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Phytochemical and Antibacterial Activities of *Newbouldia laevis* leaves (*Ogirishi*) on two Drug Resistant Bacteria

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ABSTRACT

This present study was aimed to investigate the phytochemical and antimicrobial activities of aqueous and methanolic extracts of Newbouldia laevis (Ogirishi) on *Staphylococcus aureus* and *Escherichia coli*. The phytochemical constituents of this medicinal plant were carried out using standard methods. Agar dilution method was used to determine the antibacterial activity of the plant extracts. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extracts on the test isolates were determined by the agar dilution method. Phytochemical analysis shows the presence of Phenols, flavonoids, glycosides, tanins, oxalate, terpenoids, anthraquinolones, alkaloids and tanins in both methanolic and aqueous extracts of *N. laevis*. The antibacterial activities of aqueous and methanolic leave extract of *Newbouldia laevis* shows that the mean zone diameter of inhibition for *S. aureus* on the different extracts was between the range of 9mm to 22mm while that of *E. coli* was between 8mm to 24mm. Result from this work shows a greater zone of inhibition produced by the methanolic extracts of *N. laevis* at all concentrations used compared to that produced by the positive control drug gentamicin. The MIC of different extracts of *S. aureus* was between 6.25mg/ml to 25mg/ml while that of *E. coli* was between 6.25mg/ml to 25mg/ml. The MBC of different extracts of *S. aureus* isolates was between the ranges of 50 to 100mg/ml while that of *E. coli* was also between the ranges of 50 to 100mg/ml. In conclusion, the results obtained in this work indicate that the leave extracts of *N. Laevis* possesses natural potential to inhibit the growth of *S. aureus* and *E. coli*. The observed antibacterial effects may due to the presence of secondary metabolites in the plant. Our findings justify the claim that the leave extracts of *N. Laevis* could have antibacterial properties against the stated organisms.

Keywords: *Newbouldia laevis*, Methanolic, Aqueous, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. [1] Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent. [2,1] Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. [3]

Newbouldia laevis - one of the plants with magical effects, which, is commonly, called boundary tree or 'Tree of Life.'[4] *N. laevis*is a medium sized angiosperm which belongs to the Bignoniaceae family and grows to a height of about 7-8 (up to 15) meters [5] (Usman and Osuji, 2007). It is called

'Aduruku' in Hausa;'Ogirisi' in Igbo; 'Ikhimi' in Edo and 'Akoko' in Yoruba languages. [6]

The plant is often grown as an ornamental, has shiny dark green leaves, bears large showy terminal purple flowers and easily propagated by cutting. It is more or less a sacred or symbolic tree, well planted as a fence and often permitted to grow into a stockade. [7]

Scientifically, *N. laevis* has been reported to have medicinal value ranging from anti-inflammatory, antioxidant, anti-microbial, anti-fungi, analgesic and wound healing properties. [8,9,10] Specifically, the stem bark mixed with clay and red pepper has been reported to be effective against pneumonia, fever, cold, cough and for treating different illness like bone lesions. [4] *N. 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 [11] (Arbonnier, 2004). In South Eastern part and part of the Mid-western Nigeria, the plant is used for the treatment of septic wounds and eye problems according to [5]**.** Scientific reports on the phytochemical constituents of the plant revealed the presence of alkaloids and phenylpropanoids in the root, flavonoids, and tannins in the leaf as revealed by [12]**.** In view of this, the present study is designed to analyze the phytochemical content and to study the antibacterial activity of the aqueous and methanol extracts of *Newbouldia laevis* leaf on two drug resistant bacteria.

MATERIALS AND METHODS

Collection and Identification of Test Plant

The plant *Newbouldia laevis* leaf was harvested from a farm in Ihitte, Ezinihitte Mbaise Imo State, Nigeria. The plant was authenticated at Nnamdi Azikiwe University Awka Herbarium by a Botanist, Mr. Tochukwu Egboka.

Plants Extraction

Preparation of extracts

The fresh leaf of *N. laevis* harvested was washed with distilled water to remove dust and other foreign particles. The leaf was then left to air dry at room temperature on a clean surface until well dried and ground into fine powder using a blender. Exactly 200 g of *N. laevis* was measured into a container and soaked with methanol. The mixture was allowed to stand for about 72 hours with intermittent stirring. This was followed by repeated filtration using sterile muslin cloth, non-absorbent cotton wool and Whatman filter paper No.1, in order to remove the marc. The filtrates were concentrated to dryness (semi solid) in vacuo at 40°C using a rotary evaporator (Bibby Sterlin Ltd, England, and RE. 2000). The percentage yield of each extract was determined by comparing the weight of the yield and the initial weight of the powder extracted. The extract obtained was preserved in the refrigerator at a temperature of 4°C prior to use.

Test organisms

Bacterial cultures of *Escherichia coli and Staphylococcus aureus* obtained from the laboratory section of the Department of Microbiology, Nnamdi Azikiwe University, Anambra State, Nigeria; were used as antimicrobial test organisms. Their identity was confirmed using cultural, morphological and biochemical test as previously described. [13] The bacterial isolates were maintained on nutrient agar slants at 4°C.

Biochemical Identification of the Test Organism

Escherichia coli

The *E. coli* was placed on Eosine Methylene Blue agar for 18 hours. Colonies with green metallic sheen were observed which indicate a positive result for *E. coli.* [14]

Staphylococcus aureus

The *S. aureus* was placed on Mannitol Salt Agar (MSA) for 18 hours. Smooth circular colonies with yellow colour indicate a positive result for *S. aureus.* [14]

Standardization of the Tests Organism

The test organisms (*E coli and S aureus*) were standardized by the use of 24 hours old broth cultures prepared by inoculating the test organism into 5 ml of nutrient broth and the culture was adjusted to obtain 0.5 McFarland turbidity equivalent standards. [13]

Preparation of plant material and plant extracts

Two different extracts namely aqueous and methanolic were used for plant.

Preparation of Aqueous extract

Ten grams of dried grinded leave powder was dissolved in 100 ml of distilled water for 24 hours. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was concentrated by drying at 37°C and stored at 4°C.

Preparation of methanolic extract

Ten grams of dried grinded leave powder was dissolved in 100 ml of 95% methanol for 24 hours. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off the solvent and stored at 4°C.

Extract Dilution

After preparation of the extract as described, the aqueous and the methanolic extract were reconstituted using sterile distilled H_20 to obtain concentrations of 200, 150, 100, 50, 12.5, 6.25 and 3.13 mg/ml.

Sterility test of leave extract

The leave extracts (aqueous and ethanolic) was tested for growth of contaminants. One milliliter (1ml) of standard leave extract was inoculated aseptically unto Nutrient Agar and incubated at 37^oC for 24hrs. The plates were observed for any sign of visible growth. No growth on the plates indicated/signified that the extracts were sterile.

Phytochemical Analysis (Tests)

These were carried out using standard methods. [15,16,17]

Test for alkaloids

0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. 3 ml of the filtrate was divided into three. To the first 1 ml few drops of freshly prepared Dragendoff's reagent was added. To the second, 1 drop of Meyer's reagent was added. To the third, 1 ml of Wagner's reagent was added and observed.

Test for flavonoids

Ferric chloride test

To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed. NaOH test. Some portion of the extract was dissolved in 10% aqueous NaOH solution, dilute HCl was added and observed.

Test for saponins

0.5 g of the extract was shaken with distilled water in a test tube. It was allowed to stand for 10 minutes and observed.

Test for tannins

Lead sub-acetate test

To a small portion of the extract, distilled water was added. 3-5 drops of lead acetate solution were added and observed.

Test for phenols

Ferric chloride test

To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

Test for glycosides

Legal's test

To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed.

Ferric chloride test

To a small portion of the extract, distilled water was added. A drop of ferric chloride

was added to a solution of the extract and observed.

Antibacterial Assay

The antibacterial assay of the plant leave extracts were carried out on the test isolates using Agar-well diffusion Technique. The isolates were inoculated on the surface of freshly gelled sterile nutrient agar plates by streaking using sterile swab stick. Wells were aseptically bored on each agar plate using a sterile cork borer (6mm) and wells were properly labelled. Fixed volumes (0.1 ml) of different concentrations of the extracts (aqueous and methanol) were then introduced into the wells in the plates respectively. The last two wells were used as positive control well (filled with Gentamicin) and a negative control well (filled with sterile water) respectively. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract to occur and then incubated at 37^{o}C for 24 hours. The resulting zone diameter of inhibition was measured using a transparent ruler calibrated in millimetres. The readings were taken to be the zone diameter of inhibition of the bacterial isolate in question at that particular concentration. [13]

Minimum Inhibitory Concentration

MIC was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The concentrations were determined as earlier described by [18] Volleková *et al.*, (2001) with some modification by. [5] The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, the micro-organisms were prepared using the broth dilution technique. The stock extract concentration of 100 mg/ml was made by dissolving 1 g of the extract in 10 ml of sterile distilled water and the working concentrations prepared by two-fold serial dilution technique that ranged from 0.195 mg/ml to 50 mg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 24 h. incubation at 37^{o}C, the tubes were observed for turbidity. The lowest concentrations where no turbidity were observed was determined and noted. [5]

Minimum Bactericidal Concentration

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously [18,5] by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37^{o}C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration.

Mode of action of the extracts

All plates showing no visible growth on the nutrient agar (NA) indicated bactericidal effect of the concentration of the extract used. Plates showing light growth indicated the bacteriostatic effects of the extract concentration. Concentrations of the extracts showing moderate and heavy growth were considered to have no inhibitory effect on the organism. [5]

Control

Gentamicin and distilled water were used as positive and negative control respectively.

Statistical Analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. The statistical tool employed was one-way Analysis of Variance (ANOVA) to determine if there was any significance among the solutions. Statistical significance tests included the use of *p-value* to assess for the role of chance. In this study, p-value = 0.05 was used to disapprove the null hypothesis

RESULTS

The phytochemical analysis is found on table 1. Phenols, flavonoids, glycosides, tanins, oxalate, terpenoids, anthraquinolones, alkaloids and tanins were present in both methanolic and aqueous extracts of *N. laevis*.

Table 1: Phytochemical composition of *Newbouldia laevis*

Phytochemical components,	ME,	AE,
Phenols	+	+
Flavonoids	+	+
Glycosides	+	+
Tanins	+	+
Saponins	+	+
Alkaloids	+	-
Anthraquinolones	+	+
Oxalate	+	+
Terpenoids	+	+

KEY: - = Absence ME = Methanolic extract
+ = present AE = Aqueous extract

The antibacterial activities of aqueous and methanolic leave extract of *Newbouldia laevis on S. aureus and E. coli* is found on table 2. The mean zone diameter of inhibition for *S. aureus* on the different extracts was between the ranges of 9mm to 22mm while that of *E. coli* was between 8mm to 24mm.

Table 2: Antibacterial activities of aqueous and methanolic leave extract of *Newbouldia laevis* on *S. aureus* and *E. coli*

Isolates	Mean zone diameter of inhibition (mm)						Extracts
S.aureus	18	15	12	9	18	0	AE
S.aureus	22	20	17	11	18	0	ME
E. coli	20	17	13	8	15	0	AE
E. coli	24	22	20	17	15	0	ME
	200	150	100	50	+c	-c	

Key: AE = Aqueous Extract ME = Methanolic Extract
+C = Positive control (Gentamicin)
-C = Negative control (Sterile water)

The MIC of leave extracts of *Newbouldia laevis* on *S. aureus and E. coli* is found on table 3. The MIC of different extracts of *S. aureus* was between 6.25mg/ml to 25mg/ml while that of *E. coli* was also between 6.25mg/ml to 25mg/ml.

Table 3: Minimum Inhibitory Concentration (MIC) of *Newbouldia laevis on S. aureus and E. coli*

	Concentration of Extracts(mg/ml)									
Isolates	200	150	100	50	25	12.5	6.25	3.13	Extracts	MIC
S.aureus	-	-	-	-	-	+	+	+	AE	25
S.aureus	-	-	-	-	-	-	-	+	ME	6.25
E. coli	-	-	-	-	-	+	+	+	AE	25
E. coli	-	-	+	-	-	-	-	+	ME	6.25

KEY:
Key: AE = Aqueous Extract
ME = Methanolic Extract

The MBC of leave extracts of *Newbouldia laevis* on *S. aureus* and *E. coli* are found on table 4. The MBC of different extracts of *S. aureus* isolates was between the ranges of 50 to 100mg/ml while that of *E. coli* was also between the ranges of 50 to 100mg/ml.

Table 4: Minimum Bactericidal Concentration (MBC) of *Newbouldia laevis* leaves *on S. aureus and E. coli*

	Concentration of Extracts(mg/ml)									
Isolates	200	150	100	50	25	12.5	6.25	3.13	Extracts	MBC
S. aureus	-	-	-	+	+	+	++	++	AE	100
S. aureus	-	-	-	-	+	+	++	++	ME	50
E. coli	-	-	-	+	+	++	++	++	AE	100
E. coli	-	-	-	-	+	++	++	++	ME	50

KEY:
Key: AE = Aqueous Extract
ME = Methanolic Extract

DISCUSSION

The preliminary phytochemical analysis revealed the presence of steroids, glycosides, saponin, terpenoids, alkaloids, flavonoids and tannins in both the aqueous and methanolic leave extracts. The presence of tannins, terpenoids, flavonoids, steroids and cardiac glycosides in the leaf extract was in line with the reports of [5] and [19] Several available literature reports are discordant on the phytochemical composition of the plant. [5] did not detect the presence of alkaloids and saponins in their study while Dandjesso [20] reported the absence of alkaloids, flavonoids, saponins and steroids on the leaf extract. Ejele [21] reported in their work the absence of flavonoids and steroids while Akerele[10] reported the presence of saponins and steroids in the stem extract but was not detected in this study. Differences in these reports could be due to environmental factors, time of collection and handling. [10]

Phytochemicals are secondary plants metabolites responsible for many observed bioactivity of plant extracts. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory and anti-sickling activities. [22] The presence of those metabolites no doubt is indication of the potential medicinal usefulness of the plant extracts.

Saponin has been shown to have immense significance as anti-hypercholesterol, hypotensive and cardiac depressant properties. [23] Presence of tannins as shown in the result suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent. [24] Flavonoids have been shown to have antibacterial, anti - inflammatory, anti-allergic and antiviral antineoplastic activity. [25] Many of these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chellators. [26] Steroidal com-pounds are of importance in pharmaceuticals because of their relationship with compounds used as sex hormones. [27] The terpenoids have also been shown to decrease blood sugar level in animal studies. [28] Glycosides may be crucial in the transduction of intracellular signals mediated by neurotransmitters, hormones, and neuromodulators receptors, [29] activated by certain biological enzymes through hydrolysis, resulting in the separation of the sugar portion. When activated, these molecules can act on different intracellular targets (glycoside-linked signal transduction proteins). [30]

The susceptibility exhibited by the microorganisms to the extracts may be attributed to the presence of different groups of constituents that may be acting synergistically with one another. Partitioning plant extracts in two immiscible solvents enables the separation of the plant active constituents with the eventual aim of locating where the active constituents could be residing. [31] In this work, the results obtained show that the constituents of the extract were responsible for the observed antibacterial activities and it is important to note that, for some of the organisms, the inhibitory effects became more pronounced for the aqueous extract. The overall observed antibacterial activities of the extracts could be traced to the presence of the secondary metabolites like tannins, alkaloids, flavonoids reported present in the plant material. [32]

Result from this work shows a greater zone of inhibition produced by the methanolic extracts of *N. laevis* at all concentrations used compared to that produced by the positive control drug gentamicin. This indicates the possibility that *N. laevis* could serve as a better and alternative drug to treat infections caused by *S. aureus* and *E. coli* compared to most conventional antibiotics used presently in the world. Similar result was discovered in the work of. [32]

The extract showed remarkable activities against both the Gram-negative (*E. coli*) and Gram positive bacteria (*S. aureus*). The Gram-negative bacterial organisms are generally regarded more difficult to inhibit due to the presence of a thick murein layer that tends to prevent the entry of inhibitors. [31,32] It is remarkable to note that *Staphylococci* which have a record of developing resistance rapidly and successfully to antibiotics Kloos [33] were the most susceptible to the methanol leave extract. The *E. coli* and *S. aureus* species which earlier exhibited resistance to the extracts were observed to have MIC and MBC between 6.25 to 25mg/ml and 50 to 100mg/ml respectively. These indicated that the concentrations used earlier were grossly ineffective in subduing the growth of the organisms. Also, based on these higher MIC and MBC, the Gram negatives can be said to be the most resistant to the extract.

CONCLUSION

The results obtained in this work indicate that the leave extract of *N. Laevis* possesses natural potential to inhibit the growth of *S. aureus and E. coli*. The observed antibacterial effects may due to the

presence of secondary metabolites in the plant. Also, result from this work shows a greater zone of inhibition produced by the methanolic extracts of *N. laevis* at all concentrations used compared to that produced by the positive control drug gentamicin. Our findings justify the claim that the leave extracts of *N. laevis* could have antibacterial properties against the stated organisms.

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