), they are consistent with the performance of many crude plant extracts (Gibbons, 2004). The lower MIC of the stem bark extract strongly suggests a higher concentration or more potent synergy of its active constituents, notably flavonoids and alkaloids, which are known for membrane-disruptive and protein-binding activities (Cushnie & Lamb, 2005; Khan & Wassilew, 1987).

The remarkable efficacy of the stem bark extract against *S. aureus* is of particular relevance in the context of AMR, given the clinical importance of this pathogen and its propensity for methicillin resistance (MRSA). The multi-component nature of the extract, potentially targeting cell walls, membranes, and virulence factors like biofilm formation, presents a promising strategy to circumvent existing resistance mechanisms (Cowan, 1999).

IV. CONCLUSION

This study demonstrates that the antimicrobial potency of *Azadirachta indica* is highly dependent on the plant part used. Ethanolic extract of the stem bark showed significantly greater efficacy than leaf extract against both *Staphylococcus aureus* and *Aspergillus niger*, with its activity against *S. aureus* surpassing that of a standard antibiotic at an optimal concentration. This superior performance is strongly correlated with its distinct phytochemical profile, particularly the presence of flavonoids. The findings validate the ethnomedicinal use of Neem bark and position it as a promising candidate for the development of standardized phytotherapeutic agents, especially for targeting resistant bacterial infections. Future work should focus on bioassay-guided fractionation to isolate the specific active compounds from the stem bark and evaluate their mechanisms of action and *in vivo* safety.

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