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Original Article

Inhibitory Effect of *Newbouldia laevis* and *Stereospermum acuminatissimum* on Multi-Resistant Bacteria Isolates

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ABSTRACT

Aim: Multi-drug-resistant bacteria is a growing concern worldwide. Plants have emerged as a promising avenue due to their bioactive compounds. *Newbouldia laevis* and *Stereospermum acuminatissimum* are two plants with a long history of use in traditional medicine. This study aimed at exploring the antimicrobial activity of their extracts against multi-drug-resistant bacteria. **Material and Methods:** Extracts were prepared by maceration of pulverized leaves or stem barks (250 g) in 2L dichloromethane/methanol (30/70) filtered and the filtrate was dried using a rotary evaporator. The crude extracts were then tested against five pathogenic multi-resistant bacterial strains and Minimum Inhibition Concentration (MIC) was determined using the broth dilution method. The combinations of extracts and reference drug ceftriaxone was carried out following the checkerboard technique. **Results:** The overall results showed that *Newbouldia laevis* and *Stereospermum acuminatissimum* crude extracts contain various phytochemicals such as alkaloids, flavonoids, terpenoids, and polyphenols, which were found to be associated with strong anti-bacterial effects against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*, exhibiting MICs values ranging from 100 µg/mL to 400 µg/mL. The combinatorial analysis showed a combination and strains dependent-out, including synergism, additivity, and antagonism. **Conclusion:** These findings suggest that *Newbouldia laevis* and *Stereospermum acuminatissimum* could be used as alternative sources of antimicrobial agents for treating bacterial infections.

Keywords: Antibacterial, Combination, Multi-resistance, *Newbouldia laevis*, *Stereospermum acuminatissimum*.

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INTRODUCTION

Antimicrobial resistance is a global public health threat that has been a growing concern for several decades. The emergence and spread of multi-drug resistant bacteria have made treating infectious diseases increasingly challenging, leading to increased morbidity, mortality and healthcare costs.[1] However, according to the World Health Organisation (WHO), antimicrobial resistance is a global public health threat affecting all regions of the world. The prevalence and trends of antimicrobial resistance vary by region and country, with some areas experiencing higher rates than others. In Africa, antimicrobial resistance is a growing concern due to various factors including high rate of infectious diseases, limited access to healthcare and overuse or poor use of available antibiotics. In 2018, the WHO reported that resistance to commonly used antibiotics is increasing in many African countries, especially those with high burdens of HIV/AIDS, tuberculosis, and malaria.[2-4]

Globally, around 700,000 people are dying every year due to antimicrobial resistance, inducing an economic impact of 100 trillion dollars by 2050.[5-9] This highlighted the need for increasing efforts to control and prevent the spread of antibiotic resistance in Africa. Amongst other strategies, WHO stressed that new drugs are need to tackle the actual pan-resistant strains.[10] Traditional medicinal plants have been used for centuries in the treatment of various ailments, including infections. In recent years there has been renewed interest in the use of traditional medicinal plants as potential sources of new antimicrobial agents.

Belonging to the Bignoniaceae family, *Newbouldia laevis* and *Stereospermum acuminassitimum* are two medicinal plants

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folklorically used in traditional medicine for the treatment of various ailments. *Newbouldia laevis* is a fast-growing evergreen shrub native to West Africa and distributed from Senegal to Cameroon and Gabon. The plant is commonly found in secondary and dried forests, woody savannah and deciduous forests. In pharmacopeia, its barks are utilized to cure coughs, diarrhoea, chest pain, dysentery, epilepsy, convulsion, uterine colic, dysmenorrhea, wounds, abscesses, and ulcers. A decoction of the leaves is used to treat ophthalmia and conjunctivitis, and powdered roots are used in the treatment of intestinal problems.[1,11-21] *Stereospermum acuminassitimum* is a medicinal plant native to tropical areas of India and Africa. Various parts of the plant including bark, leaves, and roots are used for the management of various pathological conditions such as respiratory disorders, skin diseases, digestive disorders, fever, and postpartum haemorrhage. Phytochemical screening and pharmacological investigations pointed out their rich phenolics, glycosides, anthraquinones, volatile oils, tannins, glycosides, steroids, alkaloids, flavonoids, and terpenoids content associated to significant antimicrobial, anti-inflammatory, and anthelmintic properties.[22-24] Based on the aforementioned, the present work was undertaken to investigate the effect of these plants on multi-drug resistant strains and the combinatory effect of the plant extracts with a reference antibiotic.

MATERIALS AND METHODS

Microbial strains

Microbial strains were clinical isolates graciously offered by the bacteriology unit of the Gynaecology and Paediatric Hospital of Douala, Cameroon. Therefore, the authors did not face patients during the study. The strains were identified by automatic colorimetric reading on VITEK 2™ (BIOMERIEUX SA, France) after plating into Eosin Methylene Blue agar and incubation at 37°C for 24 hr. Susceptibility tests were performed by dilution in cards, and automatic turbidimetric reading on VITEK 2. The isolates, site of harvest and susceptibility are presented in Table 1.

Plants Material

The stem barks and leaves of *Newbouldia laevis* were harvested in Tonga, a village situated in the Ndé Division, West region of Cameroon. *Stereospermum acuminatissimum* (leaves and bark) was harvested at Mekalat, a neighbourhood village of Ebolowa, South region of Cameroon. The taxonomic authentication was done at the Cameroon National Herbarium, Yaoundé, in comparison with voucher specimen numbers 29469/ HNC and 59169/ HNC for *Newbouldia laevis* and *Stereospermum acuminatissimum* respectively.

Extracts preparation

The freshly collected leaves and stem barks were shade-dried for 2 weeks and pulverised using and electric grinder. Then, 250 g of the powder was soaked in 2 L of DCM/methanol (3:7) in a glass container for 72 hr with multiple-daily shaking. Thereafter, the mixture was filtered using Whatman paper n°1, and the filtrate was concentrated using rotary evaporator at 65°C. The crude extracts were then left overnight in the oven at 45°C resulting in complete evaporation of the solvent. Each extract was weighted to determine the yield of extraction using the formula below: then, stored at 4°C until usage.

$$Percentage\ yield\ (\%) = \frac{crude\ extract\ (g)}{powdered\ plant\ (g)}\ \text{x}\ 100.$$

Qualitative phytochemical screening

The prepared crude extracts were qualitatively screened for their phytochemical composition following standard procedures as describe in the literature.[25] Common phytochemicals family named tannins, saponins, flavonoids, alkaloids, terpenes, steroids, phenols, coumarins, glycosides, and quinones were investigated. The tests were based on visual observation of the reaction mixture colouration or the formation of precipitates after addition of a specific reagent.

Evaluation of the antimicrobial activity of crude extracts

Determination of Minimum Inhibitory Concentrations (MICs) of extracts against bacterial species

The MICs of extracts against bacteria were determined as described by Ameja,[22] using the 96-well microtiter plate format. Fifty microliters (50 µL) of twofold diluted extracts in a nutrient broth medium were introduced into the wells of the plate. Thereafter, 50 µL of the bacterial inoculum standardized at 0.5 McFarland were added into each well containing the test substances except the blank column used for sterility control. The extracts and ceftriaxone (standard drug) concentrations ranged from 3.125 to 100 µg/mL and 0.15625 to 5 µg/mL, respectively. Plates were incubated for 24 hr at 37°C, and 10 µL of resazurin was added to confirm the cell growth. The lowest concentration inhibiting the visible growth of bacteria was recorded (here maintaining the blue colour of resazurin). Extracts with the best antibacterial activity were selected for the antioxidant and cytotoxic assays.

Determination of the Minimum Bactericidal Concentration (MBC)

The broth microdilution method as described by Santo with slight modifications was used to examine the MBC for the antimicrobials.[30] Briefly, 50 µL of samples from wells with no visible growth in the MIC assay and introduced into a microtiter plate containing 150 µL of MHB. The plate was covered with a sterile sealer and incubated for 72 hr at 37°C. 10 µL Resazurin was later added to each well of the microtiter plate and was incubated at 37°C for 30min. The wells containing bacterial growth turned

pink colour whereas the well without bacterial growth remained blue. Based on the Minimum Inhibitory Concentration (MIC) obtained, the antimicrobial activity of the extracts was considered as follows according to Kuete's scale, 2010.[31]

Significant (strong) inhibition: MIC less than 100 µg/mL;

Moderate inhibition: MIC ranges from 100 µg/mL to 625 µg/mL;

Low inhibition: MIC greater than 625 µg/mL.

Determination of combinatory parameters

The interaction mechanism was determined using Fractional Inhibitory Concentration Index (FICI) method, which consisted in combining most efficient extracts with ceftriaxone at various concentrations (two-fold dilution from 400 µg/mL). Briefly, crude extracts were serially diluted along the y-axis of the microplate, while ceftriaxone was serially diluted along the x-axis. The final concentration ranged from 3.125 µg/mL to 400 µg/mL. Each well-contained 50 µL of a unique combination of crude extract and standard antibiotic. 50 µL of bacterial suspension containing $3x10^6$ CFU/mL was added in each well. The plates were covered er and incubated overnight at 37°C. Then, 10 µL of resazurin was added into each well and the plate was incubated at 37°C for 30 min. The wells containing bacterial growth turned into pink colour whereas the well without bacterial growth remained blue which was considered as an effective MIC for the combination. The interactions between antimicrobial agents were determined and quantified by calculating the Fractional Inhibitory Concentration (FIC) index for each combination of plant extract and standard antibiotic using the following formula:

$$FICI = FICa + FICb$$

$$FICa = \frac{MIC\ of\ plant\ extract\ in\ combination}{MIC\ of\ plant\ extract\ alone}$$

$$FICb = \frac{MIC\ of\ antibiotic\ in\ combination}{MIC\ of\ antibiotic\ alone}$$

Where; **FICI:** Fractional inhibitory concentration index; **FICa:** Fractional inhibitory concentration of plant extract; **FICb:** Fractional inhibitory concentration of antibiotic.

The interaction mechanism was considered as synergistic, additivity, indifferent (non-intercative) or antagonism for FICI values≤0.75, ranged between 0.76-1.0, ranged between 1- 4 or higher than 4, respectively.

RESULTS AND DISCUSSION

Extraction yield and phytochemical screening results

Table 2 summarized the colour and extraction yield of each tested extract, which was prepared by cold maceration method using DCM/methanol as solvent. The leaves extract of *S. acuminatissimum* yielded 8.54%, and the stem bark yielded 3.4%. Similarly, the extraction yield of *N. laevis* leaves was found to be superior to the stem bark, exhibiting 5.68% and 2.73% yield respectively (Table 2).

The qualitative phytochemical analysis of these crude extracts showed that both plants are a rich source of secondary metabolites of various classes including flavonoids, tannins, and alkaloids. Steroids were present in *S. acuminatissimum* extracts (leaves and bark), and absent in *N. laevis*. Only the crude leaves of *N. laevis* contended glycosides. Coumarins and Quinone were not detected either in *S. acuminatissimum* nor *N. laevis* extracts as shown in Table 3.

Antimicrobial inhibitory parameters

Minimum Inhibitory Concentration (MIC)

Table 4 shows the MIC of the plant extracts against multi-resistant bacteria strains. It can be observed that all extracts were active on all bacteria strains. *S. acuminatissimum* stem bark exhibited significant activity on *E. coli* MDR1 and *S. aureus* at the concentration of 200 µg/mL. *N. laevis* bark extract induced significant activity on *E. coli* MDR1, *S. aureus,* and *P. aeruginosa*

Table 1: Multidrug-resistant bacteria strains.

Isolates	Place of harvest	Sensitive	Resistant
Pseudomonas aeruginosa	Wound swap	Meropenem, ertapenem, ciprofloxacine, ofloxacine.	**Ceftriaxone**, ceftazidime, cefoxitin, cefamandole, cotrimoxazole, cefuroxime.
Escherichia coli MDR1	Urine	Cefepime, cefpodoxime, chloramphenicol.	Colistin sulphate, norfloxacine, ertapenem, meropenem, levofloxacin, **ceftriaxone,** ceftazidime.
Staphylococcus aureus	Pus	Norfloxacine, levofloxacin, doxycycline, ciprofloxacine, azithromycin, pristinamycine.	Meropenem, ertapenem, cefoxitine, lincomycin.
Klebsiella pneumoniae	Urine	Norfloxacine, levofloxacine, cefoxitine, chloramphenicol, ciprofloxacine, meropenem.	**Ceftriaxone**, cefepime, ceftazidime, colistin sulphate, cefuroxime, cefpodoxime, Nitrofurantoin.
Escherichia coli MDR2	Blood	Ciprofloxacine, meropenem, ofloxacine, ertapenem.	**Ceftriaxone,** nitrofurantoin, colistin sulphate, cefoxitine.

MDR1: Multidrug resistant 1; MDR2: Multidrug resistant 2.

Table 2: Extraction yield of various parts of tested plants.

Plant species	Part used	Yield (%)	Colour
S. acuminatissimum	Stem bark	3.40	Brown
	Leaves	8.54	Dark green
N. laevis	Stem bark	2.73	Brown
	Leaves	5.68	Dark green

S. acuminatissimum: *Stereospermum acuminatissimum;* ***N. laevis:*** *Newbouldia laevis.*

Table 3: Phytochemical constituents of prepared crude extracts.

Metabolites	*S. acuminatissimum*		*N. laevis*	
	Stem bark	Leaves	Stem bark	Leaves
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Phenols	+	+	-	+
Coumarins	-	-	-	-
Saponins	+	+	+	-
Glycosides	-	-	-	+
Tannins	+	+	+	+
Terpenes	+	-	+	+
Steroids	+	+	-	-
Quinones	-	-	-	-

S. acuminatissimum*:** *Stereospermum acuminatissimum*; ***N. laevis: *Newbouldia laevis*; Present: +; Absent: - .

at 100 and 200 µg/mL. Also, *N. laevis* leaves extract showed significant activity on *E. coli* MDR1 at the concentration of 100 µg/mL. Ceftriaxone used as standard drug was found active on all bacterial strains with maximum effect on *P. aeruginosa* and *E. coli MDR2*.

Minimum Bactericidal Concentration (MBC)

As presented in Table 5, all tested plant extracts inhibited micro-organisms growth at concentrations ranged 100-400 µg/mL. However, no death was found for concentrations ≤400 µg/mL. On the other hand, ceftriaxone tested at 400 µg/mL inhibited growth and killed all the microorganisms and hence considered bactericidal.

Evaluation of the combinatory effect of *Stereospermum acuminatissimum* stem bark extract with ceftriaxone against multi-resistant bacteria

The combination of *S. acuminatissimum* stem bark extract with ceftriaxone showed a synergistic effect with a FICI index of 0.75 on *E. coli* MDR2. This combination showed indifference effect on the other bacteria strains with 1.5≥FICI≤2, which means that the combination of ceftriaxone and the extract has a greater inhibitory effect on the growth of the bacteria compared to either component alone. No synergistic or an antagonistic effect was observed as depicted in Table 6.

Evaluation of the synergistic effect of *Stereospermum acuminatissimum* leaf extract with ceftriaxone against multi-resistant bacteria

Table 7 shows the combination of *S. acuminatissimum* leaf extract with ceftriaxone against multi-resistant bacteria. An antagonistic effect was observed on *P. aeruginosa* as the MIC of ceftriaxone increases from 100 µg/mL to 400 µg/mL. this fact highlights the fact that compounds from the *S. acuminatissimum* leaf extract are modulating the response of *P. aeruginosa* to ceftriaxone.

Evaluation of the synergistic effect of *Newbouldia laevis* stem bark extract with ceftriaxone against multi-resistant bacteria

The combination of *N. laevis* stem bark extract with ceftriaxone showed a synergistic effect on *E. coli MDR2* (FICI=0.75). Table 8 highlights an indifference to all the other bacteria strains used in this study.

Evaluation of the synergistic effect of *Newbouldia laevis* leaf extract with ceftriaxone against multi-resistant bacteria

The combination of *N. laevis* leaf extract with ceftriaxone showed a synergistic on *P. aeruginosa* and *S. aureus* with FICI=0.5 and 0.75 respectively. An antagonistic effect was observed on *E. coli* MDR1 (FICI=5). Regarding *P. aeruginosa* compounds from *N. laevis* leave extracts seem to negatively modulate the natural

Table 4: The MIC of the different extracts.

Bacteria	*S.a/B* (µg/mL)	*S.a/L* (µg/mL)	*N.l/B* (µg/mL)	*N. /L* (µg/mL)	Ceftriaxone (µg/mL)
P. aeruginosa	400	400	200	400	100
E. coli MDR1	200	400	100	100	400
S. aureus	200	400	200	400	400
K. pneumoniae	400	400	400	400	200
E. coli MDR2	400	400	400	400	100

S. a: *Stereospermum acuminatissimum*; ***N. l:*** *Newbouldia laevis*; **B:** Stem Bark; **L:** Leaves.

Table 5: MBC of the plant extracts in µg/mL.

Extract	*P. a*	*E. c* MDR1	*S. aureus*	*K. p*	*E. c* MDR2	Conclusion
S. A/ B	>400	>200	>200	>400	>400	Bacteriostatic
S. A/ L	>400	>400	>400	>400	>400	Bacteriostatic
N.L/ B	>200	>100	>200	>400	>400	Bacteriostatic
N. L/ L	>400	>100	>400	>400	>400	Bacteriostatic
Ceftriaxone	-	-	-	-	-	inactive

S. a: *Stereospermum acuminatissimum;* ***N. l:*** *Newbouldia laevis*; **B:** Bark; **L:** Leaf; ***P. a:*** *Pseudomonas aeruginosa;* ***E. c:*** *Escherichia coli;* ***K. p****: Klebsiella pneumoniae.*

Table 6: Combination of *Stereospermum acuminatissimum* stem bark extract with ceftriaxone against multi-resistant bacteria strains

Bacteria	MICc S.a/B	FICa	MICc CEF	FICb	FICI	Interpretation
P. aeruginosa	400	1	100	0.5	1.5	Indifferent
E. coli MDR1	400	2	400	1	3	Indifferent
S. aureus	400	2	400	0.125	2.125	Indifferent
K. pneumoniae	400	1	200	1	2	Indifferent
E. coli MDR2	200	0.5	400	0.25	0.75	Synergy

S. a/B: *Stereospermum acuminatissimum* stem bark; **CEF:** Ceftriaxone; **MIC:** Minimum inhibitory concentration; **FIC:** Fractional inhibitory concentration; **FICI:** Fractional inhibitory concentration index. ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***E. coli MDR1:*** *Escherichia coli strain 1;* ***S. aureus:*** *Staphylococcus aureus;* ***K. pneumoniae:*** *Klebsiella pneumoniae.*

Table 7: Combination of *Stereospermum acuminatissimum* leaf extract with ceftriaxone against multi-resistant bacteria.

Bacteria	MICc*S.al*	FICa	MICc EF	FICb	FICI	Interpretation
P. aeruginosa	400	1	400	4	5	Antagonistic
E. coli MDR1	400	1	400	1	2	Indifferent
S. aureus	400	1	400	1	2	Indifferent
K. pneumoniae	400	1	400	2	3	Indifferent
E. coli MDR2	400	1	400	2	2	Indifferent

S. al: *Stereospermum acuminatissimum* leave; **CEF:** Ceftriaxone; **MICc:** Minimum inhibitory concentration in combination; **FIC:** Fractional inhibitory concentration; **FICI:** Fractional inhibitory concentration index. ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***E. coli MDR1:*** *Escherichia coli strain 1;* ***S. aureus:*** *staphylococcus aureus;* ***K. pneumoniae:*** *Klebsiella pneumoniae.*

resistance of *P. aeruginosa* to ceftriaxone. An indifferent effect was observed on the other bacteria strains as shown in Table 9.

DISCUSSION

Antibiotic resistance is a problem that continues to challenge the healthcare sector in a large part of the world in both developing and developed countries. The emergence and spread of multidrug-resistant pathogens have substantially threatened the current antibacterial therapy. This has necessitated a search for a new source of antimicrobial substances such as plants, as they produce a variety of bioactive compounds of known therapeutic properties. This study has been conducted to evaluate the antibacterial activity of *S. acuminatissimum* and *N. laevis* extracts against human pathogens.

Table 8: Combination of *Newbouldia laevis* stem bark extract with ceftriaxone against multi-resistant bacteria.

Bacteria	MICcNIb	FICa	MICc EF	FICb	FICI	Interpretation
P. aeruginosa	200	1	100	1	2	Indifferent
E. coli MDR1	200	2	400	1	3	Indifferent
S. aureus	6.25	0.031	400	1	1.031	Indifferent
K. pneumoniae	100	0.25	200	1	1.25	Indifferent
E. coli MDR2	100	0.25	200	0.5	0.75	synergy

N. lb: *Newbouldia laevis* stem bark; **CEF:** Ceftriaxone; **MICc:** Minimum inhibitory concentration in combination; **FIC:** Fractional inhibitory concentration; **FICI:** Fractional inhibitory concentration index. ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***E. coli MDR1:*** *Escherichia coli strain 1;* ***S. aureus:*** *staphylococcus aureus;* ***K. pneumoniae:*** *Klebsiella pneumoniae.*

Table 9: Combination of *Newbouldia laevis* leaf extract with ceftriaxone against multi-resistant bacteria

Bacteria	MICcNIl	FICa	MICc EF	FICb	FICI	Interpretation
P. aeruginosa	3.125	0.007	50	0.5	0.5	Synergistic
E. coli MDR1	400	4	400	1	5	Antagonism
S. aureus	100	0.25	200	0.5	0.75	Synergistic
K. pneumoniae	400	1	400	2	3	Indifferent
E. coli MDR2	400	1	400	1	2	Indifferent

Nll: *Newbouldia laevis* stem bark; **CEF:** Ceftriaxone; **MICc:** Minimum inhibitory concentration in combination; **FIC:** Fractional inhibitory concentration; **FICI:** Fractional inhibitory concentration index; ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***E. coli MDR1:*** *Escherichia coli strain 1;* ***S. aureus:*** *staphylococcus aureus;* ***K. pneumoniae:*** *Klebsiella pneumoniae.*

The yield of extraction obtained after cold maceration of *S. acuminatissimum* and *N. laevis* stem bark and leaves was found to be 2.73% and 5.68% and 3.4% w/w and 8.54% (w/w) respectively. Akerele *et al.*, obtained an inferior extraction yield (1.48 %) with stem barks using methanol as solvent. This result was at different with a study conducted by Lenta.[26] In this said study, the extraction yield gotten after maceration of the stem bark of *S. acuminatissimum* in methanol was 10% using 280g of powder. The extraction yield gotten after cold maceration of *N. laevis* stem bark and leaf was 2.37% w/w and 5.68% w/w respectively. In comparison with the 1.48%w/w of stem bark by Akerele and 7.05% w/w of leaf by Usman and Osuji.[1] This difference could be due to the plants geographical locations, harvesting period, drying duration, or temperature.[13] Further, the raw material particle size: has been proven to influence the extraction yield. In fact, smaller particle size increases the total mass transfer area per unit volume thereby, increasing the surface area of contact for better extraction.[26]

The qualitative phytochemical screening of *S. acuminatissimum* stem bark and leave extracts revealed the presence of various secondary metabolites such as flavonoids, phenols, saponins, tannins, terpenes, steroids and alkaloids. The stem bark and leave extracts of *N. lewis* showed similar phytochemical content, exception of phenols and glycoside found absent in the stem bark. Similar observations have been made by Izyani *et al.*, who noted the presence of anthraquinone, sterols, flavonoids, terpenes, tannins, and resins and phenols in *S. acuminatissimum* extract in addition to above-mentioned compounds [13]. Numerous studies have proven these phytochemicals to possess various biological potential including anti-microbial activities.[27] Akerele *et al.*,[13] reported the presence of flavonoids, tannins, saponins and alkaloids in *N. laevis* stem bark extract with significant antibacterial effects on isolates from infected wounds and eyes.[13] In this study, coumarins, and quinones were not detected either in *S. acuminatissimum* nor *N. laevis* extracts.

Regarding the Minimum Inhibitory Concentration (MIC), the MIC of our reference drug ceftriaxone ranged from 100 µg/mL to 400 µg/mL. According to Jones *et al.*,[28] the MIC of ceftriaxone ≤1 µg/mL is considered susceptible, 2 µg/mL-3 µg/mL intermediate or susceptible dose-dependent, and MIC values≥4 µg/mL are considered resistant on Gram-negative and Gram-positive bacteria.[28] In line with this, the MIC of ceftriaxone ranged from 100 µg/mL to 400 µg/mL indicating that all bacterial strains were resistant. *S. acuminatissimum* and *N. laevis* extracts exhibited significant activity against both gram-negative strains (*E. coli, P. aeruginosa, K. pneumoniae*), and gram-positive strain (*S. aureus*). This activity could be attributing to secondary metabolites mentioned above. Further, based on Kuete *et al.* scale it can be said that extract showed moderate inhibitory activity on *E. coli* MDR2, *P. aeruginosa, K. pneumoniae E. coli, and S. aureus.* Strains.[17]

Moreover, tested extract exhibited significant inhibitory effect against *E. coli* MDR1 with a (MIC: of 100 µg/mL). This observation corroborates the findings of Assob *et al.*,[29] who demonstrated the inhibitory activity of *N. laevis* against *E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae* amongst others bacteria.[29] This activity could be a step in reducing antibiotic

drug therapy resistance and promoting safe nontoxic natural remedies to bacterial infections in general.

In addition, tested plant extracts were found to be bacteriostatic at concentrations≤400 µg/mL as observed from the MBC results. The obtained Minimal Bactericidal Concentration (MBC) against *E. coli* and was 390 µg/mL. The reason for this difference could be the nature of the tested isolates; we carried out this study using multi-resistant bacteria isolates. This finding supports the traditional use of these plants for the treatment of stomach discomfort, diarrhoea, dysentery and as a remedy for wound healing whose causative agents are some of the microorganisms used in the present study.

Ceftriaxone and plant extracts were tested simultaneously in order to assess their combinatory effects. It is known that a synergistic effect occurs when two or more substances work together to produce an effect that is greater than the sum of their individual effects. Results indicated in that the plant extracts could enhance the antibiotic properties of ceftriaxone, making it more effective in treating infections. This could be due to bioactive compounds in the plant extracts that have antimicrobial properties or that can enhance the activity of antibiotics. However, further research is needed to confirm these findings, and to determine the specific mechanisms behind this potential synergistic effect. Also, identifying the most effective plant extract when combined with ceftriaxone is to be investigated.

CONCLUSION

The present study highlights that *N. laevis* and *S. acuminatissimum* extracts contained alkaloids, flavonoids, terpenes and phenols capable of inhibiting clinically isolate multi-resistant bacteria strains. Their combination with ceftriaxone (standard drug) increases the sensitivity of resistant strains indicating their usefulness in the fight against microbial resistance. This stands for further compounds isolation from *N. laevis* and *S. acuminatissimum* for their antibacterial effect against multiresistant and pan-resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CFU: Colony Forming Unit; **DCM:** Dichloromethane; **FICa:** Fractional inhibitory concentration of plant extract; **FICb:** Fractional inhibitory concentration of antibiotic; **FICI:** Fractional inhibitory concentration index; **MBC:** Minimal Bactericidal Concentration; **MDR:** Multidrug resistant; **MIC:** Minimum Inhibitory Concentration.

SUMMARY

- Antimicrobial resistance is a growing global public health concern, affecting all regions.
- Globally, around 700,000 people die annually due to antimicrobial resistance, causing an economic impact of $100 trillion by 2050.
- WHO emphasizes the need for new drugs to combat pan-resistant strains.
- Traditional medicinal plants are being explored as potential sources of new antimicrobial agents.
- S. Accuminatissimum leaves extract yielded 8.54%, while N. laevis leaves yielded 5.68% and 2.73% respectively.
- Both plants were rich sources of secondary metabolites including flavonoids, tannins, and alkaloids.

Antimicrobial Inhibitory Parameters

- All plant extracts were active against multi-resistant bacteria strains.
- The combination of *S. Accuminatissimum* stem bark extract and ceftriaxone showed a synergistic effect on *E. coli* MDR2.
- The combination of *S. Accuminatissimum* leaf extract with ceftriaxone showed an antagonistic effect on *P. aeruginosa*.
- *N. laevis stem* bark extract and ceftriaxone showed synergistic effect on *E. coli* MDR2 (FICI=0.75).
- No effect observed on other bacteria strains.
- *N. laevis* leaf extract and ceftriaxone showed synergistic effect on *P. aeruginosa* and *S. aureus* (FICI=0.5, 0.75 respectively).
- An antagonistic effect was observed on *E. coli* MDR1 (FICI=5).
- compounds from *N. laevis* leaf extracts negatively modulate *P. aeruginosa's* resistance to ceftriaxone.

REFERENCES

1. Usman H, Osuji JC. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis*. Afr J Tradit Complement Altern Med. 2007;4(4):476-80. doi: 10.4314/ajtcam.v4i4.31240, PMID 20161915.
2. World Health Organization. Antibiotic resistance.pdf. WHO; 2020. p. 6.
3. World Health Organization. Antimicrobial resistance.pdf. WHO; 2021. p. 8.
4. World Health Organization (WHO). Global action plan on antimicrobial resistance [internet]. World Health Organization; 2017. Available from: https://www.who.int/publications/i/item/9789241509763.
5. Ahmad M, Khan AU. Global economic impact of antibiotic resistance: a review. J Glob Antimicrob Resist. 2019;19:313- doi: 10.1016/j.jgar.2019.05.024, PMID 31176071.
6. Hofer U. The cost of antimicrobial resistance. Nat Rev Microbiol. 2019;17(1):3. doi: 10.1038/s41579-018-0125-x, PMID 30467331.
7. Renwick MJ, Simpkin V, Mossialos E. Targeting innovation in antibiotic drug discovery and development. Target Innov Antibiot drug Discov Dev need a one Heal-One Eur-one World Framew (June); 2016.
8. Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist. 2019;12:3903-10. doi: 10.2147/IDR.S234610, PMID 31908502.
9. Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, Gray AP. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022;400(10369):2221-48. doi: 10.1016/S0140-6736(22)02185-7, PMID 36423648.
10. World health organization. WHO publishes list of bacteria for which new antibiotics are urgently needed [internet]. 2017;38:444-5. Saudi Med J. Available from: https:/

/www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

11. Okagu IU, Ndefo JC, Agbo MO. Trado-medical uses, chemical constituents and biological activities of *Newbouldia laevis* (Bignoniaceae): a review. Pharm Sci. 2021;28(1):51-75. doi: 10.34172/PS.2021.29.
12. Noumi E, Fozi FL. Ethnomedical botany of epilepsy treatment in Fongo-Tongo village, Western Province, Cameroon. Pharm Biol. 2003;41(5):330-9. doi: 10.1076/phbi.41.5.330.15944.
13. Akerele JO, Ayinde BA, Ngiagah J. Phytochemical and antibacterial evaluations of the stem bark of *Newbouldia laevis* against isolates from infected wounds and eyes. Trop J Pharm Res. 2011;10(2):211-8. doi: 10.4314/tjpr.v10i2.66566.
14. Rashed K. Phytochemical and Biological effects of *Newbouldia laevis*: a review. Plantae Sci. 2021;4(4-5):208-13.
15. Dermane A, Kpegba K, Eloh K, Osei-Safo D, Amewu RK, Caboni P. Differential constituents in roots, stems and leaves of *Newbouldia laevis* Thunb. screened by LC/ESI-Q-TOF-MS. Results Chem. 2020;2:100052. doi: 10.1016/j.rechem.2020.100052.
16. Bwegne JGBindiN, Ayina CNA. Abraham Hermann Bagbila wend Pagnangdé. Noël Babayana Etaga, Quelie Selakong Nzekuie, Macaire Samdpawindé Ouedraogo, *et al. Newbouldia's laevis* biological effects against metabolic syndrome components: A review. World J Adv Res Rev. 2022;16(2):121-38.
17. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 2010;76(14):1479-91. doi: 10.1055/s-0030-1250027, PMID 20533165.
18. Akande EB, Oladejo BO, Oladunmoye MK, Abodunrin TF. Antibacterial effects of *Newbouldia laevis* {P. Beauv} on the bacteria isolated from the blood of hepatitis C virus positive individuals. Asian Plant Res J. 2020:26-34. doi: 10.9734/aprj/2020/v4i130077.
19. Vuuren Van S, Viljoen A, Africa S, Africa S. Plant-based antimicrobial studies-methods and approaches to study the interaction between natural products. Planta Med. 2011;77(11):1168-82. doi: 10.1055/s-0030-1250736, PMID 21283954.
20. Umeyor C, Anaka E, Kenechukwu F, Agbo C, Attama A. Development, *in vitro* and *in vivo* evaluations of novel lipid drug delivery system of *Newbouldia laevis* (*P. beauv.*). Nanobiomedicine. 2016;3:1849543516673445. doi: 10.1177/1849543516673445, PMID 29942388.
21. Agbodeka K, Gbekley HE, Karou SD, Anani K, Agbonon A, Tchacondo T, *et al.* Ethnobotanical study of medicinal plants used for the treatment of malaria in the plateau region, Togo. Pharmacogn Res. 2016;8;Suppl 1:S12-8. doi: 10.4103/0974-8490.178646, PMID 27114686.
22. Ameja LB. Qualitative phytochemical exploration guided *in vitro* antibacterial activity studies on crude stem bark extract of *Stereospermum kunthainum* (Bignoniaceae). p. 1-12.
23. Ramsay KST, Wafo P, Ali Z, Khan A, Oluyemisi OO, Marasini BP, *et al.* Chemical constituents of *Stereospermum acuminatissimum* and their urease and α-chymotrypsin inhibitions. Fitoterapia. 2012;83(1):204-8. doi: 10.1016/j.fitote.2011.10.014, PMID 22062354.
24. Awang FI, Ferdosh S, Sarker MZ, Sheikh HI, Ghafoor K, Yunus K, Zaidul I. *Stereospermum fimbriatum* as a Potential Source of Phytochemicals: a Review of Stereospermum Genus. Curr Pharm Biotechnol. 2016;17(12):1024-35. doi: 10.2174/1389201017666160919163207, PMID 27655363.
25. Harborne JB. Phytochemical methods: a GUIDE TO Modern TECHNIQUES OF PLANT ANALYSIS. second edi. Hamilton and Hardy's industrial toxicology. 6th ed. london: Chapman & Hall Ltd; 1984. 300 p.
26. Ndjakou Lenta B, Vonthron-Sénécheau C, Fongang Soh R, Tantangmo F, Ngouela S, Kaiser M, *et al. In vitro* antiprotozoal activities and cytotoxicity of some selected Cameroonian medicinal plants. J Ethnopharmacol. 2007;111(1):8-12. doi: 10.1016/j.jep.2006.10.036, PMID 17141994.
27. Compean KL, Ynalvez RA. Antimicrobial activity of plant secondary metabolites: a review. Res J Med Plants. 2014;8(5):204-13. doi: 10.3923/rjmp.2014.204.213.
28. Jones RN, Barry AL, Thornsberry C. Ceftriaxone: A summary of *in vitro* antibacterial qualities including recommendations for susceptibility tests with 30-µg disks. Diagn Microbiol Infect Dis. 1983;1(4):295-311. doi: 10.1016/0732-8893(83)90005-6, PMID 6321093.
29. Assob Nguedia JC, Mbeng P, Etoa F, Kuete V, Manfouo R, Lontsi D, *et al.* etude pharmacocinetiques de *Gladiolus gregasius* Baker et *Newbouldia laevis* seem, plantes medicinale a propri2t2 antimicrobienne. J Cameroun Acad Sci. 2005; 5(2&3):79-84.
30. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71-9.
31. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Med. 2010;76:1479-91.

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