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Original Research Article

Evaluation of Antibacterial Effects of *Newbouldia laevis* and *Dracaena arborea* Methanol Extracts against Some Multidrug Resistant 'ESKAPE' Pathogens Isolated from Hospital Environments

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ABSTRACT

Some multidrug-resistant bacteria particularly *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter*spp (ESKAPE pathogens) are associated with nosocomial infections. This study investigated the antibacterial activities of *Newbouldia laevis* and *Dracaena arborea* against some ESKAPE pathogens isolated from hospital environments. One hundred and twenty-five (125) samples were collected from wounds, urine, and hospital beds from Bishop Shanahan Hospital Nsukka. Ethical clearance was obtained before the collection of samples. Bacteria were isolated and characterized using appropriate microbiological and biochemical techniques. Plants methanol extracts were prepared using standard method. Antibiotic sensitivity testing was done using the Kirby Bauer disc diffusion method following Clinical Laboratory Standards Institute (CLSI) guidelines 2024. Multi-drug resistant isolates were randomly selected for plant extract sensitivity testing. *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumonia* isolates had a prevalence of 75%, 83.3%, and 78.6% respectively. Multiple antibiotic resistance indices (MARI) of the isolates showed mostly high values (0.5-1.0). *Newbouldia laevis* methanol extract showed varying degrees of antibacterial activity on most of the isolates at different concentrations (50, 100, 200, and 400 mg/mL). However, following the CLSI standard, only 100 mg/mL of the extract produced significant inhibition (≥ 11.0 mm) on 3/8 and 3/7 of *S. aureus* and *P. aeruginosa* susceptible isolates respectively; no inhibition was observed for *K. pneumoniae*. *Dracaenaarborea* had no activity against any of the isolates. These results buttress the need for the use of *Newbouldia laevis* extract as a promising alternative against antibiotic-resistant ESKAPE pathogens.

***Keywords*:** Antibiotic resistance, Hospital environment, Plant extracts, Antibacterial sensitivity

Introduction

The increasing number of 'superbugs,' or multidrug-resistant (MDR) bacteria, particularly in hospitals, is a serious worldwide problem affecting people, animals, and the environment.[1] The World Health Organization even ranks antibiotic resistance as one of the top three public health threats of our time.[2] Compared to infections from regular bacteria, MDR infections lead to more deaths, illnesses, longer recovery times, lower productivity, and higher overall costs.[3] The discovery of penicillin and other antibiotics, like cephalosporins, aminoglycosides, glycopeptides, and quinolones, revolutionized medicine.[4]

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These drugs were widely used, including in surgeries like orthopedic procedures, to fight infections. Unfortunately, the overuse and misuse of antibiotics have created resistant strains of bacteria, threatening successful treatment, especially for critically ill patients.[5]

The acronym ESKAPE refers to a group of highly concerning multidrug-resistant bacteria. Introduced in 2008 by the Infectious Diseases Society of America, ESKAPE signifies a growing threat. These pathogens are causing a significant and increasing number of hospital infections and are becoming resistant to available antibiotics.[6, 7] ESKAPE stands for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species. These bacteria are particularly troublesome because they are often associated with hospital-acquired infections, presenting a new set of challenges in terms of how these diseases spread, cause harm, and resist treatment.[8, 6] The World Health Organization prioritizes research into new antibiotics based on the severity of the threat posed by different pathogens. ESKAPE pathogens fall into the "critical" or "high" priority categories.[9] This urgency is driven by the emergence of strains resistant to even the strongest antibiotics; Carbapenem-resistant *Acinetobacter baumanii*and *Pseudomonas aeruginosa* with extended spectrum *B*-lactamase (ESBL) or carbapenem-resistant *Klebsiella pneumoniae* and *Enterobacter* spp are in the critical level while Methicillin-resistant, vancomycin-intermediate and resistant *Staphylococcus aureus* with *Enterococcus faecium* are in the high priority level.[10, 11] The clinical

and economic impact of ESKAPE pathogens is significant and call for serious attention to be paid to their impact. Infections caused by these bacteria are linked to high death rates, increased illness, and substantial healthcare costs. The challenge lies not only in treating these infections but also in diagnosing them accurately.[12, 13] ESKAPE bacteria possess a concerning ability to resist antibiotics. They achieve this through various mechanisms, including modifying their structure to prevent drugs from binding, breaking down antibiotics, or forming protective barriers. These mechanisms are often encodedin their genes or on mobile genetic elements, allowing them to readily share resistance traits with other bacteria.

In a bid to proffer a solution to the challenges posed by antibiotic resistance, the use of plant extracts and combination therapy has been adopted in treating infectious diseases and antibiotic-resistant bacteria.[14, 15] They consist of highly structurally diverse chemical compounds (phytochemicals) which include phenol, tannins, terpenes, saponins, polyphenols, and alkaloids. These phytochemicals are responsible for the healing abilities they exhibit. Apart from their pharmacologically important phytochemicals contents, each species has its nutrient compositions which are essential for improved body physiological functions.[16]

The use of natural products is not new as it has remained the backbone of traditional medicine in many parts of the world, and a fundamental part of history and culture, particularly in Asia, Africa, and other continents. They are also gradually increasing in developed countries due to their varying degrees of antimicrobial activity and other beneficial effects.[17, 18] Advances in pharmacology and synthetic organic chemistry have increased the science and application of nature among elites as an important source of medicinal agents.[19] Medicinal plants are very important in providing primary health care services to rural dwellers and in combating serious diseases in the world.[20, 16] The World Health Organization's recognition of traditional medicine as an important element of primary health care resulted in their slogan "save the plants which save the life".[21] which summarizes the importance of medicinal plants. Now, it is estimated that about 80% of the world's population depends on medicinal plant preparations to meet their health needs and for the treatment of various diseases,[21] owing to their relative safety, affordability, availability, and tolerance.[20, 22, 16] They exhibit different mechanisms of action including membrane disruption, complex formation with cell wall, inactivation of enzymes, intercalation with DNA, and improvement of efficacy through interaction.[23, 24]

Newbouldia laevis also known as boundary tree is a knotted branched angiosperm with shiny dark leaves and purple color which is native to tropical Africa, growing from Guinea savanna to dense forests on moist and well-drained soil.[25, 26, 27] It has been reportedly used in the treatment of both infectious and non-infectious diseases. Different parts of the plants have been used in the treatment of malaria, sexually transmitted diseases, worms, and dental caries. It has also been as a formula in dysentery and diarrhea, eye problems, wound infections, sore feet, convulsion, skin ulcers, epilepsy, peptic ulcer, constipation, hemorrhoids, pelvic pain in women, snake bite and impotence, and infertility.[28, 29, 30, 27] In Ghana and Nigeria, the cooked stem barkis used in the treatment of breast tumor, and child delivery in women.[25] In the treatment of bone lesions, pneumonia, cold, fever, and cough, it has been reported that the stem bark of *N. laevis* is more effective when mixed with lay and red pepper.[31] *Dracaena arborea* on the other hand is a perennial woody tree that is native to Africa and Asia, and is characterized by fibrous and tough leathery and densely crowded leaves that grows up to 6-15cm high.[32] Dracaenas are either shrubs or trees and are grouped into two groups based on their habitat: tree Dracaenas and shrub Dracaenas.[33] Its medicinal applications have been reported to include the treatment of venereal diseases, smallpox, chicken pox, measles, epilepsy, convulsion, heat, and kidney trouble, also used as a diuretic and sedative.[34] Studies have shown that *D. arborea* contains a wide range of secondary metabolites including flavonoids, steroidal saponin, tannin, sterols, alkaloids, lectin, phytate, and cyanide.[35,33] This research is aimed at investigating the antibacterial activities of *Newbouldia laevis* and *Dracaena arborea* against some ESKAPE Pathogens isolated from hospital environments through exposure to different concentrations of the extracts, to develop alternative treatment regimens in the face the failing effectiveness of the conventional antibiotics. The methanol extract was preferred due to the relatively high phytochemical extractability of methanol coupled with the fact that it is an in-vitro susceptibility study.

Materials and Methods

Chemical and Reagents

Reagents were procured from Sigma-Aldrich, China and they include Gram-staining reagents, Kovac's reagent, tryptophan, Oxidase reagent, and McFarland's reagent. Media used were procured from Oxoid, United Kingdom and they include Cetrimide, Mannitol Salt Agar (MSA), Cystine lactose electrolyte deficient agar (CLED), Mueller Hinton agar (MHA), Nutrient agar, Urea agar, and Simon citrate agar. All chemicals used were of analytical grades

Plant Collection and Identification

Fresh leaves of *Newbouldialaevis* and *Dracaena arborea* were collectedin June 2020 from farms Uwani-Ugwu, Umakashi in Nsukka Local Government Area of Enugu State (6.9 N, 7.4E). The plants were identified by Felix I. Nwafor, Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka and deposited at the University of Nigeria (UNN) Herbarium with the following collection details: *Newbouldia laevis* (P. Beau v.) Seem. Ex, Voucher No: UNN/11765 and *Dracaena arborea* (Wild.) Link, Voucher No: UNN/11766.

Plant Extraction

The preparation process began with washing the leaves, followed by air drying them at room temperature for two weeks. Once completely dry, the leaves were ground into a fine powder and stored in a sealed polyethylene bag for later use. Extraction involved a modified method from another source.[36] A 500 g of the powdered leaves were soaked in 1.5 liters of methanol with occasional shaking. This mixture rested at room temperature for 72 hours to ensure thorough extraction. The extracts were then filtered using Whatman No. 1 filter paper (Whatman, England). Finally, a rotary evaporator was used to concentrate the extracts under vacuum. The concentrated extracts were weighed, stored in separate airtight containers, and refrigerated at 4°C until analysis.

Sample collection

A total of 84 samples comprising 21 each, of wound, urine, bed, and floor samples from patients attending Bishop Shanahan Hospital, Nsukka, using sterile containers and swab sticks. The swab sticks were dipped and rolled on the wound to enhance the effectiveness of collecting samples. After collection, the samples were promptly delivered to the Microbiology Laboratory at UNN for analysis.

Ethical Consideration

Following the International Ethical Guidelines for Biomedical Research Involving Human Subjects, ethical approval for this study was obtained from the Medical Advisory Committee of the hospitals used with clearance number: BSHN/EC/19/001. Informed consent was also obtained from the patients before collection of the specimen.

Media Preparation

Each of the media (Cetrimide Agar, mannitol Salt agar, Cystine lactose electrolyte deficient agar, Mueller Hinton Agar, and Nutrient Agar) was prepared under aseptic conditions according to manufacturer's specifications and autoclaved at 121ºC for 15min and allowed to cool, after which they were dispensed into sterile Petri dishes, Bijou bottles or test tubes (depending on its purpose) and allowed to gel. The media plates were then labeled accordingly and kept refrigerated until needed.

Culture and Biochemical Identification of Species

Bacterial species were identified according to methods used by other authors.[37] Samples were streaked in duplicate onto three different agar media (Cetrimide agar, Mannitol Salt agar, and Cystine Lactose Electrolyte Deficient agar) to encourage bacterial growth. All the

culture plates showed visible growths of discrete colonies (100% viability). Incubation temperature varied depending on the suspected bacteria: 37°C for 24 hours for *Staphylococcus aureus* and *Klebsiella pneumoniae*, and 42°C for 24 hours for *Pseudomonas aeruginosa*. All plates displayed visible growth of distinct, separate colonies. To isolate pure cultures, single colonies from each plate were transferred (sub-cultured) onto fresh media plates. Finally, to confirm the identity of the isolated bacteria, researchers employed Gram staining and a series of biochemical tests including catalase, indole, coagulase, oxidase, urease, and citrate utilization.

Preparation and Standardization of Inoculum

The isolates were inoculated onto duplicate Mueller Hinton Agar (MHA) and were incubated at 37ºC for 24h using an incubator (Thermofisher scientific, USA) after which colonies were suspended in normal saline and allowed to stand for 15min. The turbidities of the suspensions formed were adjusted by using normal saline until it matched the turbidity of 0.5 McFarland standard (final inoculums equivalent to a bacterial count of approximately $1.5x10^8$ cfu/mL).

Antibiotic Sensitivity Testing

Antibiotic susceptibility testing was performed using a standardized inoculum spread evenly onto duplicate Mueller-Hinton Agar (MHA) plates, possibly including sheep blood for fastidious organisms, with sterile swabs. After a 15-minute incubation to allow for settling, multi-antibiotic discs containing both Gram-positive and Gram-negative targeted antibiotics were placed aseptically on the agar surface using sterile forceps. The discs were gently pressed down with a rotating motion to ensure even contact without damaging the disc. Following incubation at 37°C for 24 hours, the diameter of the clear zone (inhibition zone) surrounding each disc, excluding the disc diameter itself, was measured in millimeters and recorded. The size of the inhibition zone was then used to classify the isolates as resistant, intermediate, or susceptible to each antibiotic according to the current Clinical Laboratory Standard Institute (CLSI) guidelines (2024).[38]

Plant Extracts Sensitivity Testing

Plant extract sensitivity tests were done using a modified method described elsewhere.[39] Five different concentrations were prepared in duplicates, in test tubes by dissolving 0.8g (800mg) of the extract in 2mL of distilled water to yield 400mg/mL of the stock solution. Further dilution was performed by pipetting 2 mL stock concentration and diluting it in other test tubes containing 2 mL sterile water to form a concentration of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL. A sterile cork borer 6 mm in diameter was then aseptically made through holes, with adequate spacing between wells, which would not have overlapping zones of inhibition. Afterward, 0.1 mL of each concentration of the plant extract was added to wells and left to diffuse into the medium for 45 minutes in a 37°C incubation period for 24 hours. The plates were examined later for zones of inhibition produced by the extracts, showing the degree of susceptibility of the test organisms toward the extracts. Diameters of inhibition zones were measured and recorded. Triplicate plates for each isolate were prepared.

Statistical Analysis

Multiple antibiotic resistances of the isolates to the antibiotics, as well as their resistances to the methanol plant extracts, were analyzed with descriptive statistics using SPSS IBM version 21.

Results and Discussions

Prevalence of the Isolates

Results showed a high prevalence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* based on samples collected (Table 1). Out of 40 samples screened for *S. aureus*, 30 (75%) were positive. Similarly, 25 (83.3%) of the 30 samples screened for *P. aeruginosa* were positive while 11 (78.6%) of the 14 samples screened for *K. pneumonia* were positive. This buttresses the reports of cases of 'ESCAPE' pathogens in hospital environments in some recent studies.[40, 41, 42] Incidence of infections caused by drug-resistant organisms is increasing in hospitals and other clinical care settings with a resultant increase in duration of hospital stay and hospital costs. These infections pose diagnosis and treatment challenges and cause increased morbidity and mortality. These challenges have necessitated the increased search for cheaper alternatives with fewer side effects to improve or restore the activity of commonly used antibiotics.[43] The occurrences of these organisms in hospital environments are of public health concern as they prolong treatment duration. This may have resulted from poor hygiene in the hospital environment, inadequate care of the wound (such as improper wound dressing), and the use of medical devices such as catheter.

Antibiotics Sensitivity and Resistance Indices

The results of the tests showed that all the isolates presumptively identified as *Staphylococcus aureus* (100%) were resistant to Ceftriazone while 91.18%, 85.29%, 79.41%, 47.06%, 38.24%, and 20.59% were resistance to Ceftazidime, Cefuroxime, Erythromycin, Amoxycillin/Clavulanate, Ofloxacin and Gentamicin respectively. However, for isolates identified as *Pseudomonas aeruginosa*, 100% were resistant to Ceftazidime, Cefuroxime, Amoxycillin/Clavulanate, Nitrofuratoin, and Ampicillin while 23.33%, 16.67% and 13.33% of the isolates showed resistance to Ofloxacin, Ciprofloxacin and Gentamicin respectively. Whereas, isolates identified as *Klebsiella pneumoniae* showed 100% resistance to Cefuroxime, Amoxycillin/Clavulanate, and Ampicillin; 23.08%, 15.39%, and 7.69% showed resistance to ofloxacin, ciprofloxacin and gentamicin respectively. The Multiple antibiotic resistance indices (MARI) of the isolates ranged from 0.5 to 1.0. Two isolates of *Staphylococcus aureus* with resistance to eight antibiotics had a MARI of 1.0; eleven isolates with resistance to seven antibiotics had a MARI of 0.9; four of the isolates with resistance to six antibiotics had a MARI of 0.8; six of the isolates with resistance to five antibiotics had a MARI of 0.6 and eleven isolates with resistance to four antibiotics had a MARI of 0.5.

The MARI of *Pseudomonas aeruginosa* shows that three isolates with resistance to eight antibiotics had a MARI of 1.0; three isolates with resistance to seven antibiotics had a MARI of 0.9; three isolates with resistance to six antibiotics had a MARI of 0.8 and twenty-one isolates with resistance to five antibiotics had a MARI of 0.6. *Klebsiella pneumonia* also showed high MARI. One isolate with resistance to seven of the antibiotics had a MARI of 0.9; two of the isolates with resistance to six antibiotics had a MAR Index of 0.8 and ten isolates with resistance to four antibiotics had a MARI of 0.5. The antibiotic resistance and indices indices of the isolates are shown in Tables 2, 3 & 4.

Antibiotic susceptibility profiles of the isolates revealed high and multiple resistance to most of the antibiotics used compared to the sensitivity pattern to the different drugs used worldwide. Bacterial resistance to antibiotics has been attributed to indiscriminate use of antibiotics in both hospital and non-hospital environments. The resistant pattern cuts across different classes of antibiotics. However, there was a slight difference in the resistant pattern between the Gram-positive (*S. aureus*) and the Gram-negative (*P. aeriginosa* and *K. pneumonia*) in terms of the class of antibiotics (Tables 2, 3 & 4). This reason is not far from the intrinsic nature of the organisms.

The antibiotic-resistant index (MARI) shows that all the isolates used in this study recorded MARI ranging from 0.5 to 1.0 making them multidrug-resistant isolates. This is not unconnected with the fact that they were isolated from antibiotic-laden sources especially being that they were isolated from clinical (wounds, urine, and environmental) samples, thus agreeing with reports that antibiotic-laden environments promote resistance.[44] High MARI (> 0.2) could be attributed to ineffective treatment of infections which is an important factor in the emergence of antibiotic-resistant bacteria. Ineffective treatment could be a result of prolonged hospital stay, prior use of antibiotics at low dosages, spread of antibiotic-resistant organisms through inadequate sanitary conditions, inadequate hospital infection control procedures, prolonged usage, and regular use (abuse) of antibiotics in our society.

Plant Methanol Extract Susceptibility Test (Mean Values of Zones of Inhibition (mm)

The inhibition zones for *Newbouldia leaves* methanol crude extract varied from 5.5 mm to 18 mm, directly proportional to the concentration. At 400 mg/mL and 200 mg/mL, all the *S. aureus* isolates tested, 100%, were susceptible. At 100 mg/mL, seven isolates, 87.5 %, were susceptible, and at 50 mg/mL, five isolates, 52.5% were susceptible, respectively. For *P. aeruginosa*, all seven tested isolates, that is, 100%, were susceptible at 400 mg/mL and 200 mg/mL, while at a concentration of 100 mg/mL, six isolates, 85.7%, were susceptible. At 50 mg/mL, four isolates, 57.1%, showed susceptibility (Figure 2). In the case of *K. pneumoniae*, all the six tested isolates, 100%, were susceptible at 400 mg/mL and 200 mg/mL. At 100 mg/mL, five isolates were susceptible, and at 50 mg/mL, susceptibility was obtained in only two isolates. For the methanol crude extract of *Dracaena arborea*, no zone of inhibition was observed at any of the concentrations used (Table 5).

This study investigated the antibacterial properties of *Newbouldia laevis* leaf extracts against *Staphylococcus aureus*, Pseudomonas aeruginosa, and *Klebsiella pneumoniae*. The extract effectively inhibited the growth of these bacteria, with varying degrees of potency. The zones of inhibition ranged from 6mm to 18.5mm for *S. aureus*, 8mm to 22 mm for *P. aeruginosa*, and 5.5 mm to 14.5 mm for *K. pneumoniae* (Figures 1, 2 & 3). These findings align with previous research.[22, 45, 46] The effectiveness of the extract against these common pathogens supports the traditional use of *Newbouldia laevis* leaves in complementary medicine for treating wound infections, urinary tract infections, and other bacterial ailments.[28, 29, 30, 27] This study also revealed that *Dracaena arborea* leaf extract has no antibacterial effect against the multidrug-resistant ESKAPE organisms tested. This may be due to insufficient quantities of medicinal phytochemicals. We are not aware of any published report on the antibacterial activity of *D. arborea* exract on bacteria or fungi. The recent success of plant-based extracts in clinical trials has sparked a surge of interest in medicinal plants. These plants hold promise not only for treating diseases directly but also as sources for developing new drugs. Herbal medicine, with its long history of use, already plays a vital role in primary healthcare, particularly in rural areas.

This study found that Gram-negative bacteria were less sensitive to the crude methanol extract of *Newbouldia laevis* compared to Gram-positive bacteria. This aligns with previous research suggesting that plant extracts often have a stronger effect on Gram-positive bacteria.[47] The difference in cell wall structure likely plays a role in this observation. Gram-positive bacteria lack the outer membrane and periplasmic space present in Gram-negative bacteria. This simpler structure allows antibacterial substances to more easily penetrate the cell wall and cytoplasmic membrane of Gram-positive bacteria, leading to leakage of cellular contents and cell death. In contrast, Gram-negative bacteria have a complex outer membrane rich in lipopolysaccharides. This outer membrane acts as a barrier, hindering the entry of many antimicrobial agents.[48]

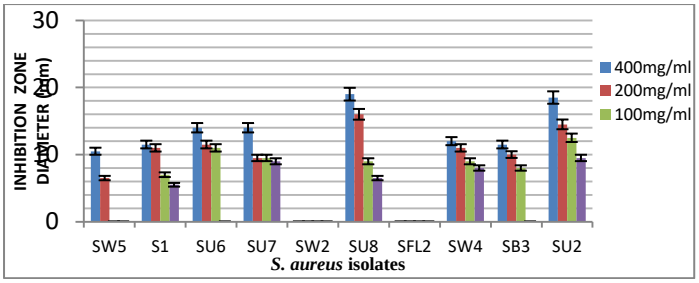

Figure 1: Selected *S. aureus* isolates susceptibility to methanol extract of *Newbouldialaevis* (mean values of zones of inhibition) (mm)

Key: SW = *S. aureus* from wound sample, SU = *S. aureus* from urine sample, SFL = *S. aureus* from floor sample, SB = *S. aureus* from Bed sample, S = Staphylococcus isolate, 1, 2, 3,… = sample numbers.

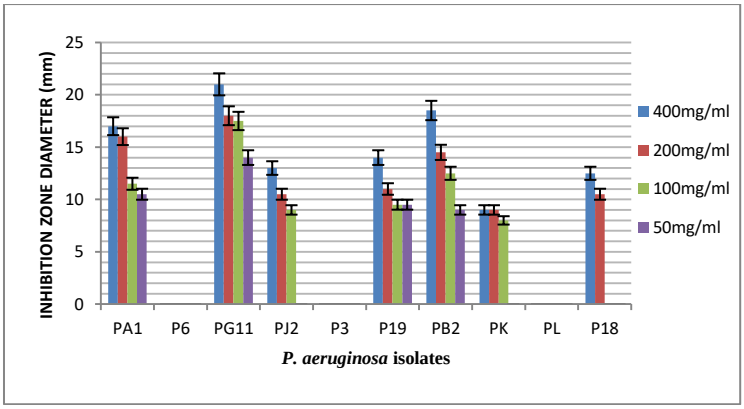

Figure 2: Selected *P. aeruginosa* isolates susceptibility to methanol extract of *Newbouldialaevis* (mean values of zones of inhibition) (mm)

Note: All P. aeruginosa isolates were gotten from wound. The lalel "PA1, P6, PG11… serves to differentiate one sample from the other

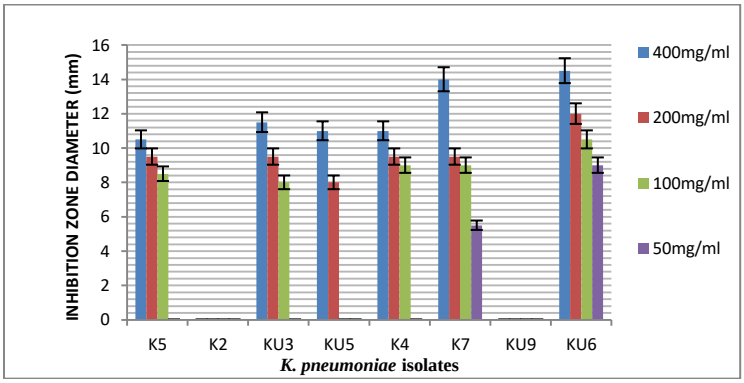

Figure 3: Selected *K. pneumoniae* isolates susceptibility to methanol extract of *Newbouldialaevis* (mean values of zones of inhibition) (mm)

Key:K = *Klebsiella pneumonia*, KU = urine isolate of *Klebsillapneumoniae*

Table 1: Percentage Prevalence of Some ESKAPE Pathogens from Different Hospital Based Samples

Growth medium	No of samples tested	Biochemical test						No positive	Suspected organism	% prevalence
		Coagulase	Catalase	Citrate	Indole	Oxidase	Urease			
MSA	40	+	+	-	-	-	-	30	*S. aureus*	75%
CA	30	-	+	+	+	+	-	25	*P. aeruginosa*	83.3%
CLED	14	-	+	+	-	-	+	11	*K. pneumoniae*	78.6%

Key: MSA= Manitol Salt Agar, CA = Cetrimide Agar, CLED Agar = Cystine Lactose Electrolyte Deficient Agar

Conclusion

This study demonstrates that extracts from the *N. laevis* leaf hold promise for treating wound infections, urinary tract infections, and infections caused by multidrug-resistant ESKAPE bacteria. The extract's effectiveness in inhibiting the growth of these harmful pathogens supports traditional uses of this plant for treating infections. Furthermore, the inhibitory properties of *N. laevis* suggest its potential as a valuable source for developing new antimicrobial drugs. These drugs could potentially combat infections caused by *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. The findings of this study can benefit both scientists and the general public. Scientists can explore *N. laevis* further for its medicinal potential, while the public can gain a broader understanding of the plant's potential health benefits, potentially validating local knowledge about its uses.

Table 2: Multiple Antibiotic Resistance Index (MARI) of *S. aureus* isolates

No. of *S. aureus*	Total No. of antibiotics tested	Total no. of antibiotics resisted	Antibiotics resisted	MARI
3	8	8	ERY,CXC,OFL,AUG,CAZ,GEN,CTR,CRX	1.0
2	8	7	ERY,CXC,OFL, CAZ, CRX, GEN, CTR	0.9
1	8	7	ERY,CXC,CAZ,CRX,GEN,CTR,AUG	0.9
3	8	6	ERY,CXC,AUG,CAZ,CRX,CTR	0.8
1	8	6	ERY,OFL,CAZ,CRX,GEN,CTR	0.8
2	8	5	ERY,CXC,CAZ,CRX,CTR	0.6
1	8	5	ERY,CXC,AUG,CRX,CTR	0.6
1	8	5	CXC, AUG, CAZ, CRX,CTR	0.6
2	8	5	ERY,CXC,CAZ,CRX, CTR	0.6
5	8	4	ERY,CAZ,CRX,CTR	0.5
4	8	4	ERY,CXC,CAZ, CTR	0.5

Key: ERY=Erythromycin; CXC=Cloxicillin; CAZ=Ceftazidime; CRX = Cefuroxime; CTR = Ceftriaxone; AUG = Clauvulanate/Amoxicillin; OFL = Ofloxacin; GEN = Gentamicin

Table 3: Multiple Antibiotic Resistance Index (MA**RI) of *P. aeruginosa* isolates**

No. *P. Aeruginosa* isolates	Total No. of antibiotics tested	Total No. of antibiotics resisted	Antibiotics resisted	MARI
3	8	8	CAZ,CRX,CPR,OFL,AUG,GEN,AMP, NIT	1.0
3	8	7	CAZ,CRX,CPR,OFL,AUG,AMP, NIT	0.9
3	8	6	CAZ,CRX,OFL,AUG,AMP, NIT	0.8
21	8	5	CAZ,CRX,AUG,AMP, NIT	0.6

KEY: CAZ=Ceftazidime; CRX=Cefuroxime; CTR=Ceftriaxone; NIT=Nitrofuratoin; AUG=Clauvulanate/Amoxicillin; OFL=Ofloxacin; GEN=Gentamicin; CIP=Ciprofloxacin

Table 4: Multiple Antibiotic Resistance Index of *K. pneumoniae* isolates

No. *K. pnuemoniae* isolates	Total No. of antibiotics tested	Total No. of antibiotics resisted	Antibiotics resisted	MARI
1	8	7	CAZ,CRX,CPR,OFL,AUG,GEN,AMP	0.9
2	8	6	CAZ, CRX, CPR, OFL, AUG, AMP	0.8
11	8	4	CAZ, CRX, AUG, AMP	0.5

KEY: CAZ=Ceftazidime; CRX=Cefuroxime; CTR=Ceftriaxone; NIT=Nitrofuratoin; AUG=Clauvulanate/Amoxicillin; OFL=Ofloxacin; GEN=Gentamicin; CIP=Ciprofloxacin

Table 5: Antibacterial activity of *D. arborea* on some members of the ESKAPE pathogens

ISOLATES	400 mg/mL	200 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL
SI	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SW4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SU6	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SU7	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PA1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PB2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PJ2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KU3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KU5	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Key: SW = *S. aureus* from wound sample, SU = *S. aureus* from urine sample, SFL = *S. aureus* from floor sample, SB = *S. aureus* from Bed sample, S = Staphylococcus isolate, 1, 2, 3,… = sample numbers,K = *Klebsiella pneumonia*, KU = urine isolate of *Klebsillapneumoniae*
Note: All *P. aeruginosa* isolates were gotten from wound. The lalel "PA1, P6, PG11… serves to differentiate one sample from the other

Conflict of Interest

The 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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