

Antibacterial activity, time-kill kinetics against multidrug-resistant *Salmonella* and toxicity of *Voacanga Africana* and *Cyperus articulatus* extracts

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Abstract

Antibiotics resistance in *Salmonella* serovars which infect humans is increasing with the emergence of multidrug-resistant (MDR) strains, necessitating the search for alternative efficacious treatments. The activity of *Voacanga africana* and *Cyperus articulatus*, used in ethnomedicine to treat a wide variety of infectious diseases, were evaluated on MDR clinical isolates of *Salmonella*. Extracts of the stem bark and leaves of *V. africana* and the rhizome of *C. articulatus* were prepared in various organic solvents and screened using disc diffusion and microdilution assays. Active extracts were tested for cytotoxicity on monkey kidney epithelial cells, acute (2000 mg/kg body weight) and subacute (80, 160, 320 and 640 mg/kg body weight) toxicity; biochemical and haematological effects were also assessed in Wistar rats. Time-kill kinetic analysis was performed on the most selective non-toxic extract. The methylene chloride and methanol extracts of *V. africana* stem bark showed high activity with high zones of inhibition (22 - 25mm) and the lowest minimum inhibitory concentrations (MIC) of 0.0625 mg/mL while the methanol extract of *C. articulatus* showed moderate activity (zones diameters = 13 - 16mm). Time-kill kinetics revealed concentration-dependent bacteriostatic and bactericidal activities for the methanol extract of *V. africana* stem bark. All active extracts were not cytotoxic and no adverse effects or mortality was recorded for the methanol extract of *V. africana* stem bark. The methanol and methylene chloride extracts of the stem bark of *V. africana* possess significant activity against MDR *Salmonella* with a low risk of toxicity and should be further investigated including *in vivo* studies.

Keywords: *Voacanga africana*, *Cyperus articulatus* Antibacterial, Toxicity, Multidrug-resistant, *Salmonella*

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Résumé

La résistance des sérotypes de *Salmonella* infectant l'homme est en augmentation avec l'émergence de souches multirésistantes, rendant nécessaire la recherche de traitements alternatifs efficaces. L'activité de *Voacanga africana* et *Cyperus articulatus*, utilisées en ethnomédecine pour traiter une grande variété de maladies infectieuses, a été évaluée sur des isolats cliniques multirésistants de *Salmonella*. Des extraits de l'écorce de tige et des feuilles de *V. africana*, ainsi que du rhizome de *C. articulatus*, ont été préparés à

l'aide de différents solvants organiques et testés par les méthodes de diffusion sur disque et de microdilution. Les extraits actifs ont été soumis à des tests de cytotoxicité, sur des cellules épithéliales rénales de singe, toxicité aiguë (2000 mg/kg de poids corporel) et subaiguë (80, 160, 320 et 640 mg/kg de poids corporel); les effets biochimiques et hématologiques ont également été évalués chez des rats Wistar. Une analyse cinétique de l'efficacité antibactérienne a été réalisée sur l'extrait le plus sélectif et non toxique. Les extraits au dichlorométhane et au méthanol de l'écorce de tige de *V. africana* ont montré une forte activité, avec de larges zones d'inhibition (22 - 25 mm) et les plus faibles concentrations minimales inhibitrices (CMI) de 0,0625 mg/mL, tandis que l'extrait méthanolique de *C. articulatus* a présenté une activité modérée (diamètres des zones = 13 - 16 mm). L'analyse cinétique a révélé des activités bactériostatiques et bactéricides dépendantes de la concentration pour l'extrait méthanolique de l'écorce de tige de *V. africana*. Tous les extraits actifs se sont révélés non cytotoxiques, et aucun effet indésirable ni mortalité n'a été observé pour l'extrait méthanolique de l'écorce de tige de *V. africana*. Les extraits méthanolique et au dichlorométhane de l'écorce de tige de *V. africana* présentent une activité significative contre *Salmonella* multirésistante, avec un faible risque de toxicité, et devraient faire l'objet d'investigations supplémentaires, notamment d'études *in vivo*.

Mots-clés : *Voacanga africana*, *Cyperus articulatus*, Antibactérien, Toxicité, Multirésistance, *Salmonella*.

Introduction

Enteric fever is caused by *Salmonella* transmitted mainly through ingestion of contaminated water or food. *Salmonella* is a Gram-negative facultative anaerobic bacterium of the Enterobacteriaceae family (Eng *et al.*, 2015). There are two species, *S. bongori* and *S. enterica*, six subspecies, with over 2600 sub-types (serovars or serotypes) of *Salmonella enterica* (Khadka *et al.*, 2021). *S. Typhi* and *S. Paratyphi* are serovars which cause enteric fever in humans, while *S. Typhimurium* is responsible for gastroenteritis. The global burden of salmonellosis ranges from 200 million to a billion *Salmonella* infections annually, with about 93 million cases of gastroenteritis and over 150,000 deaths; about 85% of the cases are associated with the consumption of contaminated food (Lamichhane *et al.*, 2024). Presently, treatment failure occurs in some cases of enteric fever due to the continuous emergence of antibiotic resistance (Sokoudjou *et al.*, 2020). The resistance challenge is further aggravated by the slow rate of development of new antibiotics whereby only two new classes of antibiotics have been introduced into clinical use in the last two decades (Hutchings *et al.*, 2019; Miethke *et al.*, 2021). Several strategies have been exploited in

the search for new antibacterials, notably natural products from fungi, bacteria and plants. Both synthetic medicinal chemistry and synthetic biology, have offered significant potentials, yet each faces its own set of limitations (Hoffman, 2020).

Plant natural products constitute a major source of new molecules which could be exploited in countering bacterial resistance as evidenced in the documented large number of such molecules with various modes of action (Khameneh *et al.*, 2019). This provides a strong basis to pursue this line of investigation of the antibacterial activity of plant natural products. Pertinent to this investigation are *Voacanga africana* and *Cyperus articulatus*, two medicinal plants that are used in ethnomedicine to treat a wide variety of infectious and non-infectious diseases. The seeds of *V. africana* for example are used to treat tooth decay and gonorrhoea (Ding *et al.*, 2018), while the leaves and roots decoction are used to treat malaria, and diarrhoea, (Liu *et al.*, 2015). The tubers, rhizomes, and roots of *C. articulatus* are used to treat diarrhoea, malaria and respiratory tract infections (Nguta *et al.* 2015; Da Silva *et al.*, 2014; Muthaura *et al.*, 2011). This research evaluated the

antibacterial efficacy of crude extracts of *V. Africana* and *C. articulatus* against characterised MDR isolates of *Salmonella* using time-kill kinetics. The active extracts were also screened for toxicological effects.

Materials and methods

Plant collection and preparation of extracts

The leaves and bark of *Voacanga africana* Stapf were harvested in Limbe in the South West region of Cameroon. *Cyperus articulatus* L. roots and rhizomes were collected from inland valleys at Sehn village, Ndu Sub-Division in the North West Region of Cameroon. Both plants were identified in the Limbe Botanic Garden by Mr. Njimba Peter, the botanist, with voucher specimen numbers SCA887 and SCA2925 respectively. The plant materials were chopped, air dried under shade at room temperature and humidity and ground into powder. Crude extracts were prepared as previously described by Mbah *et al.*, (2012), with some modifications (volume of solvent decreased to 2-3 L based on amount of powder and a single maceration). The powders (500 g of the bark and 300 g of the leaves of *V. africana*) were macerated separately for 48 hours per solvent. The extraction was carried out successively in 2 L of each solvent. The mixture was filtered and the filtrate concentrated using a rotavapor (BUCHI Rotavapor R-114, Switzerland), at 60 °C, 40 °C and 80 °C for, hexane, methylene chloride and methanol respectively. The powdered rhizomes of *C. articulatus* (500 g) was macerated in 3 L of methanol for 48 h, and the filtrate was concentrated same as above. All the extracts were kept at room temperature for 48 hours to completely evaporate residual solvent, leaving no traces of methylene chloride which is cytotoxic, and thereafter weighed and stored at 4 °C until used.

Determination of antibacterial activity of extracts

Multidrug-resistant (MDR) *Salmonella* were isolated from clinical specimens collected in the medical laboratories of some health facilities in the Southwest region of Cameroon, using standard microbiological techniques. They were characterised using antibiotic susceptibility tests, biochemical tests (API 20E kit) and molecular biology techniques including sequencing of resistance genes as reported (Ndip *et al.*, 2022). The *Salmonella* isolates used possessed multiple resistance genes and showed moderate to high resistance to cephalosporins, fluoroquinolones, chloramphenicol, antifolates and penicillins. Five control strains (*S. Typhimurium* ATCC 14028; *S. enteritidis* ATCC 13076; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Staphylococcus aureus* ATCC 25923) obtained from the American Type Culture Collection (Manassas, Virginia, USA), were included in the study. The antibacterial activity of the extracts was determined using standard methods as follows:

Disc diffusion test: this was performed on 8 multidrug-resistant *Salmonella* isolates and the control strains as previously described by Al-Rifai *et al.*, (2017), with modifications. Five (5) mm sterile Whatman filter paper No. 1 discs were impregnated with 2 mg/10 µL crude extract in 10% DMSO (Ngemenya *et al.*, 2019). A 0.5 MacFarland standard bacterial suspension (1×10^8 CFU/mL) was prepared and 75 µL spread on the surface of freshly prepared Mueller Hinton agar using a sterile spreader. The extract discs were carefully fixed on the surface of the plates and kept for 3-5 minutes before incubating. Chloramphenicol (30 µg) and ampicillin (30 µg) were used as positive control antibiotics while 10% DMSO was used as the negative control. All plates were incubated inverted at 37 °C for 24 hours.

Microdilution assay: the minimum inhibitory concentration (MIC) was determined as previously

described by Bate *et al.*, (2020), with some modification (final extract concentration of 0.0626 to 10 mg/mL). Forty (40) milligrams of crude extract (test substance, TS) was dissolved in 200 μ L of DMSO and 800 μ L of Mueller Hinton broth added to obtain a stock. The stock solution was diluted in Mueller Hinton broth to obtain concentrations from 0.125 to 20 mg/mL. Extract solutions were added followed by bacterial suspension in equal volumes (75 μ L each) into a 96-well microtitre plate, giving final concentrations of 0.0626 to 10 mg/mL extract and 1×10^5 CFU/mL. Positive (25 μ g/mL gentamycin) and negative control (broth and bacterial cells) were included, optical densities (ODs) of wells read at 595nm (Emax-Molecular Devices Corporation, USA), and the plate incubated at 37 °C for 24 hours and optical densities were read again. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\Delta OD_{NC} - \Delta OD_{TS}}{\Delta OD_{NC}} \times 100$$

The MIC was the lowest concentration with percentage inhibition $\geq 50\%$ relative to the negative control (NC). The minimum bactericidal concentration (MBC) was determined by culturing $\geq$ MIC wells on freshly prepared nutrient agar and incubated for 24 hours at 37 °C. The plates were observed for bacterial growth. MBC was considered as the plate with lowest concentration with no visible bacterial colonies.

Time kill assay: The kinetics of the antimicrobial activity of the methanol extract of *V. africana* stem bark against three clinical multidrug-resistant *Salmonella enterica* isolates was assessed following the method of Appiah *et al.*, (2017), with some modifications. Solutions of the extract were prepared at concentrations of 0.5, 0.25 and 0.125 μ g/mL corresponding to 4xMIC, 2xMIC and MIC. A volume of 1 mL of each concentration and 1 mL bacterial suspension (5×10^5 CFUs/mL final concentration) were added into a 5 mL glass test tube in triplicate with

a negative control tube included. Viable *S. enterica* colonies were determined by the plate count technique at 0, 2, 4, 6 and 24 h. This was done by transferring an aliquot (100 μ L) of the suspension per tube into a sterile test tube and diluted (1:10). Then 20 μ L of the dilution was spread on nutrient agar, incubated for 24 h at 37 °C, the colonies counted and the colony forming units (CFUs) per mL calculated. The percentage reduction at each time interval was calculated using the formula:

$$\% \text{ Reduction} = \frac{\text{count at 0hr} - \text{count at xhr}}{\text{count at 0hr}} \times 100$$

Log₁₀ reduction = log₁₀ initial count - log₁₀ at xhr **Cytotoxicity assay**

Cytotoxicity was assessed as previously described in detail by Ngemenya *et al.*, (2019) with some modification (final extract concentration of 3.91 to 1000 μ g/mL). Briefly, monkey kidney epithelial cells, (LLC-MK2, ATCC, USA) were grown in complete culture medium (CCM, containing new born calf serum, NBCS). The cells were seeded in 96 well plates at a density of 3000 cells/100 μ L of CCM and incubated at 37 °C under an atmosphere of 5 % CO₂ in humidified air for them to become fully confluent. Stock solutions (4 mg/mL) of the active extracts of *V. africana* and *C. articulatus* were prepared by dissolving in 100 μ L of DMSO and 900 μ L of CCM added and vortexed. After 3 days when the cells were fully confluent, the medium was replaced with 100 μ L of fresh CCM. The extract solution was serially diluted in CCM and 100 μ L added in triplicate giving final concentrations of 3.91 to 1000 μ g/mL. Negative control wells (2% DMSO) and positive control wells (30 μ g/mL Auranofin) were included. The plate was incubated as above for 120 hours (5 days). Thereafter, the supernatant was discarded, the wells decolourised with 100 μ L incomplete medium (no NBCS), and 100 μ L of 1 mg/mL MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) added per well, followed by incubation as above for 30 minutes.

The formazan formed was solubilized with DMSO and optical densities (OD) of wells read at 595 nm as described (Ngemenya *et al.*, 2019). The percentage inhibition of formazan formation was calculated using the following formula:

Percent inhibition (%) =

$$\frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control}} \times 100$$

The CC₅₀ (concentration which kills 50% of cells) of each extract was obtained from a plot of concentration of extract against percentage inhibition generated using Graph Pad Prism software version 8.0

Acute toxicity test

Ethical clearance was obtained from the University of Buea–Institutional Animal Care and Use Committee (UB-IACUC No 09/2022). The test was done for the methanol extract of *V. africana* stem bark on female Wistar rats following the guidelines of the Organisation for Economic Cooperation and Development (OECD, 2002), as previously described by Fotso *et al.*, 2025 with slight modification. A total of 6 rats were used with 3 in each of the test and normal control groups. The animals were maintained in cages throughout the experiment. Initially, one animal in the test group was fasted overnight (given water) for 12-14 h. The rat was weighed and then administered the extract orally by gavage at a dose of 2000 mg/kg. The animal was observed for 2 h during which it did not die and was provided food. The other two test animals were then given the same treatment. The animals were observed carefully for any behavioural changes after 4 h and later after 24 h. Both test and control groups were allowed access to food and water *ad libitum*. They were further observed daily for any adverse signs for a period of 14 days.

Sub-acute toxicity study

This was performed according to the OECD 2008 test guidelines as previously described by

Vadakkan, (2019) . Female Wistar rats (25) weighing 170-200 g were put in 5 groups of 5 rats each. The rats were fasted overnight for 12-14 h but given water and weighed. Doses of the methanol extract of *V. africana* stem bark were weighed, dissolved in distilled water and filtered to remove any particles.

The extract was administered at concentrations of 80, 160, 320 and 640 mg/kg to four groups of rats respectively daily for 28 days. The control group received distilled water. Animals were observed for any abnormal behaviour daily. Food and water intake were recorded daily and body weight weekly. At the end of the treatment period, rats were fasted overnight same as above. On day 29 the animals were anesthetized with Ketamine (90 mg/kg) and Xylazine (10 mg/kg), and blood collected by cardiac puncture. Blood was collected into an anticoagulant (EDTA) tube for haematological analysis and without anticoagulant for biochemical analysis. The animals were dissected and major organs (spleen, liver and kidneys) removed and weighed. Blood for biochemical analysis was allowed to coagulate, centrifuged at 2000g for 5 min and the serum used to test for liver (aspartate aminotransferase AST, alanine aminotransferase, ALT) and kidney (urea and creatinine) functions using test kits (Chronolab, Switzerland), according to manufacturer's instructions. Haematological parameters were determined using a haematology analyser (URIT 3000 Plus, China).

Statistical analysis

The zones of inhibition and MIC values were interpreted following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020) for antimicrobial activity of extracts and other chemicals. One-way analysis of variance (ANOVA) was done using GraphPad prism version 8.4.2 to determine significant difference between control and test groups of rats. The Dunnett's test was used to compare means

between all groups and a p-value < 0.05 indicates a significant difference

Results

Antibacterial activity of extracts

Two of the seven crude extracts, methylene chloride and methanol extracts of *V. africana* stem bark showed high activity with zones of inhibition ranging from 22 – 25 mm. The methanol extract of *C. articulatus* showed moderate activity with zones from 13 – 16 mm. The zone diameters for

clinical isolates and control strains were comparable (Table 1), including control strains of other bacterial species. The other four extracts (the hexane extract of *V. africana* stem bark and the hexane, methylene chloride and methanol extracts of *V. africana* leaves) recorded weak activity. The standard antibiotics (ampicillin and chloramphenicol) had zones of inhibition of 28 mm for chloramphenicol and 8 mm (disc size = 5 mm) for ampicillin.

Table 1 Antibacterial activity of 2 mg crude extracts of *Voacanga africana* and *Cyperus articulatus* against multidrug-resistant *Salmonella*

Salmonella Isolate/ strain	Extract Inhibition zone diameter (mm)						
	<i>V. africana</i>						<i>C. articulatus</i>
	Stem bark Hexane	Methylene chloride	Methanol	Leaf Hexane	Methylene chloride	Methanol	Rhizome Methanol
BU01	8	24	23	8	8	8	14
BU02	8	22	23	8	8	8	15
Li01	8	25	24	8	8	8	14
Li02	8	25	23	8	8	8	15
Li03	8	24	23	8	8	8	14
Ti01	8	24	25	8	8	8	14
Ti02	8	23	24	8	8	8	15
Ti03	8	25	23	8	8	8	13
1*	8	25	24	8	8	8	13
2*	8	25	25	8	8	8	13
3*	8	25	26	8	8	8	15
4*	8	23	23	8	8	8	13
5*	8	24	25	8	8	8	16

Chloramphenicol (30 µg) = 28.5 mm, ampicillin (30 µg) = 10mm (resistant). BU, Li and Ti: Multidrug-resistant *Salmonella* isolates. Control strains: 1* = *S. typhimurium* ATCC 14028; 2* = *S. enteritidis* ATCC 13076; 3* = *Escherichia coli* ATCC 25922; 4* = *Pseudomonas aeruginosa* ATCC 27853; 5* = *Staphylococcus aureus* ATCC 25923).

The MIC values of the three active extracts also showed high activity. *V. africana* recorded low values of 0.0625 and 0.125mg/mL against most isolates and control strains while *C. articulatus* recorded 0.25mg/mL against a few (Table 2). The active extracts had an MBC:MIC ratio of ≤4 against almost all tested bacteria indicating possible bactericidal activity.

Time-kill kinetic profile of *Voacanga africana* extract

The time kill assay for the *V. africana* extract showed a dose-dependent inhibition of all three MDR *Salmonella* isolates (Figure 1). The percentage inhibitions measured at the various incubation intervals ranged from 25.93% to 100% inhibition at 24 h. An inhibition greater than or equal to 99.9% was observed for all isolates at 4xMIC indicating bactericidal activity. Lower test concentrations showed less than 99.9% bacterial inhibition at all incubation intervals indicating bacteriostatic activity. The log reduction was calculated and a > 3Log₁₀ was observed at the highest concentration level for all isolates.

Table 2: Minimum inhibitory concentrations of extracts of *V. africana* and *C. articulatus* against multidrug-resistant *Salmonella*.

Bacterial isolate / strain	Extract MIC values (mg/mL)						
	<i>V. africana</i>						<i>C. articulatus</i>
	Stem bark			Leaf			Rhizome
	Hexane	Methylene chloride	Methanol	Hexane	Methylene chloride	Methanol	Methanol
BU01	10	0.0625	0.0625	10	8	10	1.0
BU02	8	0.125	0.125	8	10	10	1.0
Li01	10	0.125	0.125	10	-	-	2.0
Li02	-	0.0625	0.25	-	-	-	0.5
Li03	-	0.25	0.125	-	-	-	1.0
Ti01	-	0.125	0.0625	-	10	8	0.25
Ti02	10	0.0625	0.0625	10	8	-	0.5
Ti03	8	0.125	0.125	8	-	8	1.0
1*	-	0.125	0.0625	-	-	10	0.25
2*	-	0.0625	0.125	-	-	10	0.25
3*	10	0.0625	0.125	10	-	-	0.25
4*	-	0.125	0.125	-	-	-	2.0
5*	8	0.0625	0.25	-	10	8	1.0

MIC = Minimum inhibitory concentration. - = No MIC in the tested range (0.0626 to 10 mg/mL).

Multidrug-resistant *Salmonella* isolates and control strains are defined in Table 1

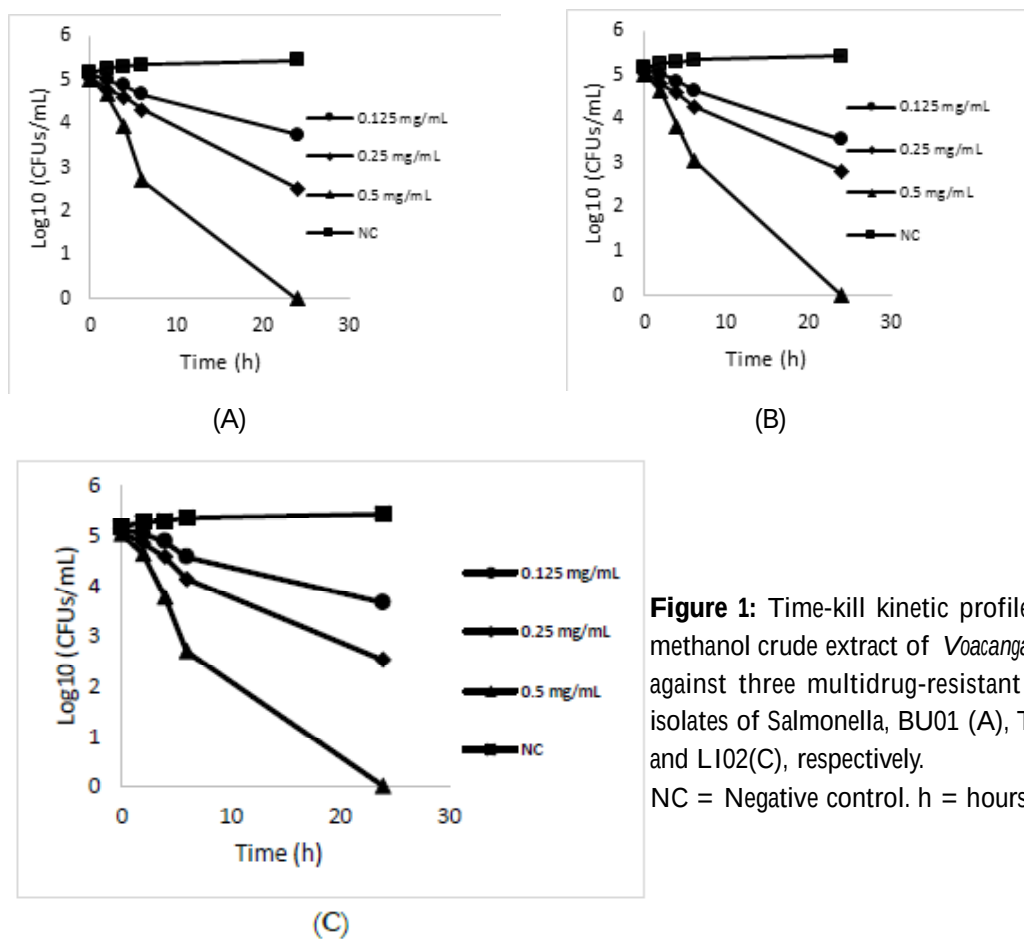


Figure 1: Time-kill kinetic profile of the methanol crude extract of *Voacanga africana* against three multidrug-resistant clinical isolates of *Salmonella*, BU01 (A), TI01 (B) and LI02(C), respectively. NC = Negative control. h = hours

Cytotoxicity of extracts

The CC_{50} values of all extracts were much higher (over 14 times) than the cut-off value of 30 $\mu\text{g/mL}$ (Ngemenya *et al*, 2019), indicating they were

not cytotoxic. The selectivity index (SI) values ranged from 3 to 14 MIC with the two extracts of having SIs of over 7 (Table 3).

Table 3: Selectivity indices of active crude extracts of *V. africana* and *C. articulatus*

Crude extract	CC_{50} ($\mu\text{g/mL}$)	MIC* ($\mu\text{g/mL}$)	Selectivity Index
<i>Voacanga africana</i>			
Methanol	894.7	62.5	14.3
Methylene chloride	441.7	62.5	7.06
<i>Cyperus articulatus</i>			
Methanol	749.9	250	3.0

*Lowest MIC

Acute toxicity of *V. africana* stem bark extract

No adverse effects and no mortality were observed for the methanol extract of *V. africana* stem bark at 2000 mg/kg. No abnormalities in fur and eye colours, salivation, touch to response, diarrhoea, anorexia and asthenia (loss of body strength or body weakness) were observed.

Sub-acute toxicity of *V. africana* stem bark extract

The crude extract of *V. africana* did not induce any significant changes or adverse effects in animals in the test groups. No mortality was observed throughout the treatment period (28 days). Food and water intake of tests and normal groups were not significantly different ($p < 0.05$).

The body weights of individual animals increased after 28 days of treatment indicating growth. The change in body weights for the 640 mg/kg test group was significantly higher compared to the control group ($p = 0.004$). The weights of individual organs were normal. There was no significant difference in relative body weight between animals in the test groups and the control group ($p > 0.05$).

The effects of the methanol extract of *V. africana* stem bark on haematological parameters are shown in Table 4. There was no significant change in the haemoglobin, haematocrit, white and red blood cells. However, the mean platelet counts in the 80, 320 and 640 mg/kg groups were higher and significant at 320 mg/kg ($p < 0.001$).

Table 4: Effects of the methanol extract of *V. africana* stem bark on haematological parameters in sub-acute toxicity study

Dose (mg/kg)	HB (g/dL)	HCT (%)	WBC ($\times 10^3/\mu\text{L}$)	RBC ($\times 10^6/\mu\text{L}$)	Platelet ($\times 10^3/\mu\text{L}$)
0	12.5 $\pm$ 0.38	28.0 $\pm$ 0.88	8.0 $\pm$ 0.50	6.7 $\pm$ 0.47	542.2 $\pm$ 16.54
80	12.7 $\pm$ 0.77	26.7 $\pm$ 1.97	8.7 $\pm$ 0.70	7.4 $\pm$ 0.57	667.6 $\pm$ 56.71
160	16.6 $\pm$ 0.5 ^a	38.7 $\pm$ 4.12 ^a	4.6 $\pm$ 1.40 ^a	9.0 $\pm$ 0.22	450.4 $\pm$ 10.6
320	13.0 $\pm$ 0.21	27.6 $\pm$ 0.55	13.2 $\pm$ 0.55 ^a	7.3 $\pm$ 0.16	845.2 $\pm$ 49.45 ^a
640	12.6 $\pm$ 0.24	27.0 $\pm$ 0.45	7.5 $\pm$ 0.97	4.8 $\pm$ 1.17	671.0 $\pm$ 31.87

Values expressed as mean $\pm$ SEM; n= 5; HB= haemoglobin; HCT= haematocrit; WBC= white blood cell; RBC= red blood cell;

^aP < 0.001: significant difference compared to negative control.

The haemoglobin and haematocrit values for 160 mg/kg ($p = 0.006$) were significantly higher than in the control animals. The white blood cell counts of the normal group were reduced significantly compared to the 160 mg/kg group ($p = 0.022$) but increased in the 320 mg/kg group ($P < .001$) parameters. There was no significance

difference in the level of liver enzymes (ALT and AST) between the control and some test groups. However, there was a significant decrease in the urea levels at 160 mg/kg extract compared to the control group ($p = 0.05$). Creatinine levels in all test groups were similar to those of the control group (Table 5).

Table 5: Effects of the methanol extract on *V. africana* stem bark on biochemical parameters in sub-acute toxicity study

Dose (mg/kg)	AST (U/L)	ALT (U/L)	Urea (mmol/L)	Creatinine (mg/dL)
0	5.8±0.58	38.2±3.94	36.4±1.21	0.8±0.09
80	5.6±1.6	75.2±9.39	35.8±3.44	0.8±0.1
160	5.4±1.54	51.8±6.58	28±1.38	0.7±0.11
320	4.2±1.02	56.8±3.43	31.4±2.82	0.8±0.14
640	4.2±1.28	59.8±10.26	33.6±1.36	0.9±0.05

Values expressed as mean $\pm$ SEM; n= 5.

Discussion

The anti-infective medicinal and pharmacological properties of *V. africana* and *C. articulatus* are well documented as highlighted above. However, their antibacterial properties on resistant bacterial strains have not been extensively explored. This study has demonstrated significant antibacterial activity of the methylene chloride and methanol extracts of the stem bark of *V. africana* against multidrug-resistant clinical isolates of *Salmonella* with no adverse toxicity or mortality in rats. To the best of our knowledge this is the first report on the activity of this plant against multidrug-resistant *Salmonella*.

The two extracts of *V. africana* showed high activity in both the disc diffusion and microdilution tests against the *Salmonella* isolates and all control strains hence showing consistency in their activity. The zones of inhibition are comparable to those of some standard antibiotics (corresponding to high susceptibility or high activity); these include penicillins, cephalosporins, antifolates, phenicols and fluoroquinolones used to treat *Salmonella* infections (CLSI, 2020). However, the lowest MICs (equivalent to 62.5 $\mu\text{g/mL}$), were relatively higher than those of these standard antibiotics.

C. articulatus showed moderate activity and should be further explored by testing extracts prepared using other solvents. The MBC:MIC ratios (≤ 4) suggest bactericidal activity; this was confirmed in the time-kill assay after 24 h incubation which revealed greater than 3 $\log_{10}$ decrease in bacterial growth, indicating a bactericidal mode of action. The antibacterial activity of the stem bark of *V. africana* has not been extensively investigated. Antibacterial activity has been reported for the seeds of this plant (Liu *et al.*, 2015), and the pure compounds from the fruits (Ding *et al.*, 2018; Yan *et al.*, 2021). Some pure compounds have been isolated from the stem bark of *V. africana*; these compounds may account for the antibacterial activity of the crude extract of the stem bark (Babiaka *et al.*, 2021).

All the active extracts were not cytotoxic (CC_{50} values $> 30 \mu\text{g/mL}$) but only the two extracts of *V. africana* stem bark had a good selectivity for *Salmonella* (Table 4); this indicates a low risk of toxicity suggesting that the stem bark is a source of a potential safe alternative treatment for resistant *Salmonella* infections. No adverse toxicity or mortality was recorded in both the acute and sub-acute tests. Several haematological parameters except red blood cells were increased at certain

doses suggesting stimulation of haematopoiesis by the extract in response to mild toxicity induced by the extract. The biochemical parameters were not significantly altered in the test rats indicating that there was no adverse effects in the vital organs, liver and kidney, assessed.

Previous studies on the cytotoxicity and *in vivo* toxicity of crude extracts of this plant are limited. However, some of compounds isolated from the stem bark of *V. africana*, notably voacangine and voacamine have been reported to possess moderate to significant cytotoxicity against various normal mammalian and cancer cell lines (Chen *et al*, 2016). These compounds may be present in the active crude extracts in low concentrations which explains the lack of cytotoxicity recorded in this study.

Conclusion: This study is the first report of significant activity of the methanol and methylene chloride extracts of the stem bark of *V. africana* against multidrug-resistant *Salmonella* with a low risk of toxicity. This plant may be considered a potential alternative treatment for antibiotic resistant clinical infections of *Salmonella*. A window has therefore been opened for further investigation, including *in vivo* studies of the extracts and isolated pure compounds of *V. africana* and *C. articulatus*.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' contributions

MNN and FC conceived, designed and supervised the study. LAA conducted the biological experiments. LAA and MJA carried out the chemistry work. LAA, RAN and TRK analysed the data and drafted the manuscript. FC, MNN and MJA reviewed the draft manuscript. All authors read and approved the final version of the manuscript.

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References

- Al-Rifai A, Aqel A, Al-Warhi T, Wabaidur M, Al Othman ZA and Badjah-Hadj-Ahmed AY, (2017). Antibacterial, antioxidant activity of ethanolic plant extracts of some *Convolvulus* species and their DART ToF-MS profiling. *Evidence-Based Complementary and Alternative Medicine*, Article ID 5694305.
- Appiah T, Boakye YD and Agyare C, (2017). Antimicrobial activities and time-kill kinetics of extracts of selected Ghanaian mushrooms. *Evidence Based Complementary and Alternative Medicine*, Article ID 4534350.
- Bate PNN, Oroch AE, Nyongbela KD, Babiaka SB, Kukwah A and Ngemenya MN, (2020). *In vitro* activity against multidrug-resistant bacteria and cytotoxicity of lichens collected from Mount Cameroon. *Journal of King Saud University-Science*, 32: 614–619.
- Babiaka SB, Simoben CV, Abuga KO, Mbah JA, Karpoormath R, Ongarora D, Mugo H, Monya E, Cho-Ngwa F, Sippl W, Loveridge EJ and Ntie-Kang F (2021). Alkaloids with anti-Onchocercal activity from *Voacanga africana* Stapf (Apocynaceae): identification and molecular modeling. *Molecules*, 26(70): pages 1-19.
- Che H-M, Yang Y-T, Li H-X, Cao Z-X, Dan, X-M, Mei L *et al*, (2016). Cytotoxic monoterpenoid indole alkaloids isolated from the barks of *Voacanga africana* Staph. *Natural Product Research*, 30(10): 1144-1149.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, M100, 30th ed. Wayne. 2020. <https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf>. Accessed 10 April, 2024

Da Silva ICM, Dos Santos WL, Leal ICR, Zoghbi MDGB, Feirhmann AC, Cabral VF, Macedo EN and Cardozo-Filho L, (2014) Extraction of essential oil from *Cyperus articulatus* L. var. *articulatus articulatus* (priprica) with pressurized CO₂. *J. Supercritical Fluids*, 88: 134-141.

Ding C-F, Ma H-X, Yang J, Qin X-J, Njateng GSS, Hao-Fei Yu H-F, Wei X, Liu Y-P, Huang W-Y, Yang Z-F, Wang X-H and Luo X-D, (2018). Antibacterial indole alkaloids with complex heterocycles from *Voacanga africana*. *Organic letters*, 20, (9): 2702–2706.

Eng S-K, Pusparajah P, Ab Mutalib N-S, Hooi-Leng Ser H-L, Chan K-G. and Lee L-H, (2015). *Frontiers in Life Science*, 8(3):284-293.

Fotso FE, Tih AE, Sama W, Ngemenya MN, (2025). Anti-onchocerca activity, toxicity and induced biochemical changes of *Allanblackia floribunda* and *Symphonia globulifera* stem bark methanolic extracts in mice. *Journal of Parasitic Diseases*. [Doi.Org/10.1007/S12639-025-01780-9](https://doi.org/10.1007/S12639-025-01780-9)

Hoffman PS, (2020). Antibacterial Discovery: 21st Century Challenges. *Antibiotics*, 9: 213.

Hutchings MI, Truman AW and Wilkinson B, (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, 51:72–80. [Doi.org/10.1016/j.mib.2019.10.008](https://doi.org/10.1016/j.mib.2019.10.008).

Khadka P, Thapaliy J and Thapa S, (2021). Susceptibility pattern of *Salmonella enterica* against

commonly prescribed antibiotics, to febrile-paediatric cases, in low-income countries. *BMC Pediatrics*, 21:38.

Khameneh B, Iranshahy M, Soheili V and Bazzaz BSF, (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*. 8:118.

Lamichhane B, Mawad AMM, Saleh M, Kelley WG, Harrington II PJ, Lovestad CW, *et al*, (2024). Salmonellosis: An overview of epidemiology, pathogenesis, and innovative approaches to mitigate the antimicrobial resistant infections. *Antibiotics*, 13 (1): 76.

Liu X, Yanga D, Liua J and Ren N, (2015). Analysis of essential oils from *Voacanga africana* seeds at different hydro distillation extraction stages: chemical composition, antioxidant activity and antimicrobial activity. *Natural Product Research* 29(20):1950- 1953.

Miethke M, Pieroni M, Weber T, Brönstrup M, Hammann P, Halby L *et al*, (2021). Towards the sustainable discovery and development of new antibiotics. *Nature Reviews Chemistry*, 5: 726-749.

Muthaura CN, Keriko JM, Derese S, Yenesew A and Rukunga GM, (2011). Investigation of some medicinal plants traditionally used for treatment of malaria in Kenya as potential sources of antimalarial drugs. *Experimental Parasitology*, 127: 609-626.

Mbah JA, Ngemenya MN, Abawah AL, Babiaka SB, Nubed LN, Nyongbela KD, Lemuh ND and Efange SMN. (2012). Bioassay-guided discovery of antibacterial agents: in vitro screening of *Peperomia vulcanica*, *Peperomia fernandopoioana* and *Scleria striatinux*. *Annals of Clinical Microbiology and Antimicrobials*, 11:10.

Ndip RA, Awah LA, Ghogomu SM, Cho-Ngwa F, Ngemenya MN, (2022). Isolation and molecular identification of Salmonella with high multidrug resistance to first line typhoid antibiotics in Southwest Cameroon. *Microbes and Infectious Diseases* 3(4): 988-997.

Ngemenya MN, Djeukem GGR, Nyongbela KD, Bate PNN, Babiaka SB, Monya E and Kanso RK, (2019). Microbial, phytochemical, toxicity analyses and antibacterial activity against multidrug-resistant bacteria of some traditional remedies sold in Buea Southwest Cameroon. *BMC Complementary and Alternative Medicine*, 19:150.

Nguta JM, Appiah-Opong R, Nyarko AK, Yeboah-Manu D, and Addo PGA, (2015). Medicinal plants used to treat tuberculosis in Ghana. *International Journal of Mycobacteriology*, 4(2): 116-123.

Organization for Economic Cooperation and Development, Test No. 423: Acute oral toxicity-acute toxic class method, in: OECD guidelines

for the testing of chemicals, OECD Publishing, Paris, 2002. Section 4.

Sokoudjou JB, Atolani O, Njateng GSS, Khan A, Tagousop CN, Bitombo AN, Kodjio N and Gatsing D, (2020). Isolation, characterization and *in vitro* anti-salmonella activity of compounds from stem bark extract of *Canarium schweinfurthii*. *BMC Complementary Medicine and Therapies*, 20:316.

Vadakkan K (2019). Acute and sub-acute toxicity study of bacterial signaling inhibitor *Solanum torvum* root extract in Wister rats. *Clinical Phytoscience* 5(19).

Yan Y, Li X, Zhang C, Lv L, Gao B and Li M, (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: *Antibiotics* 10 (3): 318.