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*Full Length Research Paper*

# The antibacterial potentials of *Nauclea latifolia*

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**Herbal medicine is readily available in diverse African vegetation with the potentials of introducing new templates into medicine worldwide. Evaluating plants from the traditional African system of medicine provides us with clues on how these plants can be used in the treatment of diseases. *In vitro* effect of *Nauclea latifolia* extract in hot water, cold water, petroleum ether and chloroform at concentrations of 200, 150, 100, 50% were tested on some pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Using agar diffusion punch hole method, both the aqueous and alcoholic extracts of the leaves and roots showed appreciable inhibitory effect when compared to the positive control on *S. aureus* and *P. aeruginosa* while *S. typhi* and *E. coli* were resistant to the extracts. Using serial doubling dilution, the minimum inhibitory concentration (MIC) was determined. The minimum bacteria concentration was determined by plating various dilutions of the extracts without turbidity. Aqueous and alcoholic extracts of *N. latifolia* showed inhibitory and bactericidal activity on the test organisms. The alcoholic extracts showed larger zone of inhibition on the test organisms. The alcohol leaf extracts showed a higher percentage of growth inhibition when compared to the positive control. The MIC ranges from 6.25 – 150 mg/ml on *S. aureus* and 12.5 – 150 mg/ml for *P. aeruginosa*. The MBC ranges from 100 – 150 mg/ml. The phytochemical analysis revealed the presence of saponin, resins, alkaloids, and carbohydrate.**

**Key words:** Antimicrobial potentials, *Nauclea latifolia,* bacterial pathogens, Nigeria.

## INTRODUCTION

Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drugs are on the rise. Traditional medicine is a comprehensive term used to refer both to traditional medicine systems such as traditional Chinese medicine, Indian ayurveda and Arabic unani medicine, and to various forms of indigenous medicine (WHO, 1978). Historically, plant has provided a good source of anti-infective agents; emetine, quinine and berberine remain highly effective instruments in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections. Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and *Garcinia biflaronones* used in traditional African system of medicine, have been found to be active against a wide variety of microorganisms.

Seeking remedies for human ailments from the environment has formed the basis for therapeutics (Potier et al., 1990). African plants constitute a rich untapped pool of natural products (Bringmann and Pokorny, 1995). Extracts of different parts of the plant *Nauclea latfolia* (e.g. fruits, leaves, stem-bark and roots) in hot water or alcohol are used in form of infusions, decoctions or concoctions (Irvine, 1961; Agoha, 1974).

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The continual search for, and the interest in natural plant products for use as medicines has acted as the catalyst for exploring methodologies involved in

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obtaining the required plant materials and probing their constituents. The plant, *N. latifolia*, is used in many African countries by traditional medical practitioners for the treatment of various ailments including bacterial diseases. It is most effective against *Corynebacterium diphteriae, Streptobacillus spp, Streptococcus spp, Neisseria spp, Pseudomonas aeruginosa, Salmonella spp* (Deeni and Hassan, 1991).

*N. latifolia* commonly known as pin cushion tree is a straggling shrub or small tree native to tropical Africa and Asia. It is a tropical plant that grows commonly in Akwa Ibom and Cross River State of Nigeria and also in most parts of the northern Nigeria. In Akwa Ibom and Cross River states, it is called "Mbom-mbog" whilst in the northern Nigeria it is called "Tabasiya". It is known as "Uche" by Igede people. It is found in the forest and fringe tropical forest. The plant is also used in the treatment of ailments like malaria (Kokwaro, 1976; Akabue and Mittal, 1982; Boye, 1990), gastrointestinal tract disorders (Maduabunyi, 1995), sleeping sickness (Kerharo, 1974), prolong menstrual flow (Elujoba, 1995), hypertension (Akabue and Mittal, 1982) and as a chewing stick (Asubiojo et al., 1982). Aqueous extracts from *N. latifolia* S.M. *(Rubiaceae*) is also a common plant used in Ivory Coast by traditional healers for the treatment of malaria. In Burkina Faso, the plant is used for the treatment of several diseases as jaundice, malaria, infant gastroenteritis, dysentery etc (Fernandez de la pradislla, 1982a,b). The plant is also used as a tonic and fever medicine, chewing stick, toothaches, dental cares, septic mouth and malaria, diarrhea and dysentery (Lamidi et al., 1995).

Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. This study was aimed at establishing the antimicrobial potentials of widely acclaimed medicinal value of *N. latifolia* extracts on bacterial pathogens isolated in Vom.

## MATERIALS AND METHODS

The plants were obtained within the premises of National Veterinary Research Institute, Vom. The botanical identity was determined and authenticated at the Department of Botany, University of Jos, Nigeria where a voucher specimen has been deposited.

### Processing of the plant

The leaves and roots of the plant were washed with tap water and rinsed in distilled water. They were dried using hot air oven at 40°C for three days. The dried plant parts were pulverized using sterile laboratory mortar to obtain the powdered form. These were stored in airtight sterile bottles until required for analysis.

### Crude extraction

The method of Junaid et al. (2006) and Okwori et al. (2007) were adopted for this study.

### Preparation of the extract

The aqueous extracts were reconstituted using sterile distilled water while chloroform and petroleum ether were reconstituted using 50% acetone to obtain concentrations of 200, 150, 100 and 50 mg/ml.

### Sources of bacterial isolates

The stock bacterial isolates used were obtained from the Bacteriology laboratory of the Federal College of Veterinary and Medical laboratory Technology, Vom and the Bacterial research Laboratory, National Veterinary research Institute, Vom. Purity plate for each of the bacterial isolates was used for this study.

### Preparation of the test bacteria

Fresh purity plates of the test bacteria were made from the isolate cultures obtained on agar slants. The isolates were sub-cultured on selective and differential solid media and re identified phenotypically (Okwori et al., 2007).

With the aid of a sterile wire loop, colonies of fresh cultures of the different bacterial isolates were picked and suspended on 5 ml nutrient broth in different well labeled sterile 10 ml bijou bottles. 1 ml of the isolate from the broth was diluted in peptone water using the two-fold dilution. For *Staphylococcus aureus* which is a slow growing organisms, 0.02 ml of ($10^{-1}$ to $10^{-7}$) dilutions was picked using a single channel pipette and plated out in triplicate on nutrient agar to obtain the mean viable count of the isolates. The same procedure was done for *Salmonella typhi, Escherichia coli* and *P. aeroginosa*, but since they are fast growing organisms, 0.02 ml of ($10^{-4}$ to $10^{-10}$) dilutions were plated out on nutrient agar and incubated at 37°C for 24 h (Miles and Misra, 1938).

After 24 h, different numbers of colonies were obtained at different dilutions for the different bacterial isolates. The mean numbers of colonies were calculated to obtain the colony-forming unit per ml (cfu/ml) for the different isolates. 1 ml of the standard inoculum of the different bacterial isolates was used in flooding sterile nutrient agar plates in the agar diffusion method of *in vitro* antimicrobial sensitivity test.

### Antimicrobial potential

A standardized inoculum of 1 – 2 X $10^7$ cfu/ml was used for the inoculation of plates. The plates were allowed to dry in the incubator for 30 min at 37°C and with the aid of a sterile standard cork borer, 6 wells were bored at equidistant. The bottoms of the wells were sealed with sterile molten nutrient agar (Biotec, Sufolk, UK) to prevent seepage of the extract under the agar. The 5 and 6th wells served as positive and negative controls. The sterile distilled water served as negative control. Ciprofloxacin (10 ug/ml) was used as the positive control. 0.2 ml of each prepared concentration of the extracts was aseptically introduced into wells 1 – 4. The plates were allowed on the bench for 40 min for pre-diffusion followed by an overnight incubation at 37°C. The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be zone of inhibition of the bacterial isolates in question at that particular concentration (Abayomi, 1982; Junaid et al., 2006).

**Table 1.** Antimicrobial activity of different solvent extracts (zone of inhibition measured in mm).

| Isolate | Conc. | HWR | CWR | DR | CR | HWL | CWL | CL | PDL |
|---|---|---|---|---|---|---|---|---|---|
| S. aureus | 200 | 16 | 16 | 20 | 20 | 18 | 17 | 20 | 18 |
| | 150 | 15 | 15 | 18 | 19 | 17 | 15 | 15 | 15 |
| | 100 | 13 | 12 | 17 | 17 | 15 | 12 | 15 | 15 |
| | 50 | 10 | 10 | 13 | 13 | 12 | 10 | 10 | 12 |
| *S. typhi* | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. aeruginosa* | 200 | 15 | 12 | 20 | 15 | 23 | 23 | 22 | 24 |
| | 150 | 14 | 11 | 20 | 15 | 22 | 22 | 20 | 22 |
| | 100 | 10 | 11 | 15 | 13 | 23 | 22 | 18 | 20 |
| | 50 | 0 | 0 | 10 | 10 | 20 | 20 | 26 | 26 |

HWR – Hot water root; CWR – Cold water root; DR – Diether root; CR – Chloroform root; HWL – Hot water leaves; CWL – Cold water leaves; PDL – Petroleum ether leaves; CL – Chloroform leaves.

**Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

MIC of the extracts was determined by dilution to various concentrations according to the macro broth dilution technique (Baron and Finegold, 1990; Akinyemi et al., 2005). Standardized inoculum was added to series of sterile tubes of nutrient broth containing two fold dilution of the extracts and incubated at 37°C for 24 h. The MIC was read as the least concentration that inhibited the growth of the test organisms. However, the MBC was determined by sub-culturing the test dilutions onto fresh drug-free solid medium and incubated further for 18 – 24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC (Okwori et al., 2007).

**Phytochemical screening**

The presence of alkaloids, resins, saponins, glycosides, tannins, flavonoids, cardiac glycoside, steroidal ring, steroidal terpenes, anthraquinone and carbonhydrates were determined as described by Akinyemi et al. (2005) and Junaid et al. (2006) and Okwori et al. (2007).

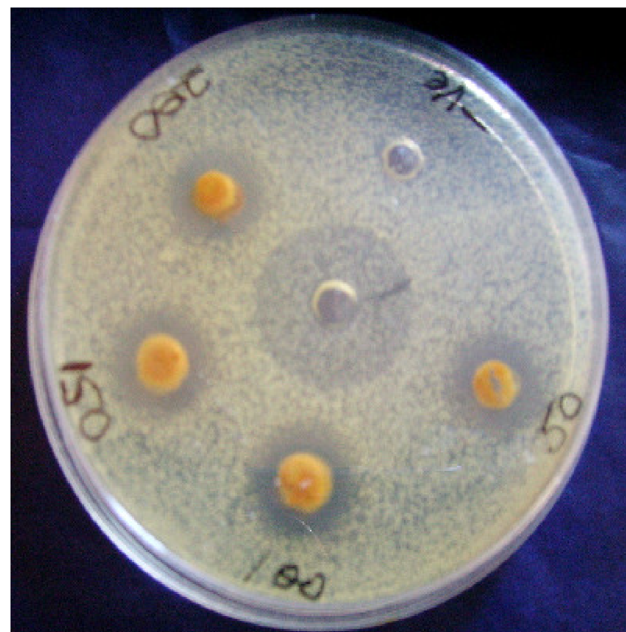

**Figure 1.** Inhibitory effect of the extracts at different concentrations on sensitivity test agar.

## RESULTS

Alcoholic and aqueous extracts of the roots and leaves extracts of *N. latifolia* were tested on four known clinical bacterial isolates using agar diffusion (punch hole method; see Figure 1). Alcoholic root extracts at concentration ranging from (200 - 50%) produced mean inhibitory zone ranging from 13 – 20 mm on *S. aureus* while alcoholic leaf extract produced mean inhibitory zone ranging from 10 – 20 mm on *S. aureus*. Alcoholic root extract produced mean inhibitory zone ranging from 10 – 20 mm on *P. aureginosa* while alcoholic leaf extract produced mean inhibitory zone ranging from 16 – 24 mm on *P. aeruginosa* which was significant compared with the control.

Aqueous root extract at concentration ranging from (200 - 50%) produced mean inhibitory zone ranging from 10 – 16 mm on *S. aureus* while aqueous leaf extract produced a mean zone of inhibition ranging from 10 – 18 mm on *S. aureus*. Aqueous leaf extract produced a significant zone of inhibition ranging from 20 – 23 mm on *P. aeruginosa* when compared with the control while the aqueous root extract produced zone of inhibition ranging from 10 – 15 mm on *P. aeruginosa* (Table 1).

*S. typhi* and *E. coli* were resistant on both alcohol and aqueous leaves and roots extract at all concentration. The alcoholic and aqueous extracts exhibited both bacteriocidal and bacteriostatic activities on the positive

**Table 2.** MIC based on bacterial turbidity at various conc. (mg/ml) using two-fold dilution.

| Isolate | Extract | 200 | 150 | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 | 0.39 | 0.19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | HWL | - | - | - | - | - | - | + | + | ++ | ++ | ++ | ++ |
| | CWL | - | - | - | - | - | - | - | + | ++ | ++ | ++ | ++ |
| | DL | - | - | - | - | - | - | - | + | ++ | ++ | ++ | ++ |
| | CL | - | - | - | - | - | - | + | + | ++ | ++ | ++ | ++ |
| | HWR | - | - | - | - | - | - | + | + | ++ | ++ | ++ | ++ |
| | CWR | - | - | - | - | - | - | + | + | ++ | ++ | ++ | ++ |
| | CR | - | - | - | - | - | - | - | + | ++ | ++ | ++ | ++ |
| | DR | - | - | - | - | - | - | - | + | ++ | ++ | ++ | ++ |
| *P. aeruginosa* | HWL | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | CWL | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | DL | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | CL | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | HWR | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | CWR | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | CR | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |
| | DR | - | - | - | - | - | - | ++ | ++ | ++ | ++ | ++ | ++ |

– = Not turbid; + = slightly turbid; ++ = very turbid.

**Table 3.** MIC based on bacterial growth.

| Isolate | Extract | Concentration | | | | | MBC |
|---|---|---|---|---|---|---|---|
| | | 200 | 150 | 100 | 50 | 25 | |
| S. aureus | HWL | - | - | + | + | + | 150 |
| | CWL | - | - | - | + | + | 100 |
| | DL | - | - | - | + | + | 100 |
| | CL | - | - | - | + | + | 100 |
| | HWR | - | - | + | + | + | 150 |
| | CWR | - | - | + | + | + | 150 |
| | CR | - | - | + | + | + | 150 |
| | DR | - | - | + | + | + | 150 |
| *P. aeruginosa* | HWL | - | - | - | + | + | 100 |
| | CWL | - | - | - | + | + | 100 |
| | DL | - | - | + | + | + | 150 |
| | CL | - | - | + | + | + | 150 |
| | HHR | - | - | + | + | + | 150 |
| | CHR | - | - | + | + | + | 150 |
| | CR | - | - | + | + | + | 150 |
| | DR | - | - | + | + | + | 150 |

– = No bacterial growth;+ = bacterial growth.

test organisms (Tables 2 and 3).

The phytochemical analysis of both root and leaf extracts revealed the presence of carbohydrate, saponins, resins, alkaloids.

## DISCUSSION

From the results obtained, both the alcohol and aqueous extracts of leaves and roots of *N. latifolia* were active against 2 out of the 4 test bacterial organisms. The exception to the rule was *E. coli* and *S. typhi,* which was found to be resistant to both, though a gram negative bacterium, has been reported to be sensitive to extracts of *N. latifolia* (Omer et al., 1998) but this was not in agreement with this work. This may be as a result of natural resistance, genetic variability or mutational changes which occur even before the introduction of

drug. The resistance can be transferred from one bacterium to another of the same species, and also between different species and sometimes, even between related general. Deeni and Hussain (1991) documented the susceptibility of *P. aeruginosa* and *Salmonella* species to plant extracts. However, our finding is in agreement with this report except for *S. typhi* and this could be as a result of the species of *Salmonella* used in the previous work.

Both the aqueous and alcohol leaf extracts showed a wide range of antibacterial activity when compared to the positive control. Umeh et al. *(2*005) suggests in his work that the antibacterial constituents of the plant are preferentially concentrated in the leaves and in this work the leaf extracts showed a higher percentage of growth inhibition.

Both aqueous and alcohol extracts of the root showed zones of inhibition. This work is also in agreement with similar reports documented by Iwu (1993) whose studies showed that the root has antibacterial activity against gram positive and negative bacteria. It was found in this work that test bacterial organisms were more susceptible to alcoholic extracts than the aqueous extracts. This may be due to its phytochemical extraction capability (Junaid et al., 2006). Similarly, the studies of Umeh et al. (2005) showed that petroleum ether and chloroform extracts of the plants studied have more inhibitory effect than methanolic and aqueous extracts, although the inhibitory effects of aqueous and methanolic extracts of medicinal plants are usually reported (Ogunlana and Ramstad, 1975; Omer et al., 1998; Olayinka et al., 1992). This report is also in line with the report of Obi and Onuoha (2000), who reported alcohol to be the best solvent for the extraction of most plant active principles of medical importance.

It was also observed from this work that the higher the concentration, the more their activity and as the concentration decreases the lower the antibacterial activity. Hence an acceptable and effective dosage can be prepared by traditional healers for the control and eradication of bacterial pathogens.

The variation in results imply that the MBC results obtained after plating various dilutions of extracts is more reliable compared to MIC results obtained usually using turbidity as an index (Junaid et al., 2006).

One of the greatest service which can be rendered to any country is to add a useful plant to its culture. It is therefore highly essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are sometimes unable to afford expensive orthodox medicine. This policy if pursued will not only preserve the scarce foreign exchange but also promote the spirit of plant conservation.

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