Antibacterial activities of different solvent extracts of Neuboldia Levis parts on some gram positive and negative uropathogenic bacterial isolates

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ABSTRACT
Considering the assertion that medicinal plants constitute a continuous source of new compounds with potential to act against multi-resistant bacteria, this present investigates the antimicrobial activity of various extracts of the leaves and stem bark of Newbouldia laevis on human urinary tract bacteria isolates. Following standard laboratory procedures, the leaves and back extracts of Newbouldia laevis were studied on Gram positive bacteria (Staphylococcus aureus) and two strains of Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The degree of antibiotic potentials varies with the extraction solvents (water, 1%HCL, ethanolic, acetone and petroleum ether) but not with the parts of the plant. S. aureus and E.coli were more sensitive to Neuboldia levis leaves and bark than P. aeruginosa. Compared to the five extraction solvents, leaf and bark extracts of Neuboldia levis in acetone and petroleum ether showed the best antimicrobial potentials. Neuboldia levis leaf and back extracts demonstrated bactericidal potential against both gram-positive and gram-negative bacteria and could be useful in the inhibition of human urinary tract biotic bacterium.

Key words: Herbs, Neuboldia levis, Antimicrobial, Extraction solvents.

INTRODUCTION
Due to indiscriminate use of synthetic antimicrobial drugs, microorganisms resistant and or multi resistant to major class of antibiotics have emerged in recent years and the situation is exacerbated too [1,2]. Moreover, high cost and adverse side effects of popular synthetic antibiotics are major burning global issues [3]. To this regards, antibiotics resistance has resulted in morbidity and mortality while the high cost and adverse side effects have increased health care costs. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines [4,5]. In another words, increasing capability of microbes to develop multidrug resistance has no doubt encouraged search for new, safe and effective bioactive agents of plant origin considering the fact by Racio et al. [6], that traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents [6].

One of such plant that has shown great therapeutic significant is the *Newbouldia laevis* commonly known as smooth Newbouldia or boundary tree and by various languages in Nigeria as ‘Aduruku’ in Hausa, ‘Ogirisi’ in Igbo, ‘Akoko’ in Yoruba and ‘Ikhimi’ in Edo. More usually a shrub of 2-3 metres, it grows to a height of about 7 - 15 meters [7]. *Newbouldia laevis* is widely used in African folk medicine for the treatment of malaria and fever, stomach-ache, coughs, sexually transmitted diseases, tooth ache, breast cancer, and constipation [8-11]. The leaf, stem and fruits have been used for Febrifuge and wound healing [9]. In the South Eastern and part of the Midwestern Nigeria, the plant is used for the treatment of septic wounds and eye problems [12].
Considering the assertion that medicinal plants constitute a continuous source of new compounds with potential to act against multi-resistant bacteria [13], this present study is aim at investigating the antimicrobial activity of various extracts of the leaves and stem bark of *Neuboldia laevis* on human urinary tract bacteria isolates.

**MATERIALS AND METHODS**

**Processing of plant samples:** Plant materials (leaves and back of *Neuboldia levis*) were collected from in and around Ekpoma, Edo State, Nigeria and authenticated by a Botanist in the Department of Botany, Ambrose Alli University, Ekpoma. The leave and back were separately weighted in tap water, rinsed in sterile distilled water and dried for 5 days and 10 days respectively at 60° C in Lab 1 of the Department of Microbiology, Ambrose Alli University, Ekpoma. The dried leave and back of *Neuboldia levis* were separately blended to powder with a clean kitchen blender (Sonik, Japan) and stored in air tight glass containers kept in laboratory cupboard, until required for preparation.

**Preparation of extracts:** 5 grams of each *Neuboldia levis* leave and back were weighed into 100 ml reagent bottle and 95 ml of extraction solvent (water, ethanol, 1% HCl, acetone and petroleum ether) was added and left to extract on a mechanical shaker overnight at room temperature. This was done using all the five extraction solvents on the different *Neuboldia levis* leave and back.

The extract solutions were filtered aseptically into another 100 ml reagent bottle using a watt-man No 1 filter paper. All the filtrate were screened for purity by inoculation unto MacConkey agar and nutrient agar plates and incubated at 37°C for 48 hours following the methods outlined in Orhue [14]. Filtrates yielding growth of any organism was re-filtered and re-screened for purity until a sterile extract solution was obtained.

**Micro organism preparation/growth:** The test organisms used were all human pathogenic organisms of clinical origin and were isolated from urinary tract of infected patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. They include one strain of Gram positive bacteria (*Staphylococcus aureus*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). They were stored in the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria, where they were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

**Determination of Minimum Inhibitory Concentration (MIC):** Using a 50 ml specific gravity bottle, the density of the extract solution was determined. In a similar manner, the density of the plain solvent was also determined. To determine the concentration of the extract, the density of the plain was subtracted from that of the extract solution. This was done for all 5 extraction solvents for *Neuboldia levis* leave and back. With the known extract concentrations and the three clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the MIC of the extract solutions of *Neuboldia levis* leave and back and standard drugs (Peflacine and Cefuroxine) were determined. The experiments were performed in 3 repetitions for each of the extraction solvents and *Neuboldia levis* leave and back and the average was calculated.

**Data analysis:** Data were keyed into SPSS (version 16) and the average of each determined MIC was then presented in suitable table for simple descriptive statistics. The MICs of the different *Neuboldia levis* leave and back in the different extraction solutions were compared with the values of the standard antibiotic drugs.

**RESULTS**

As shown in table 1 and 2, extracts of *Neuboldia levis* leaves and bark carry in them antibacterial potentials. However, the degree of antibiotic potentials varies with the extraction solvents but not with the parts of the plant. *S. aureus* and *E. coli* were more sensitive to *Neuboldia levis* leaves and bark than *P. aeruginosa*. This was seen in the degree of their differences in the minimum inhibitory concentrations (MIC).

Compared to the five extraction solvents, Leave extracts of *Neuboldia levis* in acetone and petroleum ether showed the best antimicrobial potentials followed by the ethanolic extraction, water and lastly 1% HCL. Similar fashion of antimicrobial potency was observed for the bark extracts of *Neuboldia levis* except for *P. aeruginosa* bacterial isolate where bark extracts of water and ethanol showed no observable potency.

Though lower in antibacterial sensitivity, comparatively, the MIC of petroleum ether and acetone extracts of *Neuboldia levis* were not significantly different (P>0.05) from those of standard antimicrobial drugs. However, extracts of *Neuboldia levis* in water, ethanol and 1% HCL were statistical difference (P<0.05) in the MIC compared to the standard drugs.
The microbial growth [27,28]. Similarly, quinones are known to complex irreversibly with nucleophilic amino acids. Hence, the suppression of the growth of gram positive and negative microbes under investigation by the leaf and back extracts of Neuboldia levis is probably due to the present of phenyl propanoid glycosides, naphthoquinone and alkaloids compounds from the roots [11, 23-24]. Specifically however, while Germann et al.[25], reported the presence of alkaloids and phenylpropanoids in the root, Usman and Osuji, [15], reported flavonoids and tannins in the leaf as the phytochemical constituents. Other isolated component from Neuboldia levis include; Neouboldiaquinone, 2-acetylfuro-1,4-naphthoquinone, 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde and lapachol [24].

**DISCUSSION**

Hence, the suppression of the growth of gram positive and negative microbes under investigation by the leaf and back extracts of Neuboldia levis indicates it antimicrobial potentials and as such its beneficial role as an important source of potentially useful new compounds for the development of chemotherapeutic agents against urinary tract bacterial infections. The mechanism of action of Neuboldia levis leaf and back extracts is probably due to the present of flavonoids, alkaloids and quinines. Studies have documented the effectiveness of flavonoids against a wide range of Gram-positive bacteria [26] by forming complex with cell wall components and adhesions to prevent the microbial growth [27,28]. Similarly, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to the inactivation of proteins and loss of function [29].

Conclusively, Neuboldia levis leaf and back extracts demonstrated bactericidal potential against both gram-positive and gram-negative bacteria and as such an indication that the plant can be a source antibacterial drug. Hence, leaf and back extracts of Neuboldia levis used as medicine, could be useful in the inhibition of human urinary tract biotic bacterium.

**REFERENCES**


Table 1: MIC of extract from Neuboldia levis leaves compared with standard antibiotics

<table>
<thead>
<tr>
<th>Organisms Isolated</th>
<th>Standard anti-biotic drugs</th>
<th>Extraction Solutions from Neuboldia levis leaves VNB: Ikhimwi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perflacine</td>
<td>Cefuroxime</td>
</tr>
<tr>
<td>S. aureus,</td>
<td>4.0*</td>
<td>6.0*</td>
</tr>
<tr>
<td>E.coli,</td>
<td>4.0*</td>
<td>6.0*</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>4.0*</td>
<td>6.0*</td>
</tr>
</tbody>
</table>

* signifies statistical significant in antimicrobial activities between standard drugs and Neuboldia levis leaves extraction solutions.

Table 2: MIC of extract from Neuboldia levis Bark compared with standard antibiotics

<table>
<thead>
<tr>
<th>Organisms Isolated</th>
<th>Standard anti-biotic drugs</th>
<th>Extraction Solutions from Neuboldia levis Bark VNB: Ikhimwi</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Perflacine</td>
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<td>E.coli,</td>
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<tr>
<td>P. aeruginosa</td>
<td>4.0*</td>
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* signifies statistical significant in antimicrobial activities between standard drugs and Neuboldia levis stem back extraction solutions.

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