Urama et al. *Afr. J. Clin. Exper. Microbiol.* 2025; 26 (1): 94 - 101 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jan 2025; Vol.26 No.1
AJCEM/2371. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Evaluation of *invitro* antimicrobial activity of *Nauclea latifolia* root extracts against multi-drug resistant bacterial isolates from diabetic foot ulcers

*[1]Urama, E. U., [1]Enweani-Nwokelo, I. B., and [2]Achukwu, N. O.

[1]Department of Medical Microbiology and Public Health, Faculty of Medical Laboratory Sciences, Nnamdi Azikiwe University Nnewi, Nigeria
[2]Department of Medical Laboratory Science, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria
*Correspondence to: amakaurama@yahoo.com; Tel: 08037918293

Abstract:

Background. Infections caused by multi-drug resistant organisms (MDROs) are becoming global health crisis especially the extended-spectrum beta-lactamase (ESBL) producing microbes. The trend in the occurrence of MDROs has been considered by the World Health Organization as critical and urgent need facing medical science. Herbal plants containing phytochemicals that can be synthesized into new antimicrobial agents are potential alternatives for therapy of MDROs.
Methodology: Fresh roots of *Nauclea latifolia* were harvested from the shrub and identified by botanist at the Nnamdi Azikiwe University with specimen number NAUH-215[A]. The roots were washed, chopped into smaller sizes for easy drying, air dried under shade and pulverized using a milling machine. The pulverized plant root was extracted using methanol, hexane, ethyl-acetate, and aqueous extraction. Wound swabs were collected from diabetic patients with foot ulcers and processed using conventional culture isolation and biochemical identification test scheme for bacterial isolates that were used as test organisms. Antimicrobial susceptibility test (AST) was performed by the Kirby-Bauer disc diffusion technique and multi-drug resistance (MDR) was determined for each isolate. The phytochemical composition of the plant extracts was assessed using standard methods. Antibacterial activities of the methanol root extracts and the fractions were determined at a concentration of 400mg/ml using agar well diffusion method in triplicate and the mean zone diameter of inhibition measured. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the methanol root extracts and the fractions were determined using the serial doubling dilution technique. The time-kill assay was also evaluated.
Results: The root extract yield of *N. latifolia* indicated that methanol extract produced a higher yield of 12.8%, followed by ethyl acetate root fraction at 8.0%, hexane fraction at 7.6%, and aqueous extract produced the least yield at 6.8%. The phytochemical analysis showed that methanol root extracts contain various phytochemicals which included phenols, flavonoids, tannins, glycosides, alkaloids, saponins, terpenoids, and triterpenes. The methanol root extract produced a higher mean inhibition zone diameter of 25.0±00mm against *Escherichia coli,* followed by mean inhibition zone diameter of 23.0±1.0mm against *Klebsiella pneumoniae*, 20.0±2.0mm against *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the least mean inhibition zone diameter of 18.7±1.2mm against *Pseudomonas aeruginosa.* The MIC of the methanol root extracts for *N. latifolia* ranged from 3.125 to 12.5mg/ml, and MBC ranged from 6.25 to 25.00 mg/ml. The time-kill assay of methanol extract at 1x MIC, 2x MIC, and 3x MIC showed that reduction in the viable cell count of the initial inoculum was observed within 2-8 hours of incubation at 37°C, indicating high activity.
Conclusion. The methanol root extracts of *N. latifolia* could be a potential source of antibacterial agent, which can complement conventional antibiotics currently used in the treatment of infections caused by MDR bacterial isolates.

Keywords: *Nauclea latifolia*; extracts; antimicrobial; bacteriostatic; bactericidal; time-kill assay

Received Oct 28, 2024; Revised Nov 19, 2024; Accepted Nov 21, 2024

Évaluation de l'activité antimicrobienne *in vitro* d'extraits de racines de *Nauclea latifolia* contre des isolats bactériens multirésistants provenant d'ulcères du pied diabétique

*[1]Urama, E. U., [1]Enweani-Nwokelo, I. B., et [2]Achukwu, N. O.

[1]Département de Microbiologie Médicale et de Santé Publique, Faculté des Sciences de Laboratoire Médical, Université Nnamdi Azikiwe Nnewi, Nigéria
[2]Département des Sciences de Laboratoire Médical, Faculté de Médecine, Université du Nigéria, Campus d'Enugu, Enugu, Nigéria
*Correspondance à: amakaurama@yahoo.com; Tél: 08037918293

Résumé:

Contexte: Les infections causées par des organismes multirésistants aux médicaments (MDRO) deviennent une crise sanitaire mondiale, en particulier les microbes producteurs de bêta-lactamases à spectre étendu (BLSE). La tendance à l'apparition des MDRO a été considérée par l'Organisation mondiale de la santé comme un besoin critique et urgent auquel la science médicale est confrontée. Les plantes médicinales contenant des composés phytochimiques qui peuvent être synthétisés en nouveaux agents antimicrobiens sont des alternatives potentielles pour le traitement des MDRO.
Méthodologie: Des racines fraîches de *Nauclea latifolia* ont été récoltées sur l'arbuste et identifiées par un botaniste de l'Université Nnamdi Azikiwe avec le numéro de spécimen NAUH-215A. Les racines ont été lavées, coupées en plus petites tailles pour un séchage facile, séchées à l'air libre à l'ombre et pulvérisées à l'aide d'une fraiseuse. La racine de la plante pulvérisée a été extraite à l'aide de méthanol, d'hexane, d'acétate d'éthyle et d'une extraction aqueuse. Des écouvillons de plaies ont été prélevés sur des patients diabétiques souffrant d'ulcères du pied et traités à l'aide d'un schéma de test d'isolement de culture et d'identification biochimique conventionnel pour les isolats bactériens qui ont été utilisés comme organismes de test. Le test de sensibilité aux antimicrobiens (AST) a été effectué par la technique de diffusion sur disque de Kirby-Bauer et la résistance multi-médicaments (MDR) a été déterminée pour chaque isolat. La composition phytochimique des extraits de plantes a été évaluée à l'aide de méthodes standard. Les activités antibactériennes des extraits de racines au méthanol et des fractions ont été déterminées à une concentration de 400 mg/ml en utilisant la méthode de diffusion en puits d'agar en triple et le diamètre moyen de la zone d'inhibition a été mesuré. La concentration minimale inhibitrice (CMI) et la concentration bactéricide minimale (CMB) des extraits de racines au méthanol et des fractions ont été déterminées à l'aide de la technique de dilution en double série. Le test de temps de mort a également été évalué.
Résultats: Le rendement de l'extrait de racine de *N. latifolia* a indiqué que l'extrait au méthanol produisait un rendement plus élevé de 12,8%, suivi de la fraction racinaire à l'acétate d'éthyle à 8,0%, de la fraction à l'hexane à 7,6% et de l'extrait aqueux à 6,8%. L'analyse phytochimique a montré que les extraits de racine au méthanol contiennent divers composés phytochimiques, notamment des phénols, des flavonoïdes, des tanins, des glycosides, des alcaloïdes, des saponines, des terpénoïdes et des triterpènes. Français L'extrait de racine au méthanol a produit un diamètre moyen de zone d'inhibition plus élevé de 25,0±00mm contre *Escherichia coli*, suivi d'un diamètre moyen de zone d'inhibition de 23,0±1,0mm contre *Klebsiella pneumoniae*, de 20,0±2,0mm contre *Staphylococcus aureus* et *Streptococcus pneumoniae*, et le diamètre moyen de zone d'inhibition le plus faible de 18,7±1,2mm contre *Pseudomonas aeruginosa*. La CMI des extraits de racine au méthanol pour *N. latifolia* variait de 3,125 à 12,5mg/ml, et la CMB variait de 6,25 à 25,00mg/ml. L'essai de time-kill d'extrait au méthanol à 1xMIC, 2xMIC et 3xMIC a montré qu'une réduction du nombre de cellules viables de l'inoculum initial a été observée dans les 2 à 8 heures suivant l'incubation à 37°C, indiquant une activité élevée.
Conclusion: Les extraits de racines au méthanol de *N. latifolia* pourraient être une source potentielle d'agent antibactérien, qui peut compléter les antibiotiques conventionnels actuellement utilisés dans le traitement des infections causées par des isolats bactériens MDR.

Mots-clés: *Nauclea latifolia*; extraits; antimicrobien; bactériostatique; bactéricide; essai de time-kill

Introduction:

The continued rise in infections caused by multi-drug resistant organisms (MDROs) calls for an urgent search for new antimicrobial agents that would be effective in the treatment of resistant pathogens and with little or no toxicity, preferably from plant origin. Herbal plants offer promising antimicrobial sources due to the abundance of naturally-endowed phytochemical constituents (1). Different parts of *Nauclea latifolia* are used by African traditional practitioners in the treatment of many ailments such as diarrhea and cough believed to be caused by *Corynebacterium diphtheriae, Streptobacillus, Streptococcus* species, *Neisseria* species*, Pseudomonas aeruginosa* and *Salmonella* species (3).

Nauclea latifolia, commonly known as African peach, belongs to the family of *Rubiaceae,* native to Africa and broadly distributed in Nigeria (4). It is known by the local names of Ubulu ilu and Uvuru ilu in some parts of eastern Nigeria. It has a fleshy fruit with a yellow color when unripe but red when ripe, appearing like strawberry. The shrub has a sweet-scented straggling flower.

Nauclea latifolia has been employed in the treatment of hypertension, typhoid, malaria, and other tropical diseases. Ezeagha et al., (5) investigated the antimicrobial and phytochemical properties of *N. latifolia* root and reported that ethyl acetate, methanol, n-hexane, butanol and aqueous extract contain some secondary metabolites which possess broad spectrum antimicrobial activities and can be useful as antimicrobial agents. Anowi et al., (6) reported that *N. latifolia* is a broad-spectrum antimicrobial agent that could serve as alternative to the contemporary antimicrobials in the treatment of infections caused by MDROs.

The aqueous and hydro-methanolic root extract of *N. latifolia* was also shown to

possess antimicrobial activity (7). These extracts inhibited the growth of MDR *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus.* But on the other hand, the extracts showed no efficacy on the strains of *Candida albicans* at the same doses used (7). *Nauclea latifolia* extracts also exhibited good antibacterial effect on *Salmonella* Typhi isolates including those resistant to multiple antibiotics (8).

Diabetic foot ulcers are among the main complications of patients who have uncontrolled diabetes mellitus. It is typically a result of poor glycemic control, underlying peripheral vascular disease, neuropathy, or poor foot care (9). It is also one of the common causes of osteomyelitis of the foot and amputation of lower extremities (1). Previous studies have shown that Gram negative organisms are more frequently isolated from wound culture than Gram-positive isolates (10). However, *S. aureus,* a Gram-positive bacterium has been reported to dominate the initial phase of wound infection (10).

There is a paucity of information on the *invitro* evaluation of *N. latifolia* root extracts against microbial organisms linked with diabetic foot ulcers. Hence the objective of this study is to evaluate the *invitro* antibacterial activities of the extracts of *N. latifolia* against selected clinical MDR bacterial pathogens isolated from infected foot ulcers of diabetic patients.

Materials and method:

Study setting:

This study was carried out at the Microbiology Laboratory of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. Approval was obtained from the Ethics Committee of the Hospital. Informed consent form was also obtained from participants who were sampled.

Plant collection and preparation:

Fresh roots of *N. latifolia* were harvested, identified and authenticated by a plant taxonomist at the Department of Plant Biology and Biotechnology, Nnamdi Azikiwe University, Awka. A sample of the plant was deposited in the herbarium with number NAUH–215[A]. The roots were washed thoroughly, cut into smaller pieces and spread out to dry in a well-ventilated room. The dried roots were then pulverized with a milling machine and stored separately in an airtight container for further studies.

Fig: 1 Fresh *Nauclea latifolia* root from the shrub

Isolation, identification and susceptibility testing of the test bacterial isolates:

The bacterial isolates used as test organisms in the study (*Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*) were isolated from swab samples of ulcers collected from diabetic patients using standard bacteriological culture technique and conventional biochemical identification test schemes.

Antimicrobial susceptibility test (AST) was performed on Mueller-Hinton (MH) agar plate for each isolate (except for *S. pneumoniae* that was performed on chocolate agar) against selected antibiotics using the disk diffusion method, and interpreted as sensitive, intermediate or resistant according to the CLSI guideline (11). Multiple antibiotic resistance (MAR) index of each isolate was determined using the formula, MAR = a/b, where 'a' is the number of antibiotics to which the isolate is resistant to and 'b' is the total number of antibiotics to which the isolate was tested. Multi-drug resistance (MDR) was defined as resistance of an isolate to antibiotics in more than two antibiotic classes (12).

Extended spectrum beta-lactamase (ESBL) production among the Gram-negative isolates was detected by the double disc synergy test (DDST) while methicillin resistance in *S. aureus* was detected by cefoxitin and oxacillin disc diffusion test on MH agar plate in line with the CLSI guideline (11). ESBL genes in the Gram-negative isolates and *mec*A genes in *S. aureus* were detected by conventional standard PCR assays.

Procedure for plant extraction:

The pulverized root of *N. latifolia* was extracted by the cold maceration method. One kg of the root was soaked in 6 liters of 90% methanol for 48 hours with intermittent shaking. The filtrate was dried under compact pressure at low temperature to recover the extracts. The crude methanol root extracts were then fractionated as described by Obaji et al., (13) using methanol, hexane, ethyl acetate and aqueous in order of increasing polarity. The methanol extract and the fractions were dried at 40^{0}C using an evaporator, and were labelled and kept in a stoppered sample vial at 4^{0}C. The purity of the extracts was assessed by plating the extract on MH agar and incubated overnight.

Phytochemical screening of the root extracts:

Qualitative phytochemical composition screening of *N. latifolia* root extract was performed using standard method as described by Onah et al., (14). The phytochemicals assessed included phenol, flavonoids, tannins, glucosides, alkaloids, anthrocyanin, terpenoids, aminoacids and triterpenes.

Primary screening of extract for antimicrobial activities:

Antimicrobial activity methanol extract and the fractions of *N. latifolia* were tested against the MDR *S. aureus, E. coli, P. aeruginosa, K. pneumoniae,* and *S. pneumoniae* by the agar well diffusion method as previously described by Emencheta et al., (15). Two grams of the root extract and the fractions were reconstituted with 5ml dimethyl sulphoxide (DMSO) to obtain a working stock concentration of 400mg/ml. Mueller-Hinton (MH) agar (Oxoid, USA) was prepared and poured into sterile Petri dishes and allowed to set. Standard concentration (McFarland 0.5) of the overnight cultures of the test isolates was swabbed aseptically on the agar plate and a 6mm sterile metal cork-borer was used to make wells in the agar plates.

Five hundred microliter (500µl) of the different plant extracts and the fractions as well as the controls were aseptically used to fill each well and allowed to stand for 15 min to enable the extract and fractions disperse into the agar. Levofloxacin (0.05mg/ml) served as the positive control, while MH broth without extract served as the negative control. The culture plates were incubated at 37°C for 24 hr. The inhibition zone diameter was measured and recorded to the nearest mm. The procedure was performed in triplicate and the mean inhibition zone diameter was calculated.

Minimum inhibitory and bactericidal concentrations:

The serial doubling dilution was used to determine the MIC and MBC as previously described by Achukwu et al., (16). Five milliliters of MH broth was added into test tubes and 1ml of each methanol crude extract and the fractions of the stock concentrations was added into the first tubes. A twofold dilution of the extracts and fractions was carried out to obtain various concentrations of the extract and the fractions at 400, 200, 100, 50, 25, 12.5, 6.25, 3.125,1.56,0.78…..mg/ml.

A standardized inoculum of 0.1ml of each test bacteria organism (McFarland) was placed into all the tubes and incubated at 37°C for 24 hours. The least concentration without turbidity was taken as the MIC. The tubes that showed no turbidity were inoculated on MH agar plate and incubated for 24 hours at 37°C. The MBC is the minimum concentration of the extracts that did not produce growth (visible colony) on the agar media plate.

Time kill assay:

The time-kill assay of the methanol crude extracts was determined at concentrations of 1xMIC, 2xMIC, and 3xMIC using the plating method. Approximately 1ml of the methanol extract at 1xMIC, 2xMIC, and x3MIC was added into 5ml of broth, and 0.1ml of the standardized bacterial cell suspension was added and properly vortexed. The tubes were incubated at 37°C for 24hours. An aliquot of 0.1ml of the mixture was removed from the tube every 2 hours for a minimum of 12 hours This was serially diluted and plated on MH agar, and incubated for 24 hours at 37°C. The culture plates with significant bacteria colonies were counted and recorded as log 10 CFU/ml according to the formula of Bremmer et al., (17) and compared with the control.

The time-kill curve was constructed by plotting the time of incubation against the logarithm of the number of viable cells. A 3log10 fold reduction in the original cell population, which corresponds to 99.9% death of viable cells, was regarded as the bactericidal concentration.

Statistical analysis:

The data were presented as the mean of three replicates and expressed as mean± SD and analyzed using the statistical packages for the social (SPSS) version 27.0. The level of significance was set at $p \leq 0.05$.

Results:

Extract yield and phytochemical constituents:

The percentage yield of the root extracts of *N. latifolia* shows that methanol extract produced 12.8%, ethyl acetate produced 8.0%, N-hexane produced 7.6% and aqueous extraction produced the least percentage yield of 6.8% (Table 1).

The qualitative phytochemical constituents of the methanol root extract *N. latifolia* showed the presence of phenol, flavonoids,

alkaloids, saponins, terpenoid, and triterpenes while anthrocyanin and amino acids were not detected.

Table 1: Percentage yield of methanol root extracts and the fractions of *Nauclea latifolia*

Extracts	Yield (%)
Aqueous	6.8
Ethyl acetate	8.0
N-hexane	7.6
Methanol	12.8

Antibacterial activities of the extracts:

Methanol root extract produced the highest mean inhibition zone diameter of 25.0 ±0.0mm against *E. coli*, followed by a mean inhibition zone diameter of 23.0±1.0mm against *K. pneumoniae*, 20.0±2.0 against *S. aureus* and *S. pneumoniae*, and the least mean inhibition zone diameter of 18.7±1.2mm against *P. aeruginosa* ($p \leq 0.05$). The ethyl-acetate extract produced the highest mean inhibition zone diameter of 20.0±0.6mm against *K. pneumoniae*, followed by 19.00±0.0mm and 19.0±0.2mm against *P. aeruginosa* and *S. pneumoniae*, 18.7±1.2mm against *S. aureus* and the least mean inhibition zone diameter of 17.0±0.0mm against *E. coli* ($p \leq 0.05$).

The aqueous extract produced the highest mean inhibition zone diameter of 19.00± 0.0mm against *P. aeruginosa*, 18.0±0.0mm against *S. aureus*, 18.0±0.1mm against *S. pneumoniae*, 16.7±0.6mm against *K. pneumoniae*, and the least mean inhibition zone diameter of 15.0±0.0 against *E. coli* ($p \leq 0.05$). The hexane extract produced the highest mean inhibition zone diameter of 19.3±1.2mm against *E. coli* and *K. pneumoniae*, 18.0± 0.3 mm against *P. aeruginosa*, 16±0.0mm against *S. aureus* and the least 15.0±0.6mm against *S. pneumoniae*. The positive control antibiotic, levofloxacin, exhibited high antimicrobial activity against *S. aureus, E. coli, P. aeruginosa K. pneumoniae*, at inhibition zone diameters of 25.0mm, 23.0mm, 23.0mm, and 25.0mm respectively, while the negative control, DMSO, showed no activity (Table 1).

The bacteriostatic (MIC) activities of the methanol root extracts of *N. latifolia* were observed with various concentrations ranging from 3.125 to 12.5mg/ml and the bactericidal (MBC) activities ranged from 6.125 to 50.00 mg/ml (Table 2).

Time-kill assay result:

The results of the time-kill assay at 1xMIC showed that the reduction in the viable cell count of the initial inoculum was observed at 99.9% between 6 and 8 hours of exposure. When the concentration of the extract was doubled (2xMIC), 99.9% of cell death occurred at about 4 hours of exposure and lastly when the concentration was tripled (3xMIC), 99.9% cell death was observed between 2 and 4 hrs of incubation.

The total cell death for all the isolates was observed between 2-8 hours of exposure (Fig 1). Furthermore, it was observed that the inhibitory activity of the extract used was concentration and time-dependent.

Table 2: Mean inhibition zone diameter of *Nauclea latifolia* methanol root extract and the fractions

Bacteria isolates	Methanol	Aqueous	Hexane	Ethyl acetate	Positive control (Levofloxacin 0.05mg/ml)	Negative control (DMSO)	P-value
Escherichia coli	25.0±0.0	15.0±0.0	19.3±1.2	17.0±0.0	25.0±0.0	0.00	0.00
Staphylococcus aureus	20.0±2.0	18.0±0.3	16.0±0.0	18.7±1.2	23.0±0.0	0.00	0.00
Klebsiella pneumoniae	23.0±1.0	16.7±0.6	19.0±1.2	20.0±0.6	23.0±0.0	0.00	0.00
Pseudomonas aeruginosa	18.7±1.2	19.0±0.0	18.0±0.0	19.0±0.0	25.0±0.0	0.00	0.00
Streptococcus pneumoniae	20.0±2.0	18.0±1.0	15.0±0.6	19.0±1.2	22.0±0.0	0 .00	0.00

Table 3: Minimum inhibitory and bactericidal concentrations of *Nauclea latifolia* root extracts

Test isolates	Methanol		Aqueous		Ethyl-acetate		Hexane	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	3.125	6.25	12.5	25	12.5	25	12.5	12.5
Staphylococcus. aureus	6.25	12.5	12.5	12. 5	3.125	12.5	6.25	12.5
Klebsiella pneumoniae	3.125	6.25	3.125	6.25	3.125	12.5	6.25	12.5
Pseudomonas aeruginosa	12.5	12.5	12.5	25	3.125	6.25	12.5	50
Streptococcus pneumoniae	6.25	6.25	12.5	50	3.125	6.25	12.5	50

MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration

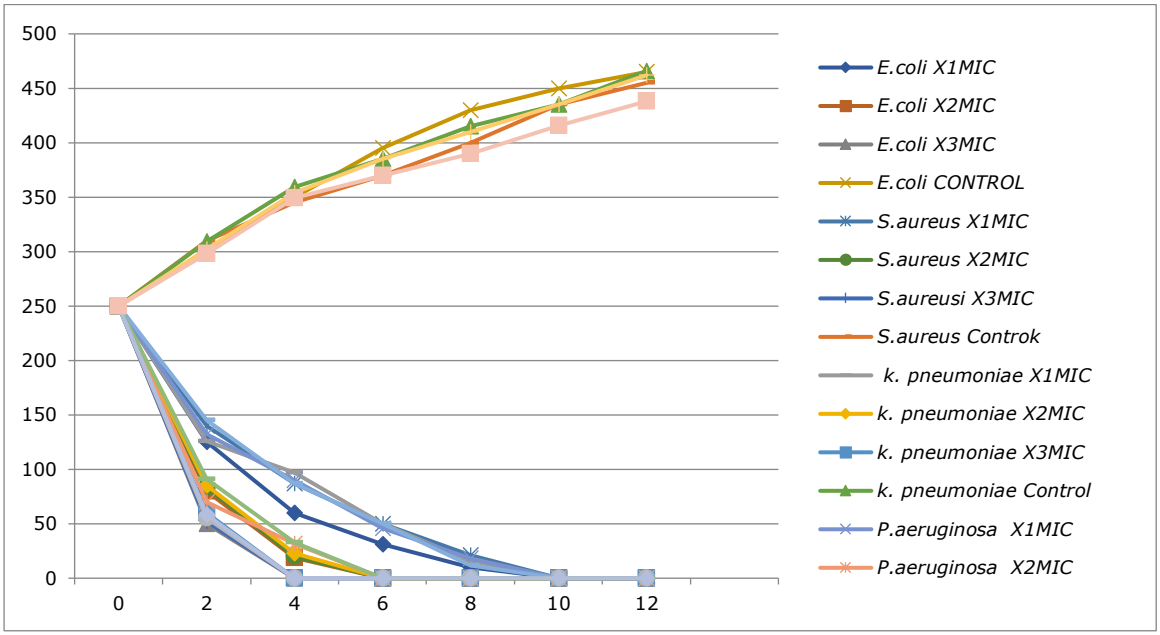

Fig 2: The time-kill curve of methanol root extract of *Nauclea latifolia* at MIC, x2MIC, and x3MIC

Discussion:

The highest *N. latifolia* root extract yield was produced by methanol, followed by ethyl-acetate while hexane and aqueous fractions produced the least yield. This might be attributed to the polarity of the solvents used and may also suggest why the indigenous use of extraction solvents in ethno-medicine is mainly aqueous or alcohol-based extraction. The qualitative phytochemical screening showed that the root extract of *N. latifolia* contained essential secondary metabolites including phenols, flavonoids, tannins, glycosides, alkaloids, saponins, terpenoids, and triterpenes. These compounds are responsible for the therapeutic usage of medicinal plants (18).

The presence of saponin and tannins might be linked to its antimicrobial activity (6). Tannin helps in coagulating the cell wall and saponin acts by causing lysis of the cells (6). Our study finding agrees with a previous study which reported the phytochemical constituents of the leaf extract of *N. latifolia* using qualitative and quantitative phytochemical analyses and spectrophotometrically established the presence of flavonoid, cardiac glycosides alka loid, anthocyanin, quinones, phenol, saponins, and terpenoids (6). Plants contain essential secondary metabolites that are well known (19), which provide antimicrobial, anti-inflammatory and anti-oxidant activity. Tannins have been shown to possess high antimicrobial and anti-inflammatory properties (20).

Comparing the various solvents used for extraction, methanol root extract produced significantly higher mean inhibition zone diameter (which compared favorably with the mean inhibition zone diameter produced by levofloxacin control antibiotic) than ethyl acetate and aqueous extracts, while n-hexane extract produced the least mean inhibition zone diameter ($p<0.05$). The high activity obtained with levofloxacin (a standard antibiotic) against the test organisms could be attributed to the fact that this is a purified and standardized antimicrobial product, with proven efficacy in the treatment of many Gram-

positive and Gram-negative bacterial infections. Our present study agrees with the those of Ezeagha et al., (6), who reported the presence of secondary metabolites and antimicrobial activity of *N. latifolia* root extracted by various solvents against bacterial and fungal isolates. Our study showed the presence of secondary metabolites and antimicrobial activity against both Gram-positive and Gram-negative bacteria. The high antimicrobial activity recorded can be attributed to the phytochemicals present in the root extracts (19).

The root extracts of *N. latifolia* produced bacteriostatic action at concentrations between 3.125 and 12.5mg/ml and bactericidal action at 6.25 to 50 mg/ml against the test isolates. The synergistic action could be attributed to their multiple target mechanisms of action, pharmacokinetics, and physiochemical activity leading to enhanced bioavailability, reabsorption, solubility neutralization rate of adverse effects and toxicity reduction (1). Our study agrees with those of Ngassaki et al., (7) that the root extract produced MIC against *S. aureus* strain and *E. coli* at concentrations of 6.25 and 12.5 mg/ml, and MBC at concentrations of 6.2 5 and 25 mg/ml respectively.

The time-kill assay showed that methanol root extract is concentration and time-dependent against the test isolates. This was observed when the test isolates were exposed to a 1xMIC concentration of root extract, it took about 8 hours for the extracts to initiate a reduction in viable cell count but when the concentration was increased to 3xMIC, it took only 2hours for a reduction in viable cell count to be initiated. The decrease in the number of viable cells count within 2 and 8 hours of incubation suggests great bactericidal properties and may be ascribed to flavonoids in combinations with the other phytochemicals that may be present in the extract.

The evaluation of the antimicrobial activity of *N. latifolia* root extract is of great importance to traditional medicine because known concentrations can be prepared. Hence it can be comfortably recommended as a complementary herbal medicine for the treatment of infections caused by MDR microbial isolates.

Conclusion:

The antimicrobial activity demonstrated by *N. latifolia* methanol root extract in our study is an indication that this could serve as alternative source for antimicrobial agent that could be used in the treatment of infections caused by MDR bacterial pathogens.

Acknowledgements:

The authors acknowledge the staff of Department of Medical Microbiology, Nnamdi Azikiwe university Teaching Hospital, Nnewi for granting us access for the use of the laboratory facilities. The authors also appreciates the management and staff of Central Research Laboratory, University of Nigeria (UNN) Nsukka Nigeria for assistance in the molecular identification of the test bacterial isolates and detection of resistance genes. The patients who consented to be recruited into the study are highly appreciated.

Contribution of authors:

UEA designed the study; ANO wrote the protocol; and contributed to the literature search; UEA EIB and ANO, performed the laboratory analysis; ANO performed statistical analysis of data; UEA and EIB contributed in discussions; ANO produced the initial manuscript draft; EIB supervised the study; UEA wrote the final manuscript; EIB proofread the manuscript and all authors approved the final manuscript submitted for publication.

Source of funds:

Authors received no fund for the study.

Conflict of interest:

Authors declare no conflict of interest.

Authors declaration:

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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