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Research Article

Phytochemical Quantification, Antibacterial Activity and Toxicity of *Azadirachta Indica* (Neem) Leaf Ethanol Extract

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Abstract

The increase in antibiotic resistance has enhanced the search for antimicrobial agents of plant origin. Azadirachta indica is a common plant used in traditional Nigerian medicine but requires scientific validation. The main objectives of this study are to quantify the major phytochemical constituents, evaluate the phytochemical profile, antibacterial activity and toxicity of leaf extract of A. indica. Phytochemical screening and quantification of total phenols, flavonoids, tannins, alkaloids and saponins of ethanol leaf extract were carried out using standard methods. Antibacterial activity against S. aureus, E. coli, S. typhi and P. aeruginosa was determined by agar well diffusion. MIC, MBC and Brine shrimp lethality assay were used to determine toxicity. The phytochemicals identified were alkaloids, flavonoids, tannins, saponins, phenols, glycosides and terpenoids. Quantitative analysis revealed high levels of phenols (89.45 ± 2.31 mg GAE/g), flavonoids (62.18 ± 1.75 mg QE/g) and tannins (45.67 ± 1.22 mg TAE/g). The extract demonstrated dose dependent inhibition with highest activity against S. aureus (18mm at 100mg/mL). MIC values ranged from 12.5 to 50 mg/mL. The LC50 was 1450 µg/mL, indicating low toxicity. The result showed that A. indica leaf extract contains bioactive compounds in appreciable quantities with potent antibacterial activities and low toxicity which justify its traditional use and potentiality for drug development.

Keywords: *Azadirachta indica, Phytochemical, Quantification, Antibacterial, Toxicity, Drug resistance.*

Introduction

According to the World Health Organization, more than 80% of the population in developing countries relies on medicinal plants for primary health care [WHO]. *Azadirachta indica* (Neem) belongs to the family *Meliaceae* and is one of the most versatile medicinal plants found in Africa and Asia. Neem leaves are traditionally used in Nigeria to treat skin infections, malaria, fever and gastrointestinal disorders. The plant is reported to contain bioactive compounds like azadirachtin, nimbin, flavonoids and tannins which are responsible for its pharmacological activities (Biswas *et al*., 2002; Alzohairy, 2016).

However, the increasing incidence of multi-drug resistant bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* necessitates scientific validation of the efficacy and safety of these plants (Cowan, 1999).

Objectives

1. To perform qualitative phytochemical screening of *A. indica* leaf extract.
2. To quantify the total phenolic, flavonoid, tannin, alkaloid and saponin contents of *A. indica* leaf extract.
3. To evaluate the antibacterial activity and determine MIC/MBC of *A. indica* leaf extract against selected clinical bacterial isolates.
4. To assess the toxicity of *A. indica* leaf extract using brine shrimp lethality assay.

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Materials and Methods

Plant Collection and Extraction

Fresh leaves of *A. indica* were collected from the School of Technology Garden, Kano State Polytechnic, authenticated and dried. 100g of powdered leaves was extracted in 500mL of 70% ethanol for 72 hours. The filtrate was concentrated using rotary evaporator at 40°C.

Phytochemical Screening

Qualitative tests were carried out for alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids and steroids using standard procedures (Harborne, 1998).

Quantitative Phytochemical Analysis

Quantification was carried out in triplicates using standard spectrophotometric methods:

1. Total Phenolic Content: Folin-Ciocalteu method, expressed as mg Gallic Acid Equivalent/g [mg GAE/g].
2. Total Flavonoid Content: Aluminum chloride method, expressed as mg Quercetin Equivalent/g [mg QE/g].
3. Total Tannin Content: Folin-Ciocalteu with PVPP, expressed as mg Tannic Acid Equivalent/g [mg TAE/g].
4. Total Alkaloid Content: Bromo cresol green method, expressed as mg Atropine Equivalent/g [mg AE/g].
5. Total Saponin Content: Vanillin-sulphuric acid method, expressed as mg Diosgenin Equivalent/g [mg DE/g].

Test Organisms and Antibacterial Assay

Four bacterial isolates *S. aureus, E. coli, S. typhi* and *P. aeruginosa* were obtained from Murtala Muhammad General Hospital, Kano State. Antibacterial activity was determined by agar well diffusion method [CLSI] using 100, 50 and 25 mg/mL. Ciprofloxacin served as positive control. MIC and MBC were determined by broth dilution method (2024).

Toxicity Test

Brine shrimp lethality assay was used (Meyer *et al*., 1982). Nauplii were exposed to 10, 100 and 1000 µg/mL of extract for 24h. LC^{50} was determined by Probit analysis.

Data Analysis

Results were expressed as Mean ± SD and analyzed using ANOVA. $P < 0.05$ was considered significant.

Results

Qualitative Phytochemical Composition

Table 1: Qualitative Phytochemical Constituents of A. indica Leaf Extract

Phytochemical	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Glycosides	+
Terpenoids	+
Steroids	-

Quantitative Phytochemical Composition

Table 2: Quantitative Phytochemical Constituents of *A. indica* Leaf Extract

Phytochemical	Content
Total Phenols	89.45 ± 2.31 mg GAE/g
Total Flavonoids	62.18 ± 1.75 mg QE/g
Total Tannins	45.67 ± 1.22 mg TAE/g
Total Alkaloids	28.34 ± 0.98 mg AE/g
Total Saponins	35.90 ± 1.40 mg DE/g

Values are Mean ± SD, n=3

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Antibacterial Activity

Table 3: Zone of Inhibition of A. indica Leaf Extract (mm)

Organism	100mg/mL	50mg/mL	25mg/mL	Ciprofloxacin
S. aureus	18 ± 0.5	14 ± 0.3	10 ± 0.2	25 ± 0.4
E. coli	15 ± 0.4	12 ± 0.3	8 ± 0.2	22 ± 0.3
S. typhi	16 ± 0.5	13 ± 0.2	9 ± 0.1	23 ± 0.5
P. aeruginosa	12 ± 0.3	9 ± 0.2	6 ± 0.1	20 ± 0.4

MIC and MBC

Table 4: MIC and MBC (mg/mL) of A. indica Leaf Extract

Organism	MIC	MBC
S. aureus	12.5	25
E. coli	25	50
S. typhi	25	50
P. aeruginosa	50	100

Toxicity

The LC^{50} of the extract was 1450 µg/mL. According to Meyer's toxicity index, extracts with LC^{50} > 1000 µg/mL are considered non-toxic.

Discussion

The qualitative screening revealed the presence of alkaloids, flavonoids, tannins and other bioactive compounds, which correlates with earlier reports on Neem (Nandal & Bhardwaj, 2012). The quantitative results showed high concentrations of phenols (89.45 mg GAE/g), flavonoids (62.18 mg QE/g) and tannins (45.67 mg TAE/g). Phenols and flavonoids are known to disrupt bacterial cell membranes and inhibit protein synthesis (Cowan, 1999). The moderate levels of alkaloids and saponins may also contribute to the broad-spectrum activity observed.

The extract exhibited dose-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria. The higher susceptibility of S. aureus may be due to lack of outer membrane (Bhowmik *et al*., 2010). MIC values ≤ 100 mg/mL indicate good antibacterial potential, and MBC/MIC ratio of 2 indicates bactericidal activity.

The LC50 of 1450 µg/mL shows low toxicity and supports the safe traditional use of Neem leaves (Ebong *et al*., 2008), and is within WHO safety guidelines.

Conclusion

This study demonstrated the presence and significant quantities of bioactive phytochemicals, potent antibacterial activity, and low toxicity of Azadirachta indica leaf ethanol extract. These findings provide scientific justification for its traditional use and potential for drug development.

Recommendations

1. Isolation and characterization of the active compounds responsible for antibacterial activity.
2. In-vivo evaluation to determine efficacy and safety in animal models.
3. Formulation of the extract into standard phytomedicines for clinical trials.
4. Collaboration with NAFDAC for standardization and regulation of Neem-based products.

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