Toxicity and Antimicrobial Activities of *Amaranthus caudatus* L. (Amaranthaceae) Harvested From Formulated Soils at Different Growth Stages

Muhali Olaide Jimoh, PhD[1,2] ![ORCID], Anthony Jide Afolayan, PhD[1], and Francis Bayo Lewu, PhD[3]

Abstract

This study examined the toxicity and antimicrobial effects of ethanol and aqueous extracts from *Amaranthus caudatus* grown on soils formulated from parent particles of silt, sand and clay in a glasshouse. Four different soils namely; sandy clay loam, loam, clayey loam and silty clay loam from were formulated were used for cultivation with the unfractionated soil which was the control. Crude extracts obtained from the plant shoots harvested at different growth stages were tested on some certain gram-negative and gram-positive bacteria and some fungi via agar dilution assay. The toxicity of the water and ethanol extracts was also examined via *Artemia salina* assay and the level of lethality was measured against Clarkson's lethality scale. All aqueous samples, as well as ethanol extracts of flowering and pre-flowering harvests of control soil tested, were non-toxic ($LC_{50} > 1$ mg/mL). At post flowering, the ethanolic extracts were highly toxic mostly in clayey loam, control, sandy-clayey loam soils ($LC_{50} < 0.5$ mg/mL). Also, antifungal effects of the plant revealed that extracts inhibited the growth of *Candida albicans* significantly with mild effect on *Candida glabrata*, *Penicillium chrysogenum* and *Penicillium aurantiogriseum* suggesting that the plant is a promising pharmacological candidate in the treatment of candidiasis. For an optimal yield of non-toxic supplement for household consumption which may also serve as pharmacological precursors, clayey loam soil is recommended for cultivation and harvesting may occur at pre-flowering or flowering stage using ethanol and water as solvents of extraction.

Keywords

Amaranthus caudatus, antimicrobial activities, clayey loam, growth stages, pharmacological precursors, soil formulation, toxicity

Introduction

From time immemorial, plants have been the mainstay of human civilization. They offer food, shelter and contribute in no small measure, to the socio-economic wellbeing of man. Traditional medicine, which has helped in the control and prevention of diseases, depends largely on plant products. Secondary metabolites are plant products made from reciprocal relations between the plant and its environment. They help plants in physiological fitness and as defense agents against predation.[1]

Many therapeutic approaches have been adopted to cure microbial infections. These include the use of antibiotic drugs in combating diseases. However, the growing occurrence of resistance against antibiotics due to indiscriminate use has frustrated anticipated success.[2] The advent of photodynamic therapy in treating microbial infections was seen as a viable alternative to antibiotics. The protocol involves the use of positively charged photosynthesizers such as porphyrins,

[1] Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, Alice, South Africa
[2] Department of Horticultural Sciences, Cape Peninsula University of Technology, Bellville Campus, Bellville, Cape Town, South Africa
[3] Department of Agriculture, Cape Peninsula University of Technology, Wellington Campus, Wellington, Cape Town, South Africa

Corresponding Author:
Anthony Jide Afolayan, Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa.
Email: aafolayan@ufh.ac.za

Table 1. Experimental Soil Formulation in Proportions Proposed by USDA Soil Texture Triangle Technique.

S/N	Soil types	% Sand	% Silt	% Clay
1	Control soil (SF$_1$)	unfractionated	unfractionated	unfractionated
2	Sandy Clay Loam (SF$_2$)	66	13	21
3	Silty Clay Loam (SF$_3$)	10	60	30
4	Clayey Loam (SF$_4$)	36	30	34
5	Loam (SF$_5$)	40	40	20

phenothiazines and phthalocyanines to stimulate photoinactivation of microbial cells at physiological pH.[3,4]

Despite these advances, antimicrobial compounds derived from plants are potent and safer. Little is known about their side threats to human health compared to synthetic drugs and other known therapies.[5,6] Also, a short live span is peculiar to chemotherapeutic agents thus, facilitating the build-up of resistance against diseases.[7] Researchers have now employed plant products as complements and alternatives to synthetic drugs to combat infectious diseases. This has now necessitated pharmacodynamic studies such as elucidation, characterization and isolation of bioactive components from medicinal plants to develop new antimicrobial remedies.[8-10]

The yield of secondary metabolites which dictate biological activities in plants has been reported to be influenced by soil types and harvest stage.[11-13] These metabolites are diverse structurally and may have been constituted during growth and development.[14,15] The synthesis of antimicrobial metabolites is instrumental to chemical barriers and signaling molecules in the defense-related process. These compounds are stowed as glucosinolates and antimicrobial peptides in dedicated tissues or organs.[16,17] Plant antimicrobial peptides are an important component of the plant defense system stored in leaves, seeds, roots, and stems of many plants. They constitute a safe class of natural antibiotic chemicals characterized by disulphide bonds support system and presence of positive charges that selectively target outer membranes of pathogenic agents.[16,18,19]

Nonetheless, naturalness does not guarantee harmlessness. Some mild adverse effects such as dizziness, diarrhea, stomach pain, vomiting, ulcers, sleep disorder and hormonal disturbance have been attributed to unguided use of herbal products due to issues arising from plant identity, contamination and overdose although affecting a smaller population.[20-22] Recently, herbs known to be harmless over the years have been proven to constitute health risks. Also, the use of advanced technology has facilitated the detection of toxic compounds in plants that were once considered safe.[23-25]

Amaranthus caudatus is a nutraceutical plant with depots of antimicrobial peptides locked up in its seeds. The peptides are capable of inhibiting microbial growth to a greater extent than chitin-binding proteins and are said to protect the seed against fungal invasion[26-28]. The dearth of literature on how soil types influence antimicrobial activities of the shoot being the most consumed part, necessitated this investigation. Thus, this study examines biological activities of extracts derived from shoots harvested at various growth stages against pathogenic microbes. Previous works have only focused on the antimicrobial property of the seed. Also, this work seeks to evaluate the toxicity of the plant on Artemia salina nauplii using brine shrimp assay to establish the impact of soil type and growth stage on lethality.

Materials and Methods

Soil Formulation

This was done according to Jimoh et al., (2019).[13] A load of topsoil obtained from the research farm of the University of Fort Hare was left to dry for 4 weeks under shade. Thereafter, the soil was sieved into primary particle sizes of sand, silt and clay and particles obtained were re-mixed to generate 4 experimental soil types used to cultivate the seeds together with the control soil. This was done following the soil texture triangle protocols of the United States Department of Agriculture[29] (Table 1).

Cultivation of Seeds and Processing of Plant Materials

This was done following Jimoh et al., (2020).[30] A. caudatus seeds were cultivated in different soils packed in almost filled plastic pots between October 2017 and January 2018. The experiment was set up and replicated in a Completely Randomised Design (CRD) in a regulated glasshouse.[13] Potted plants were watered 2 times every day (evening and morning) and the shoots were harvested at different maturity periods and sorted according to soil types and maturity stages, and oven-dried at 40 °C up to a constant weight. Plant samples were powdered and refrigerated at 4 °C for additional action.

Extraction Procedure

This was done as described by Jimoh et al., (2019).[13] The solvents used for extraction are ethanol and distilled water. This choice was made based on the pharmaceutical properties of ethanol extract and the food value of aqueous extract.[13] About 60 g each of the pulverized samples was soaked in 1000 mL of the solvents in separate glass bottles and shaken on a mechanical shaker (Gallenkamp Orbital Incubator Shaker) at 120 rpm for 48 hrs. The resulting extracts were sieved over a Whatman No. 1 filter paper fixed in a Buchner funnel which was attached to a vacuum pump to facilitate filtration. The ethanol filtrate was concentrated to parchedness in a rotary evaporator (Strike-202 Steroglass, Italy) set at 78 °C while the aqueous filtrate was lyophilized in a refrigerated circulating bath (PolyScience AD15R-40-A12E, USA; set at −40 °C) and freeze-dried with a Savant vapor trap freeze drier (RV-T41404, USA) within 24 hrs.

Antimicrobial Screening

Microbial strains. Four strains of gram-positive bacteria namely, *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 18824), *Bacillus subtilis* (KZN) and *Streptococcus pneumoniae* (ATCC 49619) and 2 gram-negative bacterial strains *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 19582) were

used for this study. The strains of fungi used were *Candida albicans* (ATCC 10231), *Penicillium chrysogenum* (ATCC 10106), *Candida glabrata* (ATCC 66032) and *Penicillium aurantiogriseum* (ATCC 16025). The microbial strains supplied by Botany Department, University of Fort Hare, were sub-cultured at 30°C for 48 h in a fresh growing medium (Sabouraud Dextrose Agar-Oxoid™) for further experiment.

Preparation of Inocula

Bacterial inoculum. This was prepared following Idris et al., (2019)[30] and with slight modifications. Freshly sub-cultured bacterial strains on sterile Muller Hinton Agar were incubated for 18 hrs at 37°C. Each bacterial inoculum was prepared by direct colony suspension technique. An inoculating loop was used to transfer identical bacterial colonies from freshly prepared Muller Hinton Agar plates into tightly covered test tubes filled with 5 mL of sterile saline solution. The suspension was vortexed and adjusted with sterile saline until a turbidity equivalent to 0.5 McFarland standards of 1×10^8 CFU (colony forming unit)/mL was obtained. The suspension turbidity was confirmed spectrophotometrically at an absorbance of 600 nm. The cell suspension was diluted in Muller Hinton Broth in a ratio 1:100 v/v to obtain an inoculum of approximate 10^4 CFU/mL. The final suspension was used for inoculation within 10 minutes of preparation. Both bacterial and fungal inocula were prepared in inoculation chamber maintained at an average temperature of 37°C using gas supplied blue flame.

Fungal Inoculum

The fungal inoculum was prepared using the method described by[31] with little modification. Fresh strains of fungi were sub-cultured at 27°C on sterile Sabouraud Dextrose Agar incubated for 72 hrs. The spores of fungi were swept into sterile saline, vortexed and later adjusted until it reached turbidity of 1×10^6 CFU/mL equivalence of 0.5-McFarland standards. The fungal suspension was diluted further with a Sabouraud Dextrose Broth to ratio 1:100 v/v until turbidity of 5×10^5 CFU/mL was achieved.

Extract Preparation

For antifungal and antibacterial activity, 200mg/mL of the extracts were respectively prepared in Sabouraud Dextrose and Muller Hinton Broth. About 100 μL of ethanol was added to the crude ethanolic extract before making up with the respective broth. Thereafter, 2-fold serial dilutions of (200, 100, 50, 25, 12.5 mg/mL) were prepared. Nystatin and ciprofloxacin prepared by double dilution method recommended by the Clinical and Laboratory Standard Institute were used as standards for antifungal and antibacterial activities respectively.

Agar Dilution Assay

This was assayed following Wiegand et al., (2008).[32] Sabouraud Dextrose and Muller Hinton Agar were respectively prepared for anti-fungal and antibacterial screening using the manufacturer's specification. 19 mL of the molten agar measured in an Erlenmeyer flask was autoclaved at 121°C. The agar was stepped down to 50°C by immersing the Erlenmeyer flasks in a water bath and 1 mL of the 2-fold serial dilutions was added, spun, dispensed in sterile Petri

dishes and left to solidify. 10 μL each of fungal and bacterial inocula were dispensed on the solidified agar and this produced an inoculum of 1×10^4 CFU/spot. Concentrations of plant samples ranged from 0.625mg/mL to 10mg/mL in both antifungal and antibacterial assays. Nystatin and ciprofloxacin were used as standard drugs for antifungal and antibacterial respectively at concentrations ranging from 1μg/mL to 16μg/mL for nystatin, and 4μg/mL to 64μg/mL for ciprofloxacin. Bacterial-inoculated plates were to an incubator kept at 37°C for 24 hrs and fungal plates were incubated for 48 hrs at 27°C under aseptic condition.

Minimum Inhibitory Concentrations (MIC)

The MIC value was evaluated as the minimum concentration of plant samples required to inhibit microbial growth on the agar dish.

Artemia Salina Assay

Cysts of *A. salina* were hatched in a beaker partly filled with seawater incubated at 27°C. After 24 hrs, a micropipette was used to transfer 10 nauplii into each petri dish having 30 mL of the 2-fold concentrations (1.0, 0.50, 0.25, 0.125, 0.0625 mg/mL) of positive control and the tested samples. The Petri dishes (arranged in triplicates) were partially enclosed and incubated at 27°C for 72 hrs under continuous illumination. Filtered seawater was used as negative control while potassium dichromate of same graded concentration as the extract was used as a positive control. Counting of active nauplii (those that showed body movement after some seconds of observation) was done every 12 hours and the percentage of mortality was estimated to assess the toxicity of the plant extracts.[33]

$$\% \text{ Mortality} = \left[\frac{Total\ nauplii\ -\ Active\ nauplii}{Total\ nauplii} \times 100 \right]$$

Statistical Analysis

A Minitab 17 statistical package distributed by Minitab, LLC, State College, Pennsylvania, was used to estimate 95% confidence interval of LC_{50} (mg/mL) values. LC_{50} is the concentration of the extracts at which 50% mortality occurred. The mean MIC values of different samples were calculated by Minitab 17 software and ranked with Fischer's Least Significant Difference (LSD) test.

Results

Antimicrobial Assay

The effects of *A. caudatus* extracts on different bacterial strains are shown in Table 2. According to the results, ethanol samples had higher bactericidal effects than water samples. The range of MIC values obtained from agar dilution for both gram-negative and gram-positive bacteria was between 2.5 and 10 mg/mL. All samples tested showed almost equal activity against bacteria except for ethanol extract of the control soil where the highest bactericidal effect was recorded at the pre-flowering stage. For *S. pyogenes*, MIC values ranged from 5 to 10 mg/mL in ethanolic and aqueous samples respectively in all soils and the 3 growth stages except in flowering harvests of clayey loam and silty clayey loam soils where MIC was

Table 2. Effect of Soil Types on Minimum Inhibitory Concentrations (MIC) of Aqueous and Ethanol Extracts of *A. caudatus* on Some Selected Bacteria Strains.

Maturity stage	Soil types	S. pyogenes		B. subtilis		S. aureus		S. pneumoniae		E.coli		P. aeruginosa	
		Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)
Pre-flowering	SF1	>5	10	>5	10	>5	10	>5	10	2.5	10	5	>5
	SF2	>5	10	>5	10	10	10	>5	10	10	10	5	10
	SF3	>5	10	>5	10	10	10	>5	10	>5	10	5	10
	SF4	>5	10	>5	10	10	10	>5	10	10	10	5	10
	SF5	>5	10	10	10	>5	10	10	10	>5	10	>10	10
Flowering	SF1	>5	10	10	10	10	10	>5	10	10	10	5	10
	SF2	>5	10	10	10	10	10	>5	10	10	10	5	10
	SF3	>5	>5	>5	10	10	10	>5	10	>5	10	5	10
	SF4	>5	>5	>5	10	10	10	>5	10	>5	10	>10	10
	SF5	>5	10	>5	10	>5	10	>5	10	>5	10	5	10
Post-flowering	SF1	>5	10	>5	10	10	10	>5	10	10	10	5	10
	SF2	10	10	>5	10	10	10	>5	10	>5	10	5	10
	SF3	10	10	10	10	10	10	10	10	10	10	>10	10
	SF4	10	10	10	10	10	10	10	10	10	10	>10	10
	SF5	10	10	10	10	10	10	10	10	10	10	>10	10

Table 3. Effect of Soil Types on Minimum Inhibitory Concentrations (MIC) of Aqueous and Ethanol Extracts of *A. caudatus* on Some Fungi Species.

Maturity stage	Soil types	P. chrysogenum		C. albicans		C. glabrata		P. aurantiogriseum	
		Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)	Ethanol (mg/mL)	Water (mg/mL)
Pre-flowering	SF_1	10	> 10	< 0.675	< 0.675	> 10	> 10	5	< 10
	SF_2	10	> 10	< 0.675	5	> 10	> 10	5	< 10
	SF_3	10	> 10	< 0.675	5	> 10	> 10	2.5	< 10
	SF_4	10	> 10	< 0.675	< 0.675	> 10	> 10	5	< 10
	SF_5	5	> 10	< 0.675	1.25	> 10	> 10	5	10
Flowering	SF_1	10	> 10	< 0.675	1.25	> 10	> 10	5	10
	SF_2	10	> 10	< 0.675	< 0.675	> 10	10	5	< 10
	SF_3	5	10	< 0.675	< 0.675	10	> 10	5	< 10
	SF_4	10	> 10	< 0.675	< 0.675	> 10	> 10	> 5	< 10
	SF_5	5	> 10	< 0.675	2.5	5	> 10	5	< 10
Post-flowering	SF_1	> 5	> 10	< 0.675	2.5	10	> 10	> 5	< 10
	SF_2	> 5	10	< 0.675	1.25	> 10	> 10	< 10	< 10
	SF_3	> 5	> 10	< 0.675	1.25	> 10	> 10	< 10	< 10
	SF_4	> 5	10	< 0.675	< 0.675	> 10	> 10	< 10	< 10
	SF_5	5	> 10	< 0.675	< 0.675	> 10	> 10	5	< 10

5 mg/mL in the aqueous extracts and post-flowering harvests of silty clayey loam, clayey loam, sandy clayey loam and loam soils where MIC was 10 mg/mL in the ethanolic samples. The bactericidal effect of aqueous extracts on *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa* and *S. pneumoniae* was felt at MIC of 10mg/mL in all soils and growth stages except in control soil where MICs of 5mg/mL was recorded in pre-flowering samples.

Results of antifungal MICs are shown in Table 3. The MIC values varied from 0.675 to 10 mg/mL. For *P. chrysogenum*, aqueous extracts of *A. caudatus* showed lower activity compared to ethanol extracts. Water extracts showed no inhibition to the growth of *P. chrysogenum* in all soil types at all concentrations of the pre-flowering samples tested whereas fungal growth was inhibited at 10 mg/mL in ethanolic samples of harvests from control, silty clayey loam, clayey loam and sandy-clayey loam. Ethanolic extracts of the plant harvested from loamy soil showed the maximum inhibition on *P. chrysogenum* at a lower concentration of 5 mg/mL. It was also observed that *C. Albicans* did not survive even the least concentration of all ethanolic samples at all maturity stages sampled. The pre-flowering aqueous extracts of control and clayey loam soils inhibited the growth of *C. albicans* at the lowest concentration (0.675 mg/mL); 1.25 mg/mL in harvest from loam and 5 mg/mL in harvests from sandy clay loam and silty clay loam. Unlike *C. albicans*, the growth of *C. glabrata* was not inhibited by both ethanolic and aqueous samples except in aqueous sample of sandy clay loam (flowering), ethanolic sample of silty clayey loam (flowering) and control soil (post-flowering) where inhibition occurred at 10 mg/mL. The growth of *P. aurantiogriseum* was inhibited at the highest concentration of the aqueous extracts (10 mg/mL) at all stages of growth while in ethanol extracts, MIC values varied between maturity stages depending on the soil types. The least MIC value (2.5 mg/mL) was observed in the pre-flowering ethanolic sample of silty clayey loam while in other soils, MICs value of pre-flowering harvest was 5mg/mL against the growth of *P. aurantiogriseum*. Also, the growth of *P. aurantiogriseum* was inhibited at an almost equal concentration of 5 mg/mL in all soil samples at flowering stage and post-flowering, inhibition was greater in control and loamy soil samples (5 mg/mL).

Artemia Salina Assay

Results from this assay showed variabilities in toxicity of *A. caudatus* extracts on brine shrimp in decreasing order; post-flowering > pre-flowering > flowering, with the highest toxicity in plants obtained from the clayey loam soil (Figure 1). Compared to the positive control potassium dichromate ($K_2Cr_2O_7$) used for toxicity test, the plant's extracts were less toxic although more toxic than saline (negative control). At post-flowering, total lethality was highest as proven by the LSD test. Samples from the control, clayey loam soils and sandy clayey loam had higher lethality than loam and silty clayey loam soils. Ethanolic extracts of plants harvested from clayey loam before flowering were below LC_{50} whereas, in other soil types, the percentage lethality ranged from 58.33% in control soil to 65.83% in silty clayey loam (Figures 2–4). Also, it was observed that the LC_{50} values of ethanolic extracts obtained at the flowering stage were below 50% (LC_{50}) in the control (Table 4), silty clayey loam and clayey loam soils whereas in sandy clayey loam and loam, percentage mortality ranged from 55.83% to 74.17% respectively.

Discussion

The growing interest in plants as fighters of pathogenic organisms has necessitated the need to examine toxicity before a
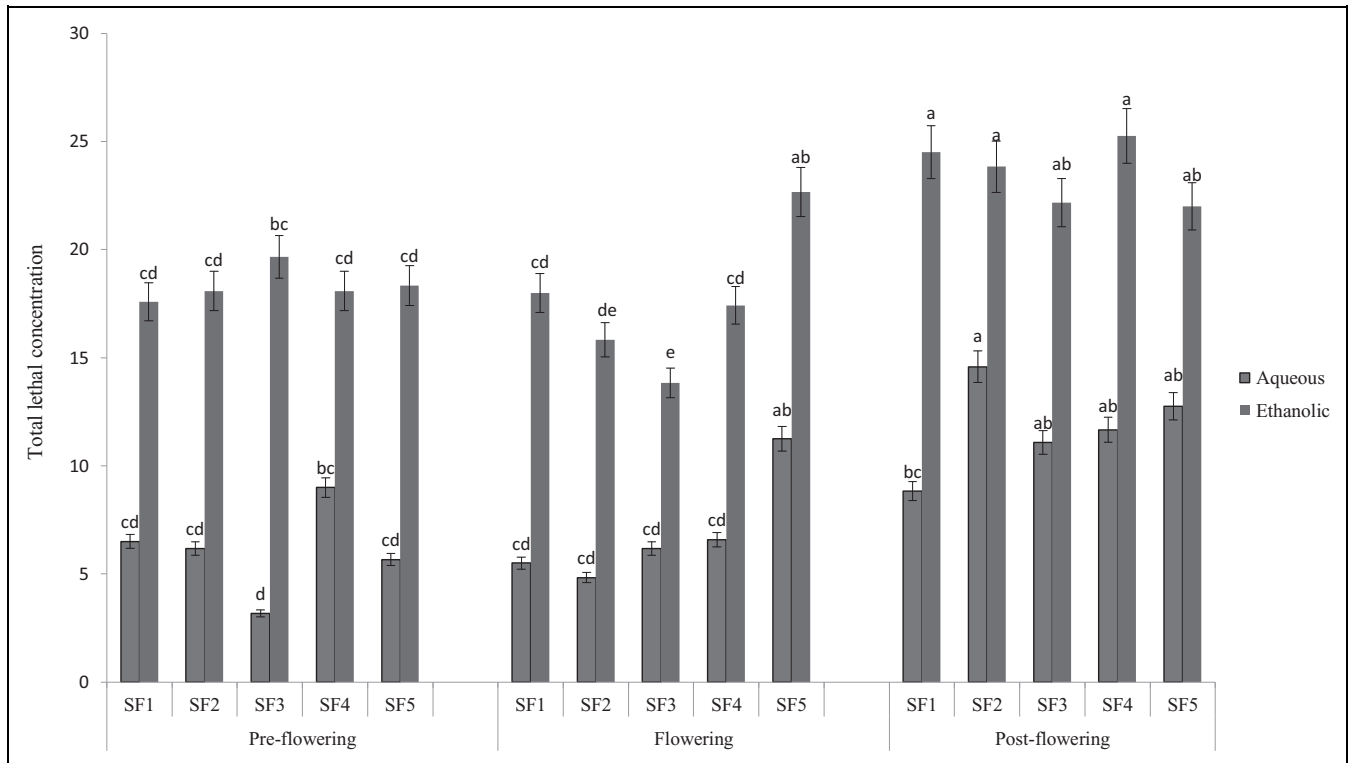
Figure 1. Total lethality concentration of *A. caudatus* harvested from different soils at different maturity stages using *Artemia salina* assay. Bars with the same alphabets are not significantly different at $p < 0.05$ across the 3 stages of harvest (pre-flowering, flowering and post-flowering).
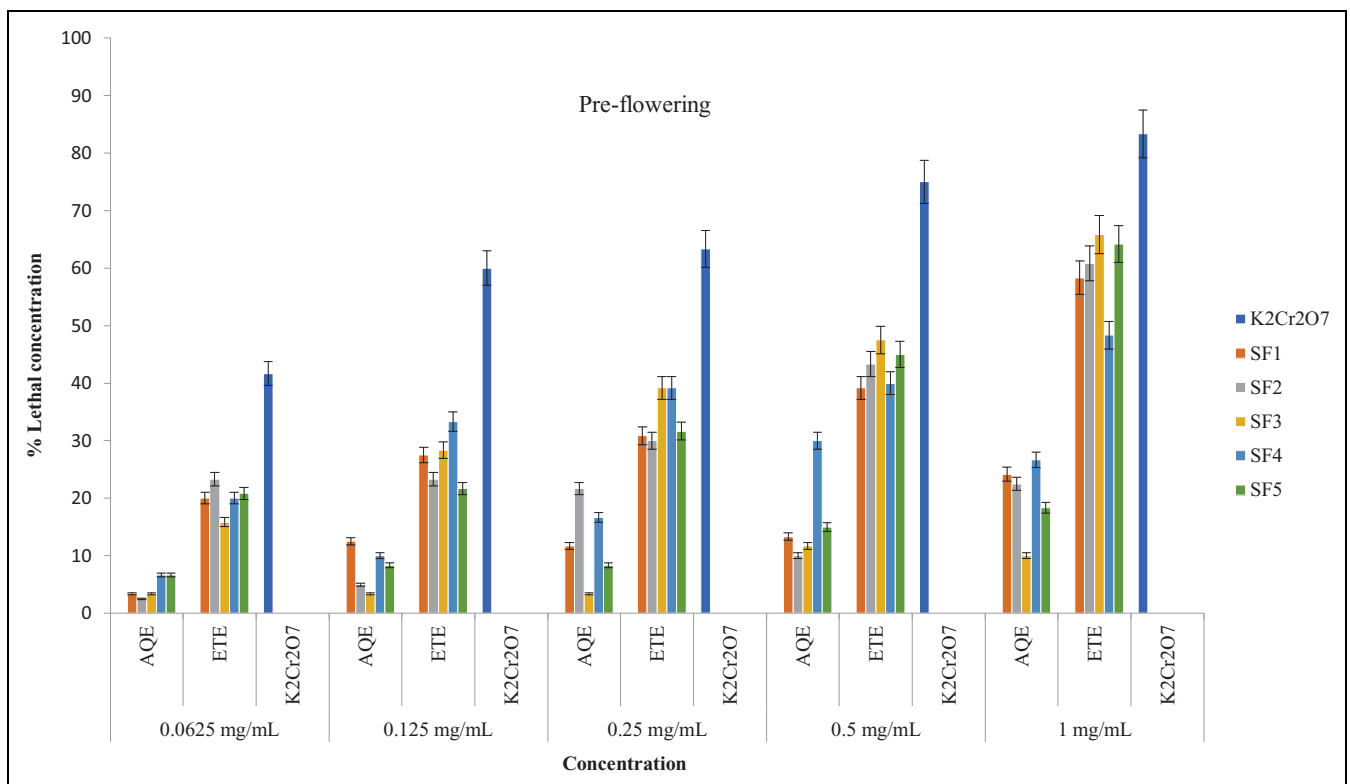
Figure 2. Percentage lethal concentration of *A. caudatus* harvested from different soils at pre-flowering.
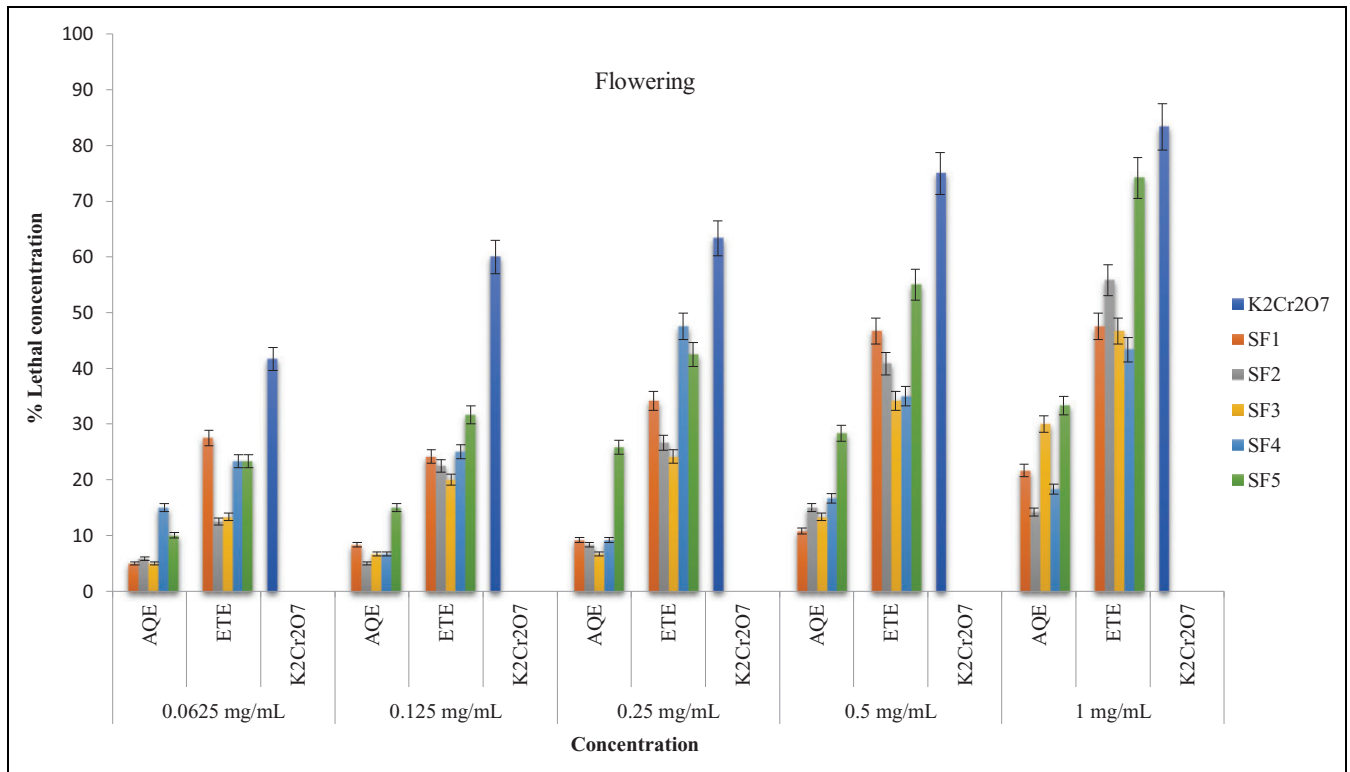
Figure 3. Percentage lethal concentration of *A. caudatus* harvested from different soils at the flowering stage.
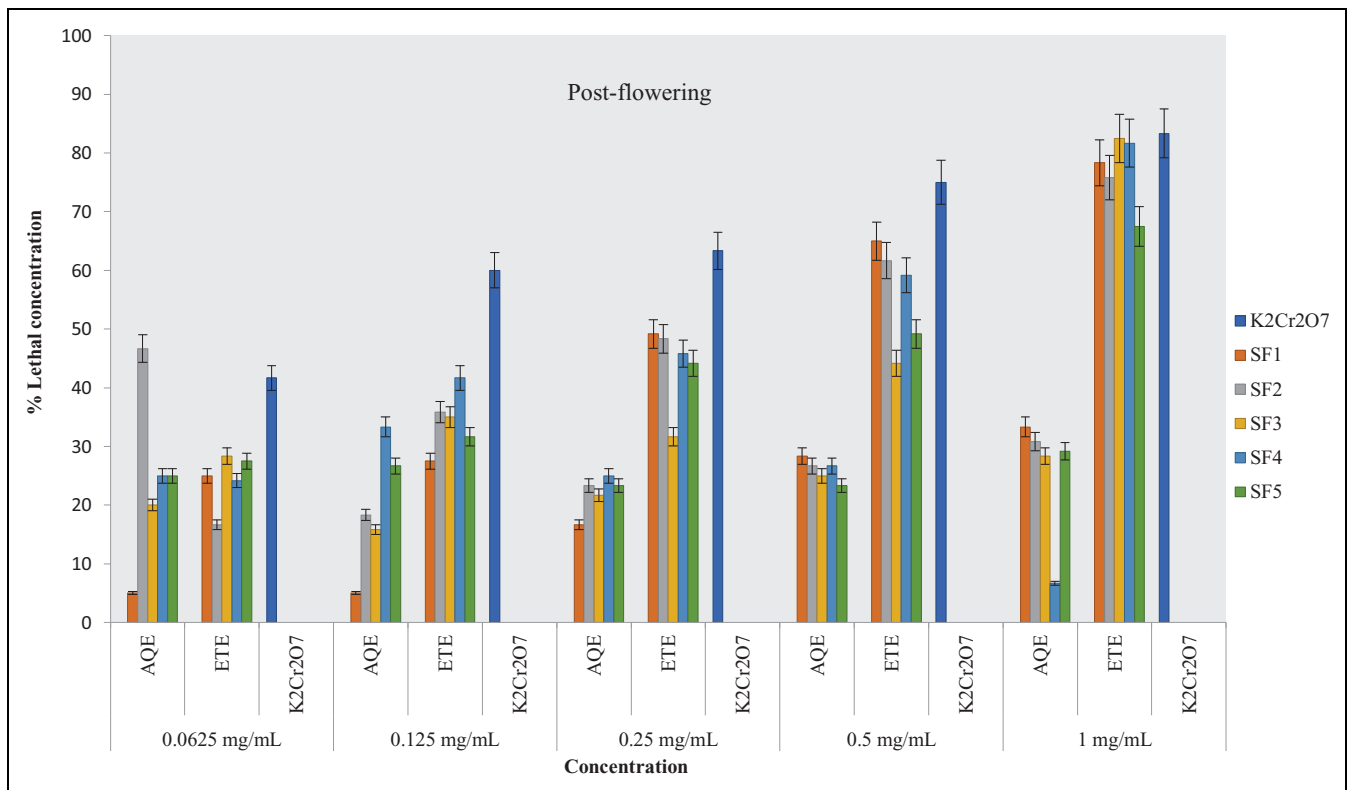
Figure 4. Percentage lethal concentration of *A. caudatus* harvested from different soils at post-flowering stage.

Table 4. LC$_{50}$ Values for the Toxicity of *A. caudatus* Harvested From Different Soils on *A. salina*.

Soil types	Pre-flowering		Flowering		Post-flowering	
	LC$_{50}$ eth-1	LC$_{50}$ aq-1	LC$_{50}$ eth-2	LC$_{50}$ aq-2	LC$_{50}$ eth-3	LC$_{50}$ aq-3
SF1	0.778	> 1	> 1	> 1	0.258	> 1
SF2	0.691	> 1	0.8	> 1	0.282	> 1
SF3	0.567	> 1	> 1	> 1	0.58	> 1
SF4	> 1	> 1	> 1	> 1	0.327	> 1
SF5	0.629	> 1	0.405	> 1	0.514	> 1

LC$_{50}$ (mg/mL) = minimum concentrations at which 50% mortality occurred, LC$_{50}$ eth-1= ethanolic pre-flowering, LC$_{50}$ aq-1= aqueous pre-flowering, LC$_{50}$ eth-2= ethanolic flowering, LC$_{50}$ eth-2= ethanolic flowering, LC$_{50}$ eth-3= ethanolic post-flowering, LC$_{50}$ eth-1= ethanolic post-flowering.

modest recommendation could be made. In many reports, efficacies of herbal products used as food supplement and medicines have been documented with sketchy information about their toxicity and other side effects.[34] This study, examined the susceptibility of some human pathogenic organisms to tested plant samples and possible adverse effects of these extracts when ingested. The MIC obtained for aqueous and ethanolic extracts suggested that harvest from control soil had the highest inhibitory effect on the bacteria listed for this experiment; particularly *E. coli* where MIC value was 2.5mg/mL in ethanolic extract. This agrees with previous studies by[35,36] that *A. caudatus* prevents infection caused by uropathogenic E. coli by decreasing the invasive and adhesive capacities of bacterial cells It was further observed that antibacterial effect of aqueous extracts was less than ethanol samples except in harvests from control soil (pre-flowering), clayey loam and sandy-clayey loam soils (flowering) where similar MIC of 5 mg/mL was recorded for *P. aeruginosa* and *S. pyogenes*.

Candida species are major pathogens responsible for fungal infections in the human bloodstream.[37,38] The activities of tested samples against the growth of some selected fungi indicated that *A. caudatus* is a good candidate for the cure of oral and vaginal candidiasis. This was further proven in *C. albicans* where fungal inoculum was not susceptible to the agar medium diluted with various concentrations of ethanolic extracts of the plant from all soil samples used in this experiment. Aqueous extracts of the plant also proved to be active against the pathogenic fungi (*C. albicans*) although with less activity. The pre-flowering harvest from clayey loam and control soils had the highest activity against *C. albicans*, followed by loam soils, then, sandy-clayey loam and silty clayey loam soils with MICs of 0.675, 1.25 and 5 mg/mL respectively (Table 3). Both aqueous and ethanolic extracts from all soils showed no activity against *C. glabrata* in pre-flowering samples; whereas, samples obtained during flowering had growth inhibition at MICs of 5 and 10 mg/mL respectively in ethanolic samples of loam and silty clayey loam soils; and 10 mg/mL in aqueous extracts derived from sandy clayey loam. Furthermore, post-flowering ethanolic sample from control soil showed activity against the pathogenic fungi at the MIC of 10 mg/mL. The trend observed is an indication of higher activity of tested samples on *C. albicans* than *C. glabrata*.

The antifungal effect of aqueous samples on *P. chrysogenum* and *P. aurantiogriseum* was considerably low particularly in *P. chrysogenum* as shown in the antifungal MIC values (Table 3). This may be due to the synergistic effect occasioned by the mutual interaction between the fungus and extracts. Instead of the extracts to inhibit colony growth, there was rather, possible cooperation or fungal growth stimulation, an indication of functional relatedness between the agar-extract medium and the fungi leading to proliferation and spatial expansion of colony perimeter as explained in the works of[39] and.[40] The MIC values of ethanol samples were higher for *P. chrysogenum* than *P. aurantiogriseum* in all soil types across the 3 harvest stages (Table 3). This implies that *P. aurantiogriseum* was more susceptible to *A. caudatus* extracts than *P. chrysogenum*; particularly, harvest from sandy clayey loam soil where the least MIC value was recorded for both fungal species at the pre-flowering stage. This agrees with[33] where lower inhibitory activity was recorded for plant samples at the pre-flowering stage.

Artemia salina assay has been a preliminary model of assessing toxic potential in plants over the years.[33,41] Also, several assays have reported a positive correlation between the LC$_{50}$ value in brine shrimp and lethal dose (LD$_{50}$) of the same plant in animals.[42] The order of toxicity stated above is an indication that the plant extracts were more toxic at post-flowering than other growth stages. Results further proved that soil type modifies lethal concentration in plants. The LC$_{50}$ values presented in Table 4 showed that aqueous samples were not toxic to the brine shrimp. Also, ethanolic samples of pre-flowering from clayey loam soil were least toxic while samples from silty clayey loam soil was highly toxic. The ethanol extracts of the plants were non-toxic in clayey loam, control and silty clayey loam soils; less toxic in sandy clayey loam soil and toxic in loam soil at flowering stage whereas at post-flowering, all ethanolic samples tested were toxic; particularly, the sample derived from control soil. This was determined following Clarkson et al., (2004)[43] lethality scale where plant extract with LC$_{50}$ < 0.5 mg/mL is considered toxic; 0.5 mg/mL $\leq$ 1 mg/mL is less toxic; and > 1 mg/mL is non-toxic. High toxicity observed in ethanol samples may not be unconnected with less polarity which facilitates degradation of toxic substances embedded in plant tissues [44-47] which aligns with the earlier

findings by[5,48] that the possibility of having higher inhibitory activity was more apparent in ethanol samples than aqueous extracts.

Conclusion

It could be established from this experiment that *A. caudatus* is a good pharmacological candidate for the treatment of pathogenic organisms. Further research on the synergistic effect of the plant with standard antibiotics is suggested to provide leads for new antimicrobial remedies that could lessen side effects attributed to synthetic drugs when used in isolation. The revelation that aqueous extracts of the plant are non-toxic regardless of growth stages and soil types corroborates the call for the reintroduction of the plant in the diet of households; particularly, low-income earners to cushion the adverse effect of hunger and malnutrition. For an optimal yield of the non-toxic harvest that may serve as pharmacological precursors, clayey loam soil is recommended for cultivation and harvesting may occur at pre-flowering or flowering stage using water extraction stabilized under anaerobic conditions.

List of abbreviations

ATCC -   American Type Culture Collection
CFU -   Colony Forming Unit
CRD -   Completely Randomized Design
$K_2Cr_2O_7$ - Potassium Dichromate
KZN -   Kwa-Zulu Natal
LC_{50} -   Lethal Concentration
LD_{50} -   Lethal Dose
LSD -   Least Significant Difference
MIC -   Minimum Inhibitory Concentration
MPED-   Medicinal Plants and Economic Development
pH -   Relative acidity and alkalinity
USA -   United State of America

Authors' Note

An ethical clearance with certificate reference number: LEW011S-JIM01 was obtained from the University of Fort Hare Research Ethics Committee before the research was carried out. MO designed and carried out the experiment. He also wrote and revised the manuscript. AJ supervised the experiment. He also provided plant seeds and laboratory resources for the research. FB supervised the experiment, perused and edited the manuscript. All authors have read and approved the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iD

Muhali Olaide Jimoh, PhD https://orcid.org/0000-0002-1183-5711

References

1. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan K. Combating pathogenic microorganisms using plant-derived antimicrobials: a minireview of the mechanistic basis. *Biomed Res Int*. 2014:761741. doi:10.1155/2014/761741

2. Tunger O, Karakaya Y, Cetin CB, Dinc G, Borand H. Review article rational antibiotic use. *J Infect Dev Ctries*. 2009;3(2): 88-93.

3. Cassidy CM, Donnelly RF, Elborn JS, Magee ND, Tunney MM. Photodynamic antimicrobial chemotherapy (PACT) in combination with antibiotics for treatment of *Burkholderia cepacia* complex infection. *J Photochem Photobiol B Biol*. 2012;106(1): 95-100. doi:10.1016/j.jphotobiol.2011.10.010

4. Ronqui MR, de Aguiar TM, De Freitas LM, Miranda ET, Fontana CR. Synergistic antimicrobial effect of photodynamic therapy and ciprofloxacin. *J Photochem Photobiol B Biol*. 2016;158:122-129. doi:10.1016/j.jphotobiol.2016.02.036

5. Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. *BMC Complement Altern Med*. 2006;6(2):1-6. doi:10.1186/1472-6882-6-2

6. Jimoh MO, Afolayan AJ, Lewu FB. Therapeutic uses of *Amaranthus caudatus* L. *Trop Biomed*. 2019;36(4):1038-1053.

7. de Souza Cândido E, Pinto MF, Pelegrini PB, et al. Plant storage proteins with antimicrobial activity: novel insights into plant defense mechanisms. *J Physiol Pharmacol*. 2011;25(Suppl1): 35-54. doi:10.1096/fj.11-184291

8. Sher A. Antimicrobial activity of natural products from medicinal plants. *Gomal J Med Sci*. 2009;7(1):72-78. http://www.ncbi.nlm.nih.gov/pubmed/12385884

9. Jaberian H, Piri K, Nazari J. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chem*. 2013;136(1):237-244. doi:10.1016/j.foodchem.2012.07.084

10. Idris OA, Wintola OA, Afolayan AJ. Phytochemical and antioxidant activities of *Rumex crispus* L. in treatment of gastrointestinal helminths in Eastern Cape Province, South Africa. *Asian Pac J Trop Biomed*. 2017;7(12):1071-1078. doi:10.1016/j.apjtb.2017.10.008

11. Jimoh MO, Afolayan AJ, Lewu FB. Micromorphological assessment of leaves of *Amaranthus caudatus* L. cultivated on formulated soil types. *Appl Ecol Environ Res*. 2019;17(6):13593-13605.

12. Makinde EA, Ayeni LS, Ojeniyi SO. Effects of organic, organomineral and NPK fertilizer treatments on the nutrient uptake of *Amaranthus cruentus* (L.) on two soil types in Lagos, Nigeria.

J Cent Eur Agric. 2011;12(1):114-123. doi:10.5513/JCEA01/12. 1.887

13. Jimoh MO, Afolayan AJ, Lewu FB. Antioxidant and phytochemical activities of *Amaranthus caudatus* L. harvested from different soils at various growth stages. *Sci Rep*. 2019;9(1):12965. doi:10. 1038/s41598-019-49276-w

14. Akula R, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav ISSN*. 2011; 6(11):1720-1731. doi:10.4161/psb.6.11.17613

15. Jimoh MO, Afolayan AJ, Lewu FB. Heavy metal uptake and growth characteristics of *Amaranthus caudatus* L. under five different soils in a controlled environment. *Not Bot Horti Agrobot*. 2020;48(1):417-425. doi:10.15835/nbha48111656

16. Bednarek P, Osbourn A. Plant-microbe interactions: chemical diversity in plant defense. *Science (80-)*. 2009;324(5928): 746-748. doi:10.1126/science.1171661

17. Vig AP, Rampal G, Thind TS, Arora S. Bio-protective effects of glucosinolates—a review. *LWT - Food Sci Technol*. 2009;42(10): 1561-1572. doi:10.1016/j.lwt.2009.05.023

18. Al-Daihan S, Al-Faham M, Al-shawi N, Almayman R, Brnawi A, shafi Bhat R. Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. *J King Saud Univ - Sci*. 2013;25(2):115-120. doi:10.1016/j.jksus.2012.11.003

19. De Lucca AJ, Cleveland TE, Wedge DE. Plant-derived antifungal proteins and peptides. *Can J Microbiol*. 2005;51(12):1001-1014. doi:10.1139/w05-063

20. Corns CM. Herbal remedies and clinical biochemistry. *Ann Clin Biochem*. 2003;40(Pt 5):489-507.

21. Elvin-Lewis M. Should we be concerned about herbal remedies. *J Ethnopharmacol*. 2001;75(2-3):141-164. doi:10.1016/S0378-8741(00)00394-9

22. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicol Appl Pharmacol*. 2010;243(2):198-216. doi:10.1016/j.taap.2009.12.005

23. Haq I. Safety of medicinal plants. *Pakistan J Med Res*. 2004; 43(4):1-8.

24. George P. Concerns regarding the safety and toxicity of medicinal plants concerns regarding the safety and toxicity of. *J Appl Pharm Sci*. 2011;1(6):40-44.

25. Idris OA, Wintola OA, Afolayan AJ. Comparison of the proximate composition, vitamins analysis of the essential oil of the root and leaf of *Rumex crispus* L. *Plants*. 2019;51(8 (3):1-15. doi:10. 3390/plants8030051

26. Paredes-Lopez O. *Amaranth: Biology, Chemistry, and Technology*. 2018th ed. P. OP-L, ed. CRC Press © Taylor & Francis Group; 1994. Updated September 27, 2018. Accessed September 20, 2018. http://www.crcpress.com

27. Jimoh MO, Afolayan AJ, Lewu FB. Suitability of Amaranthus species for alleviating human dietary deficiencies. *South African J Bot*. 2018;115:65-73. doi:10.1016/j.sajb.2018.01.004

28. Broekaert WF, Marien W, Terras FRG, et al. Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. *Biochemistry*. 1992;31(17):4308-4314. doi:10.1021/bi00132a023

29. Jimoh MO, Afolayan AJ, Lewu FB. Nutrients and antinutrient constituents of *Amaranthus caudatus* L. cultivated on different soils. *Saudi J Biol Sci*. 2020: In print. doi:10.1016/j.sjbs.2020.07.029

30. Idris OA, Wintola OA, Afolayan AJ. Evaluation of the bioactivities of *Rumex crispus* L. leaves and root extracts using toxicity, antimicrobial, and antiparasitic assays. *Evid Based Complement Alternat Med*. 2019;2019:6825297.

31. Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. *Sci World J*. 2017:9658598. doi:10.1155/2017/9658598

32. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*. 2008;3(2):163-175. doi: 10.1038/nprot.2007.521

33. Adegbaju OD, Otunola GA, Afolayan AJ. Influence of plant maturity on antimicrobial properties and toxicity of *Celosia argentea*. *J Biol Sci*. 2019;19(4):280-289. doi:10.3923/jbs.2019. 280.289

34. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014;4(January):1-10. doi:10.3389/fphar.2013.00177

35. Mohanty S, Zambrana S, Dieulouard S, et al. *Amaranthus caudatus* extract inhibits the invasion of *E. coli* into uroepithelial cells. *J Ethnopharmacol*. 2018;220:155-158. doi:10.1016/j.jep.2018. 04.003

36. Martinez-Lopez A, Millan-Linares MC, Rodriguez-Martin NM, Millan F, Montserrat-de la Paz S. Nutraceutical value of kiwicha (*Amaranthus caudatus* L.). *J Funct Foods*. 2020;65:103735. doi:10.1016/j.jff.2019.103735

37. Calderone RA, Clancy CJ. *Candida and Candidiasis*. 2nd ed. Calderone RA, Clancy CJ, eds. ASM Press; 2012. Updated September 27, 2018. Accessed October 11, 2018. https://books.google.co.za/books?hl=en&lr=&id =9u6r BAAAQBAJ&oi=fnd&pg= PT9&dq=+Calderone+RA,+Clancy+CJ.+Candida+and+Candidiasis:+ASM+Press,+Washington,+DC,+2012.&ots=g_FmAJx dUI&sig=TTCEFmgD5QVrO4w91WRizUeO-uU#v=onepage &q&f=false

38. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. *Virulence*. 2013;4(2):119-128.

39. Van Dyken DJ, Müller MJI, Mack KML, Desai MM. Spatial population expansion promotes the evolution of cooperation in an experimental prisoner's dilemma. *Curr Biol*. 2013;23:919-923. doi:10.1016/j.cub.2013.04.026

40. Unuofin JO, Otunola GA, Afolayan AJ. Polyphenolic content, antioxidant and antimicrobial activities of *Vernonia mespilifolia* Less. Used in folk medicine in the Eastern Cape Province, South Africa. *J Evidence-Based Integr Med*. 2018;23:1-9. doi:10.1177/ 2515690X18773990

41. Bastos MLA, Lima MRF, Conserva LM, Andrade VS, Rocha EMM, Lemos RPL. Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. *Ann Clin Microbiol Antimicrob*. 2009;8(16):1-6. doi:10.1186/1476-0711-8-16

42. Hamidi MR, Jovanova B, Panovska TK. Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. *Maced Pharm Bull*. 2014;60(1):9-18.

43. Clarkson C, Maharaj VJ, Crouch NR, et al. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *J Ethnopharmacol*. 2004;92(2-3):177-191. doi:10.1016/j.jep.2004.02.011

44. O'Neil MJ. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*. 15th ed. O'Neil MJ, ed. Royal Society of Chemistry (Great Britain); 2013. Updated September 27, 2018. Accessed October 28, 2018. https://pubs.rsc.org/en/content/ebook/978-1-84973-670-1

45. Lapornik B, Prosěk M, Wondra AG. Gas chromatography-mass spectrometry analysis and phytochemical screening of methanolic leaf extract of *Plumbago zeylanica*, Linn. *J Food Eng*. 2005;71:214-222. doi:10.1016/j.jfoodeng.2004.10.036

46. Azmir J, Zaidul IS, Rahman M, et al. Techniques for extraction of bioactive compounds from plant materials: a review. *J Food Eng*. 2013;117(4):426-436. doi:10.1016/j.jfoodeng.2013.01.014

47. Jimoh MA, Idris OA, Jimoh MO. Cytotoxicity, phytochemical, antiparasitic screening, and antioxidant activities of *Mucuna pruriens* (Fabaceae). *Plants*. 2020;9(1249):1-13.

48. Ohikhena FU, Wintola OA, Afolayan AJ. Toxicity assessment of different solvent extracts of the medicinal plant, phragmanthera capitata (sprengel) balle on brine shrimp (*Artemia salina*). *Int J Pharmacol*. 2016;12(7):701-710. doi:10.3923/ijp.2016.701.710