In Vitro Evaluation of Ethanolic and Aqueous Crude Extracts of Newbouldia laevis Leaves on Bacterial Isolates from Ear Infections

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Keywords: Newbouldia laevis, leaves, antibacterial activity, ear infection, extract.

Abstract. The antibacterial effects of ethanolic and aqueous crude extract of leaves of Newbouldia laevis were evaluated against pathogenic Staphylococcus aureus, Staphylococcus epidermidis, Bacillus spp, Pseudomonas aeruginosa and Escherichia coli isolated from ear infections. A concentration gradient of the ethanolic and aqueous extract (12.5 mg/ml – 100 mg/ml) was prepared and its effectiveness was tested by agar well diffusion method and nutrient broth dilution technique. The organisms tested varied in pattern of susceptibility but were more sensitive at high concentrations. The zone of inhibition of aqueous extract of Newbouldia laevis on test organisms ranged from 4.0 to 15 mm while the ethanolic extracts of Newbouldia laevis on test organisms ranged from 6.5 to 21.00 mm. The comparative susceptibility of test bacteria to Newbouldia and ciprofloxacin showed that there was a significant difference in the antibacterial activity of leaf extract and the antibiotic standard. The MIC values ranged from 12.5 mg/ml to 25 mg/ml. The extract showed a higher antibacterial activity against E. coli, Bacillus spp and S. epidermidis. The result of the study suggests that the leaf extract of N. laevis has the potential and could be used as a source for new broad spectrum antibiotics to treat ear infections caused by test organisms.

Introduction

Ear infection is one of the most common diseases occurring throughout the world. About 65–330 million people suffer from ear infection worldwide and 60% of them have significant hearing impairment [1]. If left untreated long term consequences of persistent severe ear infection can arise including speech disorder, hearing loss and lower overall quality of life [2, 3]. Many studies have reported high level of resistance pattern to different antibiotics from bacterial isolates from ear infections [4, 5]. Thus due to aforementioned reasons, it is pertinent to look for affordable and convenient alternative medicine with a view to providing better protection and treatment. Newbouldia laevis (P. Beauv) seem is a medicinal plant that belongs to Bignoniaceae family. It is popularly known as the tree of life’ and African Border Tree’. Its local Nigerian names include Aduruku in Hausa, Ogirisi in Igbo, Akoko in Yoruba language, Kontor in TIV, Ikhim in Bini and Ogiriki (Urhobo). It grows to a height of about 7–8 (up to 15) m, more usually as shrub of 2–3 m, many stemmed forming clumbs of gnarled branches [6, 7]. It is native to tropical Africa and grows on moist and well drained soils; extends from Guinea Savannas to the dense forest zone.

In Nigeria, it has been found useful as a remedy for earache, sore feet, chest pain, epilepsy and children’s convulsion [8]. Anti-bacterial activity of the leaf extract have also been studied and reported [7, 9]. As it has been asserted that Newbouldia laevis have potential activity against bacteria hence this work was undertaken at investigating the antibacterial potential of ethanolic and aqueous extracts of leaf of N. laevis against bacteria isolates from ear infections.
Materials and Methods

Collection of Plant Materials

Fresh leaves of *N. laevis* were collected in the month of June, 2016 from Calabar Street, Aba, Abia State, Nigeria. The plants were botanically identified in the herbarium section of the Department of Biology/Microbiology, Abia State Polytechnic, Aba.

Preparation of the Plant Materials

The leaves were washed with distilled water; air dried at room temperature for 2 weeks and finally grounded using a mechanical blender. The powdered sample was kept in an air tight container until required for further experiments.

Extraction Procedures

The method described by Akerele et al. [10] with slight modification was used for the extraction procedure 250g of the powdered leaf was extracted in 100ml (70%) ethanol and water respectively using maceration method for 48 hours. The crude extract was decanted and filtered using Whatman No. filter paper. The filtrate was concentrated to a semi solid residue using water bath at 75.5°C after which the solid residue was stored in an air tight bottle in a refrigerator. Different concentrations of the extracts at 100, 50 and 25 mg/ml were freshly prepared by re-dissolving the dried power in the same solvent which was used for the extraction.

Preparation of Antibiotic Dilution

The antibiotic ciprofloxacin was reconstituted by dissolving 0.5mg of powder in 100ml of distilled water so as to get a concentration of 30µg/ml. The prepared dilution of the antibiotic was used for subsequent antibacterial test and served as a positive control.

Collection of Clinical Specimens

Patients attending private and primary health care centers in Aba presenting with ear infections were enrolled for the study. Ethical clearance was gotten from ethics committee of the various health centers and permission sought from management of private hospitals used. Patients consent was first obtained before collection of specimens which were done with assistance of hospital staff. Using a sterile swab stick moistened in normal saline, the inner surfaces of the infected ear were gently swabbed and then the swabs promptly taken to the Microbiology laboratory of Abia State Polytechnic, Aba for further analysis.

Isolation and Identification of Bacteria

50 ear swab samples were cultured on MacConkey agar, blood agar and chocolate agar plates and then incubated aerobically at 37°C for 24 hours. After incubation for 24 hours, individual well separate colonies were picked and further sub cultured so as to obtain pure colonies. Pure isolates were identified using standard microbiological procedures as described by Cheesbrough [11].

Preparation of Inoculum

The standardization of culture was done according to the method of Clinical and Laboratory Standard Institute [12]. 2mm diameter colonies of the 18h culture of an organism were picked with a sterile wire loop and immersed into a sterile bottle containing Mueller Hinton Broth (Hi media) and was incubated for 5h – normal saline was added gradually to it so as to compare the turbidity to that of 0.5 McFarland standard corresponding to approximately 1.0 x 10^8 cfu/ml.
Antibacterial Test

Antibacterial activities of ethanolic and aqueous extract of *Newbouldia laevis* was carried out using agar well diffusion method. 20ml of sterilized nutrient agar was poured into pre-labeled sterile petri-dishes and was allowed to solidify. 0.1ml of the overnight culture of bacterial isolates adjusted to 0.5 McFarland standard were introduced into each plates, it was spread evenly with the aid of a spreader and then four holes of 6mm in diameter was made in each plate using a sterile cork borer. The extracts were then introduced into the hole according to concentration (100%, 50%, and 25%). The plate was allowed to stand on a flat bench for 30min to allow diffusion into the agar before incubation at 37°C for 24 hours. The experiment was done in triplicates and mean zone of inhibition was measured using meter rule. Diameters of zones of inhibition ≥ 10mm were considered active [13].

Determination of the Minimum Inhibitory Concentration (MIC)

1ml of the reconstituted crude extract at a concentration of 100 mg/ml was added to 1ml of sterile Mueller Hinton broth. 1ml of this extract concentration was transferred to another test tube and this dilution continued until an 8th test tube was reached, giving extract concentrations of 100, 50, 25, 12.5, 6.25, 3.17, 1.58 and 0.79 mg/ml in different test tubes. Then 1ml of an 18h culture of bacteria previously adjusted to 0.5 McFarland Standard (1.0 x 10^8 cfu/ml) was inoculated into each of the test tubes and the contents thoroughly mixed. The tubes were incubated at 37°C for 24 hours. The 9th test tube contained 1ml of distilled water but no extract and served as a negative control. The test tube with the lowest concentration of the extract that did not show any detectable growth was taken as the MIC [14].

Data Analysis

The results were expressed as mean standard error of mean of three measurements followed by one-way Analysis of Variance (ANOVA) using the statistical package SPSS version 20. Differences in mean (SEM) were considered at p<0.05 significant level.

Results

A total of 50 samples were collected from patients suffering from ear infections. The organisms isolated in the study were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas spp*, *Escherichia coli* and *Bacillus spp*. This is presented in Table 1. From the results all organisms were sensitive to ciprofloxacin. *Pseudomonas spp* was the most sensitive 28mm followed by *E. coli* 26mm, *S. epidermidis* 25mm, *S. aureus* 23mm and *Bacillus spp* was least sensitive with 18mm. The zones of inhibition produced using the ethanolic and aqueous extracts are presented in Table 2. Ethanol extracts produced larger zone diameters, between 6.7–21mm while the aqueous extracts had a zone diameter range 4.3–19.5mm. Table 3 shows the antibiotic susceptibility test of bacterial isolates to standard drug ciprofloxacin. The MIC of crude seed extracts are presented in Table 4.
### Table 1. Morphological and Biochemical Characteristics of Bacterial Isolates

| Isolates | Micro-morphology | Biochemical Tests | Sugar Fermentations | Probable Organism |
|----------|------------------|-------------------|---------------------|-------------------|
|          |                  |                   |                     |                   |
| 1. Cocci | + + - + - - - -  | A/- A/- A/- A/-   | S. aureus           |
| 2. Cocci | + + - + - - - -  | A/- A/- A/- A/-   | S. epidermidis      |
| 3. Rods  | - + + - + - - -  | A/G A/- A/- A/-   | Pseudomonas spp     |
| 4. Rods  | - + + + + - - +  | A/G A/G A/- A/-   | E. coli            |
| 5. Rods  | + + - - - - + +  | A/- A/G A/- A/-   | Bacillus spp        |

**KEYS:** A= acid production, G= gas production, + = present, - = absent

### Table 2. Antibacterial activity of ethanolic and aqueous extracts of *Newbouldia laevis* on test organisms

| Test Organism   | Concentration (%) | Ethanol Extract Mean Zone of Inhibition | Aqueous Extract Mean Zone of Inhibition |
|-----------------|-------------------|----------------------------------------|----------------------------------------|
| S. aureus       | 100               | 13.5 ± 0.30<sup>e</sup>                | 10.0 ± 0.17<sup>ef</sup>               |
|                 | 50                | 10.3 ± 0.00<sup>f</sup>                | 7.2 ± 0.20<sup>h</sup>                 |
|                 | 25                | 6.7 ± 0.10<sup>h</sup>                 | 4.3 ± 0.26<sup>i</sup>                 |
| S. epidermidis  | 100               | 20.1 ± 0.98<sup>ab</sup>               | 11.7 ± 0.43<sup>cd</sup>               |
|                 | 50                | 15.2 ± 0.35<sup>d</sup>                | 9.2 ± 0.00<sup>g</sup>                 |
|                 | 25                | 10.0 ± 0.79<sup>f</sup>                | 7.1 ± 0.10<sup>h</sup>                 |
| Pseudomonas spp | 100               | 15.0 ± 1.37<sup>d</sup>                | 12.6 ± 0.87<sup>bc</sup>               |
|                 | 50                | 10.0 ± 1.05<sup>f</sup>                | 9.1 ± 0.17<sup>g</sup>                 |
|                 | 25                | 8.3 ± 0.36<sup>g</sup>                 | 6.6 ± 0.00<sup>h</sup>                 |
| E. coli         | 100               | 19.5 ± 1.48<sup>b</sup>                | 16.0 ± 1.00<sup>a</sup>                |
|                 | 50                | 15.5 ± 0.87<sup>d</sup>                | 13.5 ± 0.79<sup>b</sup>                |
|                 | 25                | 10.4 ± 0.00<sup>f</sup>                | 11.0 ± 0.35<sup>de</sup>               |
| Bacillus spp    | 100               | 21.0 ± 0.95<sup>a</sup>                | 15.0 ± 0.26<sup>a</sup>                |
|                 | 50                | 17.2 ± 0.35<sup>c</sup>                | 11.3 ± 0.74<sup>cd</sup>               |
|                 | 25                | 13.1 ± 1.06<sup>e</sup>                | 8.5 ± 1.49<sup>g</sup>                 |

Key: mean with different superscript differs significantly (p < 0.05)
Table 3. Antibacterial activity of standard drug against isolated test organisms

| Antibiotic (5ug/ml) | Test Organism       | Zone of Inhibition |
|---------------------|---------------------|--------------------|
| Ciprofloxacin       | S. aureus           | 23                 |
| Ciprofloxacin       | S. epidermidis      | 25                 |
| Ciprofloxacin       | Pseudomonas spp     | 20                 |
| Ciprofloxacin       | E. coli             | 26                 |
| Ciprofloxacin       | Bacillus spp        | 28                 |

Table 4. Minimum Inhibitory Concentration (MIC) of ethanolic and aqueous leaf extract of Newbouldia laevis on test organisms

| Test Organism     | Zone of inhibition Ethanoic | Zone of inhibition Aqueous |
|-------------------|-----------------------------|-----------------------------|
| S. aureus         | 25                          | 25                          |
| S. epidermidis    | 12.5                        | 25                          |
| Pseudomonas spp   | 25                          | 25                          |
| E. coli           | 12.5                        | 25                          |
| Bacillus spp      | 12.5                        | 25                          |

Discussion

The results of this study have shown that N. laevis seem leaf extracts had varied antibacterial activity against the organisms tested. The pattern of susceptibility varied but higher efficacy was recorded at higher concentrations. The zone of inhibition produced by standard antibiotic disc (ciprofloxacin) against test organisms were found to be appreciable in relation to those activities of extracts produced by most organisms studied and it was statistically significant. This conforms to studies conducted by Usman and Osuji [7], Odunbaku and Amusa [15] and Akerele et al. [10].

The leaf extracts of N. laevis exhibited a broad spectrum activity since it was active against both gram-positive and the gram negative organisms tested. This broad spectrum activity of crude Newbouldia laevis extracts could be linked to the presence of bioactive compounds notably tannins, alkaloids, terpenoids, flavonoids, saponins, steroidal and cardiac glycosides [7, 16] which make the N. laevis plant useful for the treatment of various infectious conditions including those of the ear. Comparison of the zones of diameters produced from the ethanolic and aqueous extracts showed that ethanolic extract of N. laevis showed a higher antibacterial activity, no significant differences were observed. These results are in consonance with reports of other investigations which deployed similar extraction procedures.

The standard antibiotic ciprofloxacin demonstrated highest activity than the crude extracts. This is because the antibiotic in its pure state has undergone some refining processes that have established it as a standard antibiotic [14]. The observed difference in efficacy may also be due to the fact that extracts were in a crude form and would contain some inert substances which do not have any antibacterial activity [14].

The minimum inhibitory concentration of the test organisms ranged between 12.5mg/ml – 25mg/ml for aqueous extract. The study showed that Staphylococcus aureus, S. epidermidis and Pseudomonas spp had higher MIC values, meaning that higher concentration of the extracts are required to inhibit the growth of these bacteria while E. coli and Bacillus spp had lower MIC values and would require low concentration to inhibit their growth. The fact that the extracts produced inhibitory activities against almost all test organisms confirms the traditional therapeutic claims for these herbs to treat infections. It is therefore suggested that isolation and possible characterization of bioactive constituents from the extracts of this plant species be explored as a potential antibacterial agent.
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