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Phytochemical and Antimicrobial Screening of Root of *Newbouldia laevis* (P. Beauv)

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ABSTRACT

This study investigated the phytochemical composition and antimicrobial activities of ethanol, water and n-hexane root extracts of *N. laevis* against some clinical pathogens. The ethanol, water and n-hexane extracts were prepared by soaking 20g each of the powdered root in each solvent and placed in a Soxhlet extractor. Heat was applied to a round bottom flask containing a particular solvent. The extracts were removed and placed on a water bath at a specified temperatures for evaporation of the solvents. The antimicrobial activities of the ethanol, water and n-hexane root extracts were carried out using agar well diffusion method. The phytochemi al analysis was done using standard techniques. Data were analysed using Analysis of Variance (ANOVA) to test for significance. Means were separated using Duncan's Multiple Range Test (DMRT). The results of the antimicrobial activity revealed that ethanol extract showed highest inhibition of *S. aureus* (14.00) and lowest inhibition of *P. aeruginosa* (2.00). Water extract showed highest inhibition of *E.coli* (16.50) and lowest inhibition of *A. flavus* (14.18). N-hexane showed highest inhibition of *P. aeruginosa* (13.36) and lowest inhibition of *S. typhi* (1.52). There was a significant difference in the inhibitory activities of the plant extracts against all the test organisms ($P<0.05$). The results of this study affirmed the use of the plant for medicinal purposes.

Keywords: Phytochemical, Antimicrobial, Screening, Root, *Newbouldia laevis*

INTRODUCTION

The practice of traditional medicine is as old as the origin of man [1]. The use of plants in traditional medicine referred to as herbalism or simply botanical medicine [2] falls outside the mainstream of the Western or orthodox medicine. The active ingredients for a vast number of pharmaceutical derived medication contain components originated from phytochemicals in plant. These substances that contain the healing property known as the active principles are found to differ from plant to plant. Generally, the active principles found in medicinal plants can be extracted in different forms which include infusions, syrups, concoctions, decoctions, infused oils, essential oils, ointments and creams [3]. Among these plants are the vegetables whose part(s) are eaten as supporting food or main dishes and which could be aromatic, bitter or tasteless [4]. *Newbouldia laevis* (P. Beauv) or boundary tree called variously as; "Aduruku" in Hausa, "Ogirisi" in Igbo and "Akoko" in Yoruba languages

[5] is a medium sized angiosperm which belongs to the Bignoniaceae family. It grows to a height of about 7-8 (up to 15) metres, more usually a shrub of 2-3 metres, many-stemmed forming clumps of gnarled branches [6]. *Newbouldia laevis* is native to tropical Africa and grows from Guinea Savannahs to dense forests on moist and well-drained soils. It inhabits the secondary forest extending from Senegal to Cameroon, Gabon, Democratic Republic of Congo, Angola [6]. In Nigeria, the bark is chewed and swallowed for stomach pains, diarrhea and toothache [7]. The plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile and as a vermifuge to round worms. It has also been found useful for earache, sore feet, chest pain, epilepsy and children's convulsion [8]. The leaf, stem and fruits have been used for febrifuge; wound dressing and stomach ache [9]. Earlier studies on the leaves and bark of Congolese *Newbouldia laevis* revealed the absence of flavonoids, saponins, quinones, terpenes or steroids [10]. Recent phytochemical studies on the root, root bark and stem of this plant revealed the presence of alkaloids, quinoid and phenylpropanoid among others [11]; [12]; [13]. This study investigated the phytochemical and antimicrobial screening of root of *Newbouldia laevis*.

Materials and Methods

Collection of Sample

The root of *N. laevis*, used in this study was collected from Uli, Ihiala Local Government Area, Anambra State, Nigeria. The analysis was carried out at Central Service Laboratory, National Root Crops Research Institute, Umudike, Abia State, Nigeria. The botanical identity of the plant was authenticated by a botanist, Mr. Donatus Ogbonnaya, the head of laboratory technologist of the institute.

Preparation of Sample for Analysis

The fresh root of *N. laevis* was washed with tap water and sundried for several days and ground into fine powder using grinding machine after which the ground sample was sieved to obtain powdered processed sample used for analysis.

Extraction of Plant Material

The ethanol, water and n-hexane extracts were prepared by soaking 20g each of the powdered root in each solvent and placed in a Soxhlet extractor. Heat was applied to a round bottom flask containing a particular solvent connected to the base of the Soxhlet extractor. The extraction temperatures for ethanol, water and n-hexane were 67^{0}C, 102^{0}C and 66^{0}C respectively. Heat was applied for 18 hours to complete extraction of active ingredients. After that, the extracts were removed and placed on water bath at the corresponding temperatures for evaporation of the solvents.

Qualitative Determination of Phytochemicals in the root of *N. laevis*

Qualitative tests were conducted to evaluate the presence or absence of phytochemicals of interest. It was conducted using standard methods described below. The extracts were screened for the following phytochemical compounds: alkaloids, saponins, flavonoids, tannins, terpenoids, cardiac glycosides and steroids [14].

Alkaloids Determination

The presence of alkaloid in the samples were investigated using Meyer's colourimetirc method described by [15]. Ethanolic extract of the samples were obtained by shaking 2g of the samples in 20ml of ethanol for 30mins before filtrating over the funnel using Whatman no 1 filter paper in 100ml beaker. 2.5ml of each filtrate was taken and poured in the test tube labelled A, B, C and D, placed in a test tube rack. Few drops of Meyer's reagent were added to each of the test tube respectively. Formation of orange precipitate/colour shows the presence of alkaloid.

Saponins Determination

The froth test and emulsion test described by [15] were used to determine the presence of saponin. 5ml of distilled water was used to dissolve 1g of powdered samples in 250ml conical flask. Each of them was shaken and placed in water bath for 5mins. They were filtered hot over the funnel using Whatman no 1 filter paper in 100ml beaker. 2.5ml of each cooled filtrate was poured into the test tube labelled A, B, C and D, and placed in a test tube rack. 10ml of distilled water was used to dilute each of the tube respectively.

Froth Test

Each of the flask was shaken vigorously for few minutes and observed. A stable forth (foam) upon standing indicates the presence of saponin.

Emulsion Test

Two drops of olive oil was added to the four test tubes respectively and shaken vigorously. The formation of emulsion indicates the presence of saponin.

Tannins Determination

The presence of tannins was determined using the [15] method. 1g of powdered samples were boiled with 5ml of distilled water in a water bath for 5 minutes. They were filtered hot with Whatman no 1 filter paper folded over a funnel in 100ml beakers. Four test tubes labelled A, B, C and D, was positioned in a test tube rack. 1ml of the cooled filtrates was added to each test tube accordingly. 10ml of ferric chloride was added to each of the test tube and observed. A greenish brown precipitate was observed which indicates the presence of tannin.

Flavonoids Determination

The presence of flavonoid in the samples was determined using the [15], [3] methods. 1g of powdered samples were dissolved with 10ml of distilled water in 250ml conical flask, shaken and placed in water bath for 5mins. They were filtered hot using Whatman no 1 filter paper folded over the funnel in 100ml beaker. The filtrates were allowed to cool. Two drops of 20% NaOH was added to 1ml of each of the filtrates in a test tubes labelled A, B, C and D. A yellow amber colour was observed in tubes A and D, light yellow in B and C. To each of the test tube was also added two drops of one normal sulphuric acid and observed. No colour change was observed in all the samples after addition of two drops of sulphuric acid. It implied that flavonoid is absent in the plant due to no formation of colourless solution.

Cardiac Glycoside Determination

5ml of each of the aqueous extracts was taken using 5ml syringe into the test tubes labelled A, B, C and D. 2ml of glacial acetic acid was added to each of the tube. One drop of ferric chloride was also added respectively. 1ml of concentrated sulphuric acid was added to the four tubes and observed for brownish or greenish precipitate. Flask A, C and D was found to contain cardiac glycoside as the colour being evident while flask B has clear solution.

Terpenoids Determination

5ml of aqueous extracts from each of the samples was poured into the test tube labelled A, B, C and D. 2ml of chloroform was added to each of the test tube. 1ml of concentrated sulphuric acid was also added to each of the flask to form a layer. A reddish brown precipitate at the interface indicates positive.

Steroids Determination

0.5ml extract dissolved in 3ml of chloroform was added and filtered. To the filtrate, conc. sulphuric acid was added by the sides of the test tubes which formed a layer. A reddish brown colouring with slight greenish fluorescence was taken as the presence of steroids.

Quantitative Determination of Phytochemicals in the root of *N. laevis*

Determination of Alkaloids

2g of each sample was analysed in accordance with the alkaline precipitation gravimetric method [15]. The weighed samples were soaked in 100ml of 10% acetic acid solution in ethanol and allowed to stand for 4 hours at room temperature before filtering using Whatman no 1 filter paper. The filtrates were reduced to a quarter of their original volume by evaporation over a steam bath. Alkaloids in the extracts were precipitated by drop wise addition of concentrated NH_4OH solution until full turbidity was obtained. The precipitate was recovered by filtration using weighed filter papers and then washed with 1% NH_4OH solution, dried in the oven at 100°C for an hour. They were cooled in desiccator and reweighed. By difference, the weight of alkaloids present in the samples were determined and expressed as percentage for the samples and analysed using the formula

% Alkaloid = W2-W1×100/Weight of sample

Where;

W1= weight of empty filter paper

W2= weight of paper + alkaloid precipitate

Determination of Saponins

Saponin content of the samples were determined by double solvent extraction gravimetric method [15] 2g of the powdered samples were mixed with 50ml of 20% aqueous ethanol solution. The mixtures were heated with periodic agitation in water bath for 30mins at 55°C. They were filtered, the residues were extracted with 50ml of ethanol and both extracts were put together. The combined extracts were reduced to about 40ml at 90°C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Reaction was carried out until the aqueous layer became clear. The saponins were extracted with 60ml of normal butanol. The combined extracts were washed with 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. They were dried at 60°C in the oven and reweighed. The saponin content was calculated as percentage of original sample as;

% Saponin = W2-W1×100/Weight of sample

Where;

W1= weight of evaporating dish

W2= weight of dish + sample

Determination of Tannins

The Follins – Dennis spectrophotometric [16] was used. 2g of the powdered samples were dispensed into 50ml of distilled water in a conical flask and shaken for 30mins in a shaker. The mixtures were filtered. 5ml of the filtrates were measured into 50ml volumetric flask and then diluted with 35ml of distilled water. Also, 5ml of standard tannic acid solution and 5ml of distilled water were measured with separate flasks to serve as standard and blank respectively. They were diluted with 35ml of distilled water separately. 1ml of Follins – Dennis Reagent was added to each of the flask followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was filled to mark level with distilled water and incubated for 90mins at room temperature. The

absorbance of the developed colour was measured at 76nm wavelength with the reagent blank at zero. The tannin content was calculated as shown below;
% Tannin = 100/w×AU/As×VF/VA×D
Where;
W = weight of the sample analysed
AU = absorbance of test sample
AS = concentration of standard in mg/ml
C = total volume of extract
VF = volume of filtrate analysed
D = dilution factor (where applicable)

Determination of Flavonoids

Flavonoids determination was done using Bohamand Kocipai method. 10g of the plant samples were extracted repeatedly with 100ml of 80% aqueous methanol in conical flask at room temperature. The whole solution was filtered using a weighed Whatman no 1 filter paper. The filtrates were transferred into crucible and dryness in the oven, cooled in the desiccator and weighed. The percentage flavonoid was expressed as the weight of sample analysed using the formula;
% Flavonoid = W2-W1×100/Weight of sample
Where;
W1 = weight of empty filter paper
W2 = weight of filter paper + flavonoid precipitate

Determination of Terpenoids using Ferguson (1996) method

10g of plant sample powder were taken separately and soaked in alcohol for 24hrs and filtered. The filterate was extracted with petroleum ether. The ether extract was taken as total terpenoids.

Determination of Cardiac Glycosides

5g of the sample was soaked in 100ml of distilled water. 1ml of the extract was measured into a test tube. 2mls of DNS reagent was added to the test tube in a beaker of water and boiled for 10-15 minutes and allowed to cool. 10ml of distilled water was added and absorbance was read at 540nm.

Determination of Steroids

This was determined by the method described by [2]. A known weight of each sample was dispersed in 100ml of distilled water and homogenate filtered and the filterate was eluted with normal ammonium hydroxide. 2ml of the elute was put in a test tube and mixed with 2ml of chloroform. 3ml of ice cold acetic anhydride was added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added to obtain a standard sterol solution. The absorbance of standard and prepared sample was measured in a spectrophotometer at 420nm.

Preparation of the Plant Extract

1, 2, 3, 4 and 5mg of the concentrated extracts were dissolved in 10ml of distilled water to obtain 5-serial dilutions of each of the extracts separately corresponding to 0.1, 0.2, 0.3, 0.4 and 0.5mg/ml which were used as the extracts for antibacterial/antifungal test.

Preparation of Bacterial/Fungal Suspension (Inoculum)

The following organisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Aspergillus flavus*, *Aspergillus nige*r and *Candida albican*) were cultured. The bacterial cultures were inoculated into nutrient broth and incubated for 24hrs at 37^{0}C and the growth was observed microscopically. The turbidity of the medium indicated the growth of bacterial organisms. The fungal cultures were inoculated into potato dextrose agar and allowed to incubate at 25^{0}C for 48hrs.

Antimicrobial Sensitivity Test of Crude Extracts

The well agar diffusion method was employed for the determination of antimicrobial activity of the extracts. I ml of the extracts was poured into the different agar wells using sterile micro pipettes. The plates were allowed to stand for few minutes for proper absorption/diffusion of the extracts into the agar. The plates were incubated with the base upward at 37^{0}C for 24 hours for bacteria and at 25^{0}C for 48 hours for fungi. After the incubation, the plates were removed and zone of inhibition measured using a pair of caliper and meter rule and recorded in millimeter.

Statistical Analysis

Data were analysed using analysis of variance (ANOVA) via statistical Analysis System (SPSS) of Version 21 and means were separated with Duncan's Multiple Range Test (DMRT) at $P<0.05$.

RESULTS

Qualitative Determination of Phytochemical Composition of the Root of *N. Laevis*

The results of the qualitative phytochemical analysis of the root of *N. laevis* showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids and cardiac glycosides. Tannins and saponins were moderately present in the root of *N. laevis* (Table 1).

Table 1: Qualitative phytochemical composition of root of *N. laevis*

Phytochemicals	*Root of N. laevis*
Alkaloids	+
Flavonoids	+
Tannins	++
Saponins	++
Terpenoids	+
Steroids	+
Cardiac Glycosides	+

Key: + means present; ++ means moderately present

Quantitative Determination of Phytochemical Composition of the Root of *N. Laevis*

The results of the quantitative phytochemical analysis of the root of *N. laevis* revealed that saponins had the highest composition (11.00) while the lowest was flavonoids (0.60) (Table 2).

Table 2: Quantitative phytochemical composition of root of *N. laevis*

Phytochemicals	*Root of N. laevis*
Alkaloids	4.00^{a}
Flavonoids	0.60^{b}
Tannins	10.00^{c}
Saponins	11.00^{c}
Terpenoids	1.26^{b}
Steroids	4.87^{a}
Cardiac Glycosides	3.06^{a}

Mean values with the same letter in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT)

Antimicrobial Sensitivity Test of Crude Extracts

The results of the inhibitory activity of water, ethanol and n-hexane root extracts of *N. laevis* against the test organisms at 0.5mg/ml concentration revealed that ethanol extract showed highest inhibition of S. aureus (14.00) while the lowest inhibition was observed on *P. aeruginosa* (2.00). The water extract showed highest inhibition of *E. coli* (16.50) while the lowest inhibition was noticed on *A. flavus* (14.18). The n-hexane showed highest inhibition of *P. aeruginosa* (13.36) while the lowest inhibition was observed on *S. typhi* (1.52). There was a significant difference in the inhibitory activity of the plant extracts against all the test organisms (P<0.05) (Table 3).

Table 3: Zone of inhibition (mm) of test organisms by the water, ethanol and n-hexane root extracts of *N. laevis* at 0.5mg/ml concentration

Plant Extracts	*S. typhi*	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. flavus*	*A. niger*	*C. Albican*
Water	4.88^{a}	12.62^{b}	16.50^{b}	6.40^{b}	4.18^{a}	5.44^{c}	12.58^{b}
Ethanol	12.00^{b}	14.00^{c}	10.80^{b}	2.00^{d}	7.20^{a}	8.00^{a}	9.12^{c}
N-hexane	1.52^{d}	5.32^{a}	8.16^{c}	13.36^{b}	11.29^{b}	6.62^{a}	4.32^{c}

Mean values with the same letter in the same column are not significantly different at P>0.05 using Duncan's Multiple Range Test (DMRT)

DISCUSSION

This study showed that both ethanol, water and n-hexane root extracts of N. *laevis* were able to inhibit the growth of human pathogens tested. This is probably as a result of the synergistic actions of the active components present in the plant root. According to [17], the presence of bioactive substances has been reported to confer resistance to fungi, bacteria and pests. This therefore, explains the demonstration of antifungal activities by the extracts used in this study. Thus, the antifungal properties of the plant extracts are probably due to the presence of phytochemicals which are antimicrobial agents [18], and inhibitory to the growth of these pathogens [19]. The study revealed the fungitoxic potentials of ethanol, water and n-hexane extracts of root of *N. laevis* at 0.5mg/ml concentration could be further developed to produce natural fungicides in the control of pathogenic microorganisms that cause damage to agricultural crops.

CONCLUSION

This study has confirmed that the ethanol, water and n-hexane root extracts of *N. laevis* were able to inhibit the growth of test organisms such as *S. typhi*, *S. aureus*, *E. coli*, *P. aeruginosa*, *A. flavus*, *A. niger*, and *C. albican*. It is recommended that the root extracts of *N. laevis* be used as natural antibiotic in place of the chemically combined ones. This will reduce the cost and allergy often associated with synthetic drugs.

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