# The Antimicrobial Potency of Neem (*Azadirachta indica*) Leaves and Root Extracts

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**Abstract:** The ethanol, petroleum ether and aqueous extracts of Neem (*Azadirachta indica*) at varying concentrations, were potent on pathogenic *Pseudomonas*, *Staphylococcus epidermidis*, *Proteus*, *Escherichia coli*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* and *Yeast*. The bacterial isolates were resistant to nalixidic acid, nitrofurantoin, amoxicillin, tetracycline, cloxacillin, augmentin and erythromycin. They were however susceptibility to ofloxacin and gentamicin. The Neem extracts seem to compare favourably with ofloxacin and gentamycin.
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**Key words**: Neem; *Azadirachta indica*; Antimicrobial; Extract

## 1. Introduction

Neem (*Azadirachta indica*) is used in Nigeria, West Africa in the treatment of various ailments and is locally called ''Dongoyaro'' by the Yoruba tribe of the Southwest Nigeria (Ekanem, 1978). It is also known as Indian Lilac and belongs to the Division Magnoliophyta of the Kingdom Plantae (Dutta, 2007). Neem is a key ingredient in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides (Beaulieu, 2013). Reports by Cowman (1999) have revealed its antimicrobial potentials.

In the current investigation, root and leaves extracts of Neem (*Azadirachta indica*) was assessed for efficacy and antimicrobial potency on certain pathogenic strains of microorganisms. This was with a view to comparing its potentials with some regular commercially available antibiotics.

## 2. Materials and Methods

### 2.1 Selection of Plant

The plant Neem (*Azadirachta indica*) was purchased at Bode market, Ibadan, Nigeria. The roots were identified and authenticated at the Department of Microbiology, Lead City University, Ibadan, Nigeria. The roots were separated, washed in clean water, and dried at room temperature. The dried plants were milled to a fine powder and stored in the dark at room temperature in closed containers until required.

### 2.2 Preparation of Extracts

**(a) Petroleum Ether Extract**

20 g of dried root powder of Neem (*Azadirachta indica*) was weighed. 200 ml of petroleum ether was added and kept for 48 h with periodic shaking then filtered.

**(b) Chloroform Extract**

50g of dried dried root of *Azadirachta indica* was weighed. 200ml of chloroform was added and kept for 48 h with periodic shaking and then filtered.

**(c) Ethanol Extract**

20g of dried dried root of Neem was weighed. 200 ml of ethanol was added and kept for 48 h with periodic shaking.

**(d) Aqueous Extract**

20g of dried dried root of *Azadirachta indica* was weighed. 200 ml of distilled water was added and kept for 48 h with periodic shaking and then filtered.

### 2.3 Microorganisms

The Pathogenic isolates, *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris and Klebsiella sp* obtained from the Microbiology Laboratory of the University College Hospital, Ibadan, and *Aspergillus niger, Aspergillus flavus, Penicillium,* and *Rhodotulla* obtained from the Microbiology Laboratory of Lead City University, Ibadan were used.

### 2.4.1 Antimicrobial screening

### 2.4.2 Agar disc diffusion method

Antibiotic impregnated disc were used. Zone of inhibition of bacterial growth around each disc was measured and the susceptibility determined (Perez *et al*., 1990).

### 2.5 Determination of Minimum Inhibitory Concentration (Micro-dilution Assay)

The minimum inhibitory concentration, defined as the lowest concentration of the compound that will inhibit the growth of microorganisms, was determined. The minimum inhibitory concentration values were determined by broth dilution assay with micro-dilutions. Varying concentrations of the extracts (200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml) were prepared. 0.1ml of standardized test organism of controls was equally set up by using

solvents and test organisms without extract. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration (Olutiola *et al.*, 1991).

## 3. Results

The anti-bacterial effects of different concentrations of root extracts of *Azadirachta indica* on different isolates are presented (Table 1). The effects of the root extracts on fungal isolates are presented in Table 2. The effects of the leaves extracts of *Azadirachta indica* on bacterial isolates are presented in Tables 3 - 7.The effects of the leaves extracts on fungal isolates are presented in Tables 8 - 11. Some of the conventional antibiotics and their sensitivity on specific isolates are presented in Table 12.

**Table 1: Effects of different concentrations of extract of the root of Neem (*Azadirachta indica*) using different solvents on bacteria**

| S/N | Microbial isolate | Solvent | Undiluted Extract | 66.6mg/ml | 50mg/ml | 40mg/ml | Control |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| **1** | *Pseudomonas* | Ethanol | 28mm | 28mm | 14mm | 12mm | 10mm |
| | | Pet Ether | ___ | ___ | ___ | ___ | ___ |
| | | Water | ___ | ___ | ___ | ___ | ___ |
| | | Chloroform | ___ | ___ | ___ | ___ | ___ |
| **2** | *Staph epidemidis* | Ethanol | 32mm | 24mm | 24mm | 20mm | 16mm |
| | | Pet Ether | 26mm | 12mm | 10mm | ___ | 10mm |
| | | Water | ___ | ___ | ___ | ___ | ___ |
| **3** | *Proteus* | Ethanol | 22mm | ___ | 31mm | 28mm | 23mm |
| | | Pet Ether | 36mm | 39mm | 20mm | 14mm | 12mm |
| | | Water | ___ | ___ | ___ | ___ | ___ |
| **4** | *Klebsiella* | Ethanol | 38mm | 30mm | 26mm | ___ | 10mm |
| | | Pet Ether | 26mm | 22mm | 18mm | 16mm | 10mm |
| | | Water | ___ | ___ | ___ | ___ | ___ |
| **5** | *E.coli* | Ethanol | | | | | |
| | | Pet Ether | 30mm | ___ | ___ | ___ | ___ |
| | | Water | ___ | ___ | ___ | ___ | ___ |

**Table 2: Effect of different concentrations of extract of the root of Neem (*Azadirachta indica*) using different solvents on fungi**

| S/N | Microbial isolate | Solvent | Undiluted Extract | 66.6mg/ml | 50mg/ml | 40mg/ml | Control |
|---|---|---|---|---|---|---|---|
| 1 | **Aspergillus niger** | Ethanol | 16mm | RR | 12mm | 10mm | 9mm |
| | | Pet Ether | RR | RR | RR | RR | RR |
| | | Water | RR | 12mm | RR | RR | |
| | | Chloroform | RR | RR | RR | RR | RR |
| 2 | **Aspergillus flavus** | Ethanol | RR | RR | RR | RR | RR |
| | | Pet Ether | RR | RR | RR | RR | RR |
| | | Water | RR | RR | RR | RR | |
| | | Chloroform | RR | RR | RR | RR | |
| **3** | **Penicillium** | Ethanol | 15mm | 18mm | 8mm | 17mm | 9mm |
| | | Pet Ether | RR | RR | RR | RR | RR |
| | | Water | RR | RR | RR | RR | |
| | | Chloroform | RR | RR | RR | RR | RR |
| **4** | **Yeast** | Ethanol | 16mm | 14mm | 15mm | 24mm | 12mm |
| | | Pet Ether | RR | RR | RR | RR | RR |
| | | Water | 18mm | 16mm | 14mm | RR | |
| | | Chloroform | RR | RR | RR | RR | RR |

**Table 3: Antibacterial activity of Neem (*Azadirachta indica*) leaves extracts on *Pseudomonas***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 28.00 ± 1.63 | 14.00 ± 3.27 | 14.00 ± 0.82 | 12.00 ± 2.45 | 10.00 ± 3.27 |
| **Petroleum Ether** | ---- | ---- | ---- | ---- | ---- |
| **Water** | ---- | ---- | ---- | ---- | ---- |
| **Chloroform** | ---- | ---- | ---- | ---- | ---- |

Values represent the mean ± standard deviation obtained from three replicates

**Table 4: Antibacterial activity of Neem (*Azadirachta indica*) leaves extracts on *Staph epidermidis***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 32.00 ± 8.16 | 24.00 ± 1.63 | 24.00 ± 4.90 | 20.00 ± 2.45 | 16.00 ± 4.03 |
| **Petroleum Ether** | 26.00 ± 3.27 | 13.00 ± 2.45 | 10.00 ± 2.45 | ---- | 10.00 ± 1.63 |
| **Water** | ---- | ---- | ---- | ---- | ---- |
| **Chloroform** | ---- | ---- | ---- | ---- | ---- |

Values represent the mean ± standard deviation obtained from three replicates

**Table 5: Antibacterial activity of Neem (*Azadirachta indica*) leaves extracts on *Proteus***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 31.00 ± 7.35 | 28.00 ± 3.27 | 23.00 ± 2.45 | ---- | 22.0 ± 2.45 |
| **Petroleum Ether** | 35.33 ± 7.41 | 29.00 ± 0.82 | 20.00 ± 2.45 | 14.00 ± 0.82 | 12.00 ± 2.45 |
| **Water** | ---- | ---- | ---- | ---- | ---- |
| **Chloroform** | ---- | ---- | ---- | ---- | ---- |

Values represent the mean ± standard deviation obtained from three replicates

**Table 6: Antibacterial activity of Neem (*Azadirachta indica*) leaves extracts on *Klebsiella***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 38.00 ± 11.43 | 25.00 ± 2.45 | 26.00 ± 1.63 | ---- | 10.00 ± 5.72 |
| **Petroleum Ether** | 26.00 ± 1.63 | 22.00 ± 6.53 | 18.00 ± 0.82 | 16.00 ± 1.63 | 10.00 ± 1.63 |
| **Water** | ---- | ---- | ---- | ---- | ---- |
| **Chloroform** | ---- | ---- | ---- | ---- | ---- |

Values represent the mean ± standard deviation obtained from three replicates

**Table 7: Antibacterial activity of Neem (*Azadirachta indica*) leaves extracts on *E. coli***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 48.00 ± 3.26 | 46.00 ± 11.43 | 38.00 ± 9.80 | 36.00 ± 1.63 | 32.00 ± 8.15 |
| **Petroleum Ether** | 30.00 ± 4.89 | ---- | ---- | ---- | ---- |
| **Water** | ---- | ---- | ---- | ---- | ---- |
| **Chloroform** | ---- | ---- | ---- | ---- | ---- |

Values represent the mean ± standard deviation obtained from three replicates

**Table 8: Activity of Neem (*Azadirachta indica*) leaves extract on *Aspergillus niger***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 11.33 ±2.62 | 6.67 ± 3.09 | RR | RR | RR |
| **Petroleum ether** | RR | RR | RR | RR | RR |
| **Water** | RR | RR | RR | RR | RR |
| **Chloroform** | RR | RR | RR | RR | RR |

Values represent the mean ± standard deviation obtained from three replicates

**Table 9: Activity of Neem (*Azadirachta indica*) leaves extracts on *Aspergillus flavus***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 12.67 ± 3.09 | RR | 8.33 ± 2.49 | 8.33 ± 0.94 | 7.33 ± 1.25 |
| **Petroleum ether** | RR | RR | RR | RR | RR |
| **Water** | 14.00 ± 2.49 | 14.33 ± 6.02 | 11.33 ± 2.49 | 6.67 ± 1.89 | 4.33 ± 1.89 |
| **Chloroform** | 14.33 ± 6.13 | 12.33 ± 4.03 | 11.33 ± 3.300 | 10.00 ± 2.93 | 7.00 ± 2.166 |

Values represent the mean ± standard deviation obtained from three replicates

**Table 10: Activity of Neem (*Azadirachta indica*) leaves extracts on *Penicillium***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | RR | RR | RR | RR | RR |
| **Petroleum ether** | RR | RR | RR | RR | RR |
| **Water** | RR | RR | RR | RR | RR |
| **Chloroform** | RR | RR | RR | RR | RR |

Values represent the mean ± standard deviation obtained from three replicates

**Table 11: Activity of Neem (*Azadirachta indica*) leaves extracts on*Yeast***

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Ethanol** | 15.33 ± 1.25 | 10.33 ± 2.62 | 7.33 ± 2.36 | 8.33 ± 2.05 | 4.00 ± 1.41 |
| **Petroleum ether** | RR | RR | RR | RR | RR |
| **Water** | RR | RR | RR | RR | RR |
| **Chloroform** | RR | RR | RR | RR | RR |

**Table 12: Conventional Antibiotics sensitivity and isolates**

| | | S/N | Disc Name | Code | Diameter Inhibition |
|---|---|---|---|---|---|
| | | **1** | Nalixidic acid | NAL | R |
| | | **2** | Nitorfurantoin | NIT | R |
| | | **3** | Amoxicillin | AMX | R |
| | | **4** | Tetracycline | TET | R |
| | | **5** | Augumentin | AUG | R |
| | | **6** | Erythromycin | ERY | R |
| | | **7** | Cloxacillin | CXC | R |
| | | **8** | Streptomycin | STR | R |

| S/N | Microbial Isolate used | Disc | Code | Diameter |
|---|---|---|---|---|
| **1** | *E.coli* | ___ | ___ | ___ |
| **2** | *Klebsiella pneumonia* | Ofloxacin | Ofl | 10mm |
| **3** | *Pseudomonas aeruginosa* | Ofloxacin | Ofl | 8mm |
| **4** | *Proteus mirabilis* | Ofloxacin | Ofl | 10mm |
| **5** | *Staphylococcus* | Gentamycin | Gen | 15mm |

In our conducted study, the ethanol, chloroform, petroleum ether and aqueous extracts of Neem were screened for their activity on bacterial strains (*Escherichia coli, Pseudomonas, Proteus, Klebsiella pneumoniae* and *Staphylococcus epidermidis*) and fungal strains (*Aspergillus niger, Aspergillus flavus and Penicillium* and *Yeast*). The extracts showed antimicrobial activity on both the test bacteria and fungi with the ethanol extracts having the highest activity, followed by the petroleum ether extracts, while the water extracts had the least activity. The water and chloroform extracts did not show any reasonable activity on all the isolates. The ethanol extracts were active on all of the isolates. Zone of inhibition observed for *Pseudomonas*- 14mm; *Staph epidermidis* – 24mm diameter zone of inhibition, *Proteus*- 23mm diameter zone of inhibition; *E. coli* – 38mm zone of inhibition; and *Klebsiella* -26mm diameter zone of inhibition, at 50 mg/ml concentration. The petroleum extracts were active on the bacteria: *Staph epidermidis* – 10 mm diameter zone of inhibition; *Proteus*- 20mm diameter zone of inhibition; and *Klebsiella* -18mm diameter zone of inhibition at 50 mg/ml concentration. The bacterial

isolates showed resistance to nalixidic acid, nitrofurantoin, amoxicillin, cloxacillin, tetracycline, augmentin, erythromycin in this investigation. The isolates were susceptible to ofloxacin and gentamycin (Table 12).

## 4. Discussion

Different solvents have various degrees of solubility for different phyto-constituents (Majorie, 1999). Synergistic effect of some phyto-constituents on some antibiotics- resistant isolates had earlier been reported (Nascimento *et al.,* 2000). The antibiotics gentamycin, erythromycin, oflaxacin, augumentin, tetracycline, cloxacillin, amoxillin and streptomycin were used as control checks and to compare the antibacterial activity with different crude extracts.

From the the review of literature, the qualitative phytochemical investigation of Neem revealed that the extracts contained some phyto-constituents. Saponins, tannins, alkaloids and flavonoids are present in the extracts of Neem. These bioactive components including thiocynate, nitrate, chloride and sulphates, beside other water soluble components which are naturally occurring in most plant materials, are known to be bacteriocidal, pesticidal and fungicidal in nature thus conferring the anti-microbial property to the plant (Lutterodt *et al*., 1999; Pretorius *et al*., 2002; El-Astal *et al*., 2005).

The demonstration of activity of Neem on both gram-negative and gram-positive bacteria and fungi is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The Neem extracts seem to compare favourably with ofloxacin and gentamycin.

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