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Antibacterial Effect of *Newbouldia laevis* Leaf Extract on Vancomycin and Methicillin Resistant Bacterial Isolates from Federal Medical Center, Owo

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study and wrote the protocol. Author OAF performed the statistical analysis and wrote the first draft of the manuscript. Authors OAF and IIA managed the analyses of the study. Author POA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Antibacterial effect of Methanol extract of *Newbouldia laevis* {Tree of life} leaf on vancomycin and methicilin resistant bacteria isolates obtained from urine samples from Federal Medical Center, Owo was assayed. The leaves of *N. laevis* was collected from Ikole Ekiti and authenticated in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The bacterial isolated from the samples namely: *E. coli*, *K. pnuemoniae*, *P. aeruginosa*, *S. aureus* and *P. mirabilis* were subjected to antibiotic sensitivity tests using disc diffusion method. Methanol extract of *N. laevis* was obtained using the cold extraction method. Phytochemical screening of the extract was assayed and inhibitory activity of the extract against the isolated bacteria was carried out using agar well diffusion technique. The concentration of 500 mg/ml was used and the zones of inhibition recorded. The isolates were resistant to vancomycin and methicilin. The extract revealed the presence of flavonoids, tannins, terpenes, alkanoids, phenolics, saponin and cardiac glycosides. Antibacterial activity revealed that Methanol extract exhibited highest potency against

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K. pnuemoniae with 29.03±0.01f and the least activity against *E. coli* with 22.90±0.17a. This study has shown that, isolated bacteria were resistant to vancomycin and methicillin. The phytochemical investigation of *N. laevis* leaf extracts revealed the presence of constituents which could be the basis for their medicinal potency against methicillin and vancomycin resistant organisms.

Keywords: Vancomycin; methicilin; phytochemical; resistance.

1. INTRODUCTION

The wide spread of diseases of microbial origin remains alarming, very high and particular of serious concerns in the tropics and sub-tropics. Microbial infections can lead to serious and lethal complications. The high cost of newer and effective drugs and other factors, has made the choice of traditional herbal remedies against these infections unavoidable and more economical [1]. Nosocomial infection is an infection whose development is favored by hospital environment. It may develop in a hospitalized patient without having been present or incubating at the time of admission, or it may be acquired in hospital but only appears after discharge. Since ancient times, plants have been found to be an essential source of natural products for maintaining human health. There are numerous compounds in plants that have efficient effects on animals and posses' high therapeutic properties, which can be utilized in the treatment and care of human and other animal diseases [2]. Modern day medicine at the turn of the millennium has been characterized by breath-taking advances that are revolutionizing the practice of medicine. However, invasive devices—especially intravascular devices of all types and urinary catheters—continue to be essential for the management of critically ill patients. Permitting lifesaving treatment and physiologic monitoring, these ubiquitous devices, unfortunately, are a major cause of institutionally acquired infections, especially in intensive care units. These infections have fueled the advancing crisis in antibiotic resistance and have major economic consequences in the form of extended length of hospital stay and attributable mortality.

The risk of device-related infection, more than anything else, has forced medicine to accept the necessity for nosocomial infection control. In general, invasive devices of all types are far more important in determining susceptibility to nosocomial infection than are underlying diseases.

The emergence and spread of microbes that are resistant to cheap and effective first-choice drugs has become a common occurrence. The problem is even more evident in bacterial infections which contribute most to the global infectious disease burden such as diarrheal, respiratory tract, meningitis, sexually transmitted infections, and tuberculosis [3].

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. Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent [4][5]. 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 [6].

1.1 Geographical Distribution of *Newbouldia laevis*

N. laevis - one of the plants with magical effects, which, is commonly, called 'Tree of Life' [7]. *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 [8]. 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 [9].

1.2 Medicinal Uses of *N. laevis*

Scientifically, *N. laevis* has been reported to have medicinal value ranging from anti inflammatory, antioxidant, anti-microbial, anti-fungi, analgestic and wound healing properties [10,11,12,13]. 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 [7].

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 [13]. In South Eastern and part of the Midwestern Nigeria, the plant is used for the treatment of septic wounds and eye problems according to [8]. 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 [14].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Test Plant

The plant *Newbouldia laevis* leaf was harvested from Ikole Ekiti, Ekiti State, Nigeria. The plant was authenticated at the Crop, Soil and Pest Department, Federal Univerrsity of Technology, Akure, Ondo.

2.2 Sources of Microorganisms Used

Following receipt of permission from the authorities of the Federal Medical Centre Owo, Nigeria, urine samples were obtained from hospitalized patients. All the bacterial organisms isolated were identified using conventional biochemical techniques [15]. The bacterial isolates were maintained on nutrient agar slants at 4°C and subcultured at intervals of three weeks to ensure their viability and purity.

2.3 Phytochemical Analysis (Tests)

These were carried out using standard methods [16,17,8].

2.3.1 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.

2.3.2 Test for flavonoids

2.3.2.1 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.

2.3.3 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.

2.3.4 Test for tannins

2.3.4.1 Lead sub-acetate test

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

2.3.5 Test for phenols

2.3.5.1 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.

2.3.6 Test for glycosides

2.3.6.1 Legal's test

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

2.3.6.2 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.

2.4 Plants Extraction

2.4.1 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.

2.4.2 Antibacterial assay or sensitivity

The pour plate method described by [18] was adopted for this test. About 1 ml of Nutrient broth containing the test organism was introduced into sterile petri dish, and sterile Mueller Hinton which has cooled to about 45°C was poured on it. The plate was gently agitated and the agar was allowed to solidify. Afterwards, wells were dug in the plates with the aid of a sterile cork borer of 8 mm in diameter. The plates were inoculated with standardized innocula (1.5x108 cells/mL) and 0.15 ml of the plant extracts were dispensed into each well as described by [19]. The extract was allowed to diffuse into the medium for one hour at room temperature. This was then incubated at 37°C for 24 hours after which the zones of growth inhibition were measured and recorded in millimeter. The control was set up in a similar manner with 30% DMSO and commercial antibiotic (ciprofloxacin) respectively.

2.4.3 Antibiotic sensitivity test

Discs were impregnated with the crude extract at the concentration of 5 mg/ml, these were aseptically mounted on a seeded agar and thereafter incubated at 37°C for 24 h. The inhibition zone was observed and then recorded in millimeters using a transparent metre rule. The tests were conducted in triplicates and results presented as mean ± SEM. Standard antimicrobial disc used were Amoxicillin (30 µg), Cotrimazole, Tetracycline, Vancomycin, Methicillin, Ofloxacin (5 µg) and Cloxacillin (5 µg).

2.4.4 Purification of extract using column chromatography

The purification of the extract was carried out using column chromatography as described by [20,21]. Petroleum ether, chloroform and methanol in ratio 3:1:1 v/v was used as eluting solvent. The column was packed with silica gel (60-120 mesh). The sample to be separated was then added on top of the packed column and eluted with the solvent at the flow rate of a drop per 2 seconds. A collecting conical flask was placed at the bottom of the column to collect the eluted fractions. The collected elute was distilled, leaving the purified fractions. The fractions obtained were reconstituted with 30% DMSO and spotted on TLC plates. Fractions with the same retention factor (R_f) were pooled together.

2.4.5 Plasmid isolation and profiling

Plasmid isolation was done using a commercial plasmid isolation kit (Plasmid Miniprep Kit, Zymogen Co. Ltd. UK) according to the manufacturer instructions. Genomic DNA of the bacterial isolates was extracted using Thermo Scientific GeneJET Plasmid Miniprep Kit method as described by [22,23]. Broth cultures of the bacterial isolates (2.0 ml) were centrifuged at 14,000 rpm at 25°C for 1 min to pellet the cells. RNase A was added to resuspension solution. The supernatant was carefully discarded and the pellets were re-suspended in 250 µl of Resuspension Solution containing RNase A. The cell suspensions were transferred into a microcentrifuge tube and vortexed completely. 250 µL of lysis solution was added, mixed thoroughly by inverting the tubes 4-6 times until the solution becomes viscous and slightly clear. A 350 µL of neutralizing solution was added and mixed immediately. The tube was centrifuged for 5 minutes to pellet cell debris and chromosomal DNA. The supernatant was transferred to the supplied GeneJET Spin Column by decanting. This was centrifuged for 1 minute. Flow-through was discarded and the column was placed back into the same collection tube. Wash solution of 500 µL was added and centrifuge for 30-60 seconds and flow-through was discarded. The wash procedure was repeated using 500 µL of the Wash Solution. The flow-through was discarded and centrifuge for an additional 1 minute to remove residual ethanol in plasmid preps. The GeneJET Spin Column was transferred into a fresh 1.5 mL microcentrifuge tube. Elution buffer of 50 µL was added to the centre of the GeneJET spin column membrane to elute the plasmid DNA. It was incubated for 2 minutes at room temperature and centrifuge for 2 minutes. The DNA was electrophoresed on 1% agarose gel containing 0.5 µg/ml ethidium bromide. The DNA was visualized by UV transilluminator and photographed.

Plasmid DNA was isolated by the method of [24]. Isolation was carried out from mini preparations. Cells pellet from 1.5 ml O/N culture was collected by centrifugation and dissolved in 50 mM Glucose, 25 mM Tris-HCl and 10 mM EDTA that

were used for cell wall disruption. 0.2 N NaOH, 1% SDS were used as lysis buffer to lyse cell wall and potassium acetate and glacial acetic acid were used to stop the reaction. Supernatant was collected by centrifugation and extracted with phenol: chloroform: isoamyl alcohol (25:24:1) followed, by a precipitation with double volume absolute ethanol. The pellet was dried in vacuum drier and dissolved in 40 µL of TE.

2.4.6 Data analysis

Data were subjected to one way analysis of variance and was used to analyze data. $P< 0.05$ was considered significant difference between means (New Duncan's Multiple Range Test).

3. RESULTS

Table 1 shows the morphological and biochemical characterization of the isolated Bacteria from urine samples. In the urine samples, six organisms were isolated. *E. coli* had the highest percentage of occurrence 38.03% followed by *K. pneumoniae* with 23.31%, *P. aeruginosa* with 19.01%, *S. aureus* with 11.04% and *Proteus sp* with 8.5%. Biochemical tests showed that four out of the six isolated organisms were Gram-negative, while two were Gram-positive shown on Table 1. Table 2 shows the qualitative analysis of the methanolic extract of *N. laevis* and this revealed the presence of flavonoids, tannins, terpenes, steroidal and cardiac glycosides; alkaloids and saponins were found to be present. The antibacterial screening presented in Tables 3 shows the susceptibility of the Vancomycin and Methicillin resistant isolates to the extracts. This exhibited considerable level of inhibition against all the test organisms at 500 mg/ml with the highest activity on *Klebsiella* with 29.03 ± 0.01^{b} zones of inhibition. The positive control, ciprofloxacin had the highest activity against *P. aeruginosa* (37.00 ± 0.30^{a}). Table 4 shows antibacterial activity of purified extracts of *N. laevis* on the isolated pathogenic organisms, using different fractions of methanol. The highest activity was shown by fractions 3 and 4 of methanol extract; (30.01 ± 0.01, 31.11 ± 0.01) respectively. The lowest activity was against *P. aeruginosa* (22.96 ± 0.33^{a}). Table 5 shows Pre and Post sensitivity curing of multiple antibiotics resistant bacteria isolate from urinary tracts patients. Antibiotics sensitivity test were carried out on the bacterial isolates from urine samples using conventional sensitivity discs containing vancomycin (30 mg) (VAN), methicillin (MET) (30 µg), Cotrimoxazole (25 µg) (COT), Ampiclox (APX) (25 µg), Ciprofloxacin (CPX) (25 µg), Tetracycline (30 µg) (TET), Amoxacillin (30 µg) (AMC) and Ofloxacin (5 µg) (OF), CIP (25 µg).

4. DISSCUSION

Table 1 shows the morphological and biochemical characterization of the isolated bacteria from urine samples. The pathogens isolated; *E. coli* had the highest occurrence followed by *K. pneumonia, P. aeruginosa, S. sp* and *Proteus mirabilis* respectively. This is in agreement with the study of [25] that stated that, 80% of urinary tract infections are caused by *E. coli* followed by *Klebsiella* sp (11.7%) and also in accordance with the report of [26] who reported *E. coli* as the most common isolated organism from urine and *Klebsiella* as the second most common pathogen.

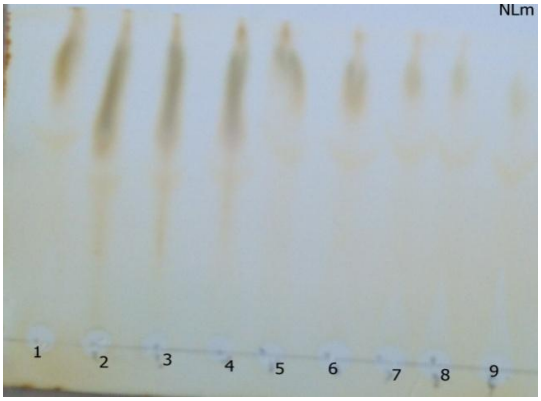

Plate 1. Developed TLC plate showing spotted fractions from methanol extract
Key: NLm = N. laevis methanol extract

Plate 2. Photograph showing plasmid profile of some multiple antibiotics resistant bacterial isolates from patients with urine infections

Table 1. Morphological and biochemical characterization of isolates

ORG	COL	GRA	SH	CAT	MR	MOT	COA	VP	OXI	IN	CI	SUC	GLU	LAC	MAL	GAS
E.coli	circular	-ve	Short rod	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
S. aureus	round	+ve	Cocci	+ ve	-ve	-ve	+ve	+ve	-ve	-ve	D	+ve	+ve	+ve	+ve	-ve
P.mirabilis	spiral	-ve	Rod	+ve	+ve	+ve	-ve	[-]	-ve	+ve	D	-ve	+ve	-ve	+ve	+ve
K. pneumoniae	raised	-ve	Rod	-ve	[-]	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
S.pyogenes	raised	+ve	Cocci	- ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
P.aeruginosa	flat	-ve	Rod	+ve	-ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve

Key: -ve = negative, +ve = positive

ORG: Organism; SHA: Shape; SUC: Sucrose; MAL: Maltose; COL: Colony; AT: Catalase; GLU: Glucose; GAS: Gas; GRA: Gram reaction; MOT: Motility; OA: Coagulase; LAC: Lactose; OXI: Oxidase; IND: Indole; D: delay; MR: Methy-red; VP: Vogues Proskauer; CI: Citrate

Table 2.The qualitative phytochemical constituent of *N. laevis* leaf extracts

Constituents	Cold	Methanol
Alkanoids	+	+
Flavonoid	+	+
Phenolics	+	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+
Cardic Glycoside	+	+

+= presence; - = absence

The phytochemical screening of the methanolic extract of *N. laevis* revealed the presence of flavonoids, tannins, terpenes, steroidal cardiac glycosides; alkaloids and saponins. This slightly varies from the study of [8] who reported the absence of alkaloids and saponins in methanolic extract of *N. laevis* and Table 7 shows the appearance of methanol extract on TLC pre-coated plate. This may be due to environmental differences. These classes of compound are known to show curative activity against several pathogens and therefore could explain its use traditionally for the treatment of wide array of illnesses according to [27]. In this study, urinary tract infections (UTI) were more in females than males; this may be due to shortness of the female urethra and its proximity to the anus. Several reports show that females are more prone to having UTI than their males' counterpart as also shown by [28,29,30,26,31]. This possibility to develop UTI more in females than in male could be explained on the basis of certain behavioral factors such as delay micturition (urination), sexual activity, the use of diaphragms and spermicides. Also the length of the urethra, the dried environment surrounding the meatus, and the antibacterial properties of prostatic fluid contribute to a lower rate of infection in males according to [26].

The result of antibacterial screening showed considerable level of inhibition against all the test organisms at 500 mg/ml concentration with highest zone of inhibition recorded by *Klebsiella* (29.03+0.01f) while the lowest shown by *E. coli* (22.90+0.17a). Studies showed that, *Klebsiella* causes different health care-associated infections including pneumonia, blood stream infections, wound or surgical site infections and meningitis. Earlier research by Usman and Osuji using methanol extract reported the same trend. Zones of inhibition with diameters ≥10mm were considered active and this shows the plant has a strong medicinal potential. This is in agreement with [8].

The result of the antibacterial activity of purified methanol extract is shown on Table 4, with the highest inhibition shown by fraction 3(F3) against *Klebsiella* with zone of inhibition (30.01 ± 0.01^{f}) and the lowest against *E. coli* with (22.99 ± 0.17^{a}).

Table 4 shows the result of the antibacterial activity of purified methanol extract against the test organisms, with the highest activity against *K. pnuemoniae* (31.11 ± 0.01^{f}). This result obtained showed higher potency than the antibacterial effect of vancomycin and methicillin obtained after curing of multiple antibiotics resistant bacteria isolate from urinary tracts patients. The shown on Table 5. The

Table 3. Antibacterial activity of *N. laevis* at 500 mg/ml on isolates zone of inhibition (mm)

Pathogen	ME	VAN 30 µg	MET 30 µg	CIP 25 µg	DMSO 30%
KB	29.03 ± 0.01^{f}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	36.0 ± 0.3^{e}	0.00 ± 0.00^{a}
PA	25.86 ± 0.32^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	37.0 ± 0.3^{e}	0.00 ± 0.00^{a}
PM	27.93 ± 0.11^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	23.0 ± 0.3^{b}	0.00 ± 0.00^{a}
EC	22.90 ± 0.17^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	30.0 ± 0.3^{d}	0.00 ± 0.00^{a}
SA	27.00 ± 0.50^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	28.0 ± 0.3^{d}	0.00 ± 0.00^{a}
SP	24.03 ± 0.95^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	31.0 ± 0.2^{d}	0.00 ± 0.00^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: PA: Pseudomonas aeruginosa; SA: Staphylococcus aureus; SP: Streptococcus pyogenes; EC: Escheria coli; KB: Klebsiella pneumonia; PM: Proteus mirabilis; ME: Methanol extract; CIP: Ciprofloxacin; VAN: Vancomycin ; MET: Methicillin; DMSO: Dimethylsulfoxide; HWE: Hot water extract; CWE: Cold water extract; CHL: Chloroform extract

plasmid analysis shows that some multiple resistant bacteria isolates harbor one or more plasmid with different molecular weights. Therefore, the genes for multidrug resistance might be located on plasmid DNA orchromosomal DNA as shown by [32] Plasmid curing converts all the isolates which were initially resistant to vancomycin and methicillin to susceptible form thereby indicating that, the resistance was plasmid mediated. Table 6 shows the resistance pattern of the bacterial isolates to conventional antibiotics. While Table 7 shows the appearance and the R_f of the extract on TLC paper.

Table 4. Antibacterial activity of purified *N. laevis* at 500 mg/ml on isolates

Pathogen	Methanol extract	
	F3	F4
KB	30.01±0.01^{f}	31.11±0.01^{f}
PA	26.76±0.32^{c}	27.86±0.53^{c}
PM	27.93±0.11^{e}	28.00±0.19^{e}
EC	22.99±0.17^{a}	24.09±0.67^{a}
SA	27.12±0.50^{e}	27.92±0.68^{e}
SP	25.63±0.95^{b}	26.66±0.98^{b}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: PA: *Pseudomonas aeruginosa; SP: Streptococcus pyogenes; EC: Escherchia coli; KB: Klebsiella pneumonia;* SA: *Staphylococcus aureus; PM: Proteus mirabilis; F3: Fraction thre; F4: Fraction four*

Table 5. Pre and post sensitivity curing of multiple antibiotics resistant bacteria isolate from urinary tracts patients

Antibiotics	Zone of Inhibition (diameter in mm)					
	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Proteus mirabilis***	***Streptococcus pyogenes***
VAN bf	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
VAN af	23.00±0.01^{e}	27.93±0.03^{g}	26.01±0.04^{f}	34.01±0.01^{e}	28.01±0.00^{f}	27.01±0.00^{f}
MET bf	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MET af	22.01±0.03^{e}	23.0±0.06^{f}	27.01±0.78^{f}	22.02±0.09^{d}	23.02±0.03^{e}	21.01±0.3^{e}
CPX bf	23.23±0.25^{e}	20.87±0.70^{e}	22.13±0.71^{e}	18.93±0.21^{d}	24.52±0.43^{e}	13.57±0.40^{d}
CPX af	23.34±0.01^{e}	21.05±0.04^{e}	23.23±0.01^{e}	20.01±0.34^{d}	24.54±0.98^{e}	15.08±0.11^{d}
COT bf	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
COT af	0.00±0.00^{a}	12.01±0.02^{c}	3.00±0.04^{b}	0.00±0.00^{a}	10.00±0.06	0.00±0.00^{a}
APX bf	14.57±0.15^{c}	14.79±0.18^{d}	10.63±0.40^{c}	14.15±0.13^{c}	12.60±0.20^{c}	6.98±0.24^{b}
APX af	15.56±0.45	14.67±0.12	12.01±0.05^{c}	13.12±0.02^{c}	15.01±0.05^{d}	8.04±0.56^{b}
TET bf	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
TET af	0.00±0.00^{a}	3.40±0.01^{b}	2.00±0.01^{b}	3.00±0.09^{b}	5.01±0.04^{b}	0.00±0.00^{a}
AMX bf	7.09±0.16^{b}	2.83±0.25^{b}	3.05±0.05^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
AMX af	7.10±0.90^{b}	4.45±0.01^{b}	3.01±0.04^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
OFL bf	15.30±0.30^{d}	12.80±0.20^{c}	14.50±0.20^{d}	12.27±0.21^{c}	11.37±0.31^{c}	12.80±0.26^{c}
OFL af	17.05±0.00^{d}	22.76±0.04^{e}	23.09±0.32	20.09±0.32^{d}	21.02±0.23^{d}	23.12±0.12^{f}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: Cot- Cotrimoxazole; Tet- Tetracycline; Van- Vancomycin; Ofl- Ofloxacin; Amx- amoxicillin; Met- Methicillin

Table 6. Antibiotics sensitivity pattern of isolated organisms

Antibiotics	Zone of Inhibition (diameter in mm)					
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Streptococcus pyogenes*
VAN	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
MET	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
CPX	23.23±0.25[e]	20.87±0.70[e]	22.13±0.71[g]	18.93±0.21[d]	24.52±0.43[d]	13.57±0.40[d]
COT	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
APX	14.57±0.15[c]	14.79±0.18[d]	10.63±0.40[c]	14.15±0.13[c]	12.60±0.20[c]	6.98±0.24[b]
TET	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
AMX	7.09±0.16[b]	2.83±0.25[b]	3.05±0.05[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
OFL	15.30±0.30[d]	12.80±0.20[c]	14.50±0.20[d]	12.27±0.21[b]	11.37±0.31[b]	12.80±0.26[c]
CIP	30.0±0.3[d]	28.0±0.3[d]	37.0±0.3[e]	36.0±0.3[e]	23.0±0.3[b]	31.0±0.2[d]

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: Cot- Cotrimoxazole; Cxc- Cloxacillin; Ofl- Ofloxacin; Met- Methicillin; Tet- Tetracycline; Van- Vancomycin; Amx- amoxicillin

Table 7. Appearance and refractive factor of fractions obtained from leaf extract spotted on the TLC plates (Methanol extract)

No of fractions	No of spot(s)	Colour	RF value
1	2	Light green	0.16
			0.83
2	3	Light green	0.51
			0.64
			0.86
3	3	Green	0.55
			0.64
			0.84
4	3	Green	0.54
			0.65
			0.86
5	3	Deep green	0.69
			0.83
			0.86
6	2	Deep green	0.63
			0.79
7	2	Deep green	0.64
			0.84
8	2	Deep green	0.65
			0.86
9	2	Deep green	0.64
			0.63

5. CONCLUSION

The phytochemical investigation of *N. laevis* leaf extracts and the assay of its functional groups revealed the presence of constituents which could be the basis for their medicinal potency against methicillin and vancomycin resistant organisms. The leaf extracts showed broad spectrum activities and higher antibacterial activities than most of the commercial antibiotics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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