Noxious to ecosystems, but relevant to pharmacology: four South African alien invasive plants with pharmacological potential

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Highlights

• Four South Africa alien invasive plants have pharmacological relevance.

• Extracts inhibited many bacterial, fungal and mycobacterial strains tested.

• Bioactive phytochemicals in the plants were present at significant levels.

• The extracts had low cytotoxicity against Vero cells and no genotoxicity.

• Excellent selectivity index values were obtained for some extracts.

Abstract

Alien invasive plants pose a huge threat to natural and semi-natural ecosystems in their introduced ranges thereby compromising ecosystem integrity. However, anecdotal and empirical evidence suggests that some invasive alien plants are used in traditional medicine due to their pharmacological activities. Here, we evaluated the antimicrobial activity of 70% ethanol, dichloromethane, acetone and hot water extracts of four invasive alien plants in South Africa viz. *Dolichandra unguis-cati*, *Cardiospermum grandiflorum*, *Chromolaena odorata* and *Gomphrena celosioides* against pathogenic and non-pathogenic microbes. The test organisms included *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), *Salmonella* Dublin, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium aurum*, *M. fortuitum*, *M. smegmatis*, *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. Phytochemicals that may be responsible for antimicrobial activity were determined using standard phytochemical methods. A further objective was to investigate the safety of these plants by conducting cytotoxicity and genotoxicity tests. All solvent extracts of plants investigated exhibited a broad spectrum of antibacterial activity with minimum inhibitory concentration (MIC) values ranging from 0.039 to 2.5 mg/ml, with the acetone and dichloromethane extracts showing better activity against *E. coli*, *K. pneumoniae* and *E. faecalis* (MIC between 0.039 and 0.078 mg/ml). Of all extracts tested, only the ethanol extracts of *C. grandiflorum* showed good antimycobacterial activity with MIC of 0.078 mg/ml against *M. smegmatis*. In contrast, *C. grandiflorum* only showed moderate antifungal activity, while dichloromethane and acetone extracts of the other three plants were very effective against *C. neoformans* and *A. fumigatus* with MIC values ranging from 0.019 to 0.078 mg/ml. All four plants moderately inhibited *C. albicans* at MIC of 0.156 mg/ml. The plant species were rich in phenolics, flavonoids and tannins in varying amounts and had relatively low levels of cytotoxicity and none was mutagenic. Promising selectivity index values (between 10 and 50) highlight the potential of these plant species as sources of antimicrobial remedies. Despite the ecological noxiousness of these alien invasive plants, our findings suggest that they possess some antimicrobial properties that are too pharmacologically relevant to ignore.

Keywords: Alien invasive plants; Antimicrobial; Antifungal, Antimycobacterial; Toxicity; Phytochemical

1. Introduction

The economic burden caused by alien invasive plants due to their negative impact on agriculture, livelihoods and biodiversity in different parts of Africa cannot be over-emphasized. Pimentel et al. (2005) estimated economic loss caused by the impact of invasive species affecting crops in the United States to be thirty-three billion dollars annually. However, Africa lacks such records, as the economic loss caused by invasive species has not been thoroughly studied. In July 2016, The National Environmental Management: Biodiversity Act (NEMBA, 2016) listed 379 plants as invasive in South Africa. Alien plants such as *Cardiospermum grandiflorum* Swartz (Sapindaceae) (native to Asia, the Caribbean and Americas); *Gomphrena celosioides* Mart (Amaranthaceae) (native to the Americas), *Dolichandra unguis-cati* (= *Macfadyena unguis-cati*) (L.) A.H. (Bignoniaceae) (native to Central and tropical South America); and *Chromolaena odorata* (L.) King and Robinson (Asteraceae) (native to the Americas) are invasive in many tropical and subtropical countries on the African continent including South Africa (Holm et al., 1977; Grierson and Long, 1984; Henderson, 2001; Acevedo-Rodriguez, 2005; Carroll et al., 2005; Kriticos et al., 2005 McKay et al., 2010; King et al., 2011).

Cardiospermum grandiflorum, popularly called balloon vine, is a perennial, woody climber that reproduces mainly by seeds which are dispersed by wind and it also has the ability to regrow from root fragments (Simelane et al., 2011). Though there is conflicting information about its nativity to the tropical regions of Africa (McKay et al., 2010), many sources consider it to be alien to South Africa (MacDonald et al., 2003). The plant is invasive in South African provinces such as Gauteng, Limpopo, Mpumalanga, Eastern Cape and KwaZulu-Natal (Henderson, 2001; Simelane et al., 2011). The sprawling herb *G. celosioides*, commonly called bachelor's button, produces seeds which are distributed by ants. Although

seen along grassy roadsides and upland rice field areas in South Africa, detailed reports on its distribution range are scarce. *Dolichandra unguis-cati,* also known as cat's claw creeper, is a perennial vine invasive in Gauteng, Mpumalanga, Limpopo and North West provinces and in some areas in KwaZulu-Natal (Henderson, 2001; Starr and Starr, 2008). The plant invades cultivated orchards, plantations, riparian corridors, natural forest remnants and disturbed areas such as roadsides and abandoned lands (Williams, 2002). *Chromalaena odorata*, an herbaceous perennial flowering shrub has two biotypes in Africa (Kriticos et al., 2005; Omokhua et al., 2016). The SAB, which is only present in southern Africa (Paterson and Zachariades, 2013), invades agricultural lands and animal grazing grounds and water courses (Mbane, 2007; Omokhua et al., 2016). The spread of the weed is linked to human movements and other anthropogenic factors such as regional trade and road construction (Uyi and Igbinosa, 2013). The plant is present in KwaZulu-Natal, Mpumalanga, Limpopo and Eastern Cape provinces (Goodall and Erasmus, 1996; Kriticos et al., 2005).

Although several control measures such as mechanical, chemical and biological controls have been initiated against these invasive plants, they seem to be negligible in terms of success rate (King et al., 2011; Simelane et al., 2011; Zachariades et al., 2011). However, these plants are exploited as sources of medicine in some of their introduced ranges (Kubmarawa et al., 2007; Kokwaro, 2009; Soladoye et al., 2013; Chauke et al., 2015). In some parts of Africa, the leaves of *C. grandiflorum* are used for the treatment of dermatological troubles, fever and chest problems (Burkill, 1995; Kubmarawa et al., 2007). *Gomphrena celosioides* finds traditional use in different parts of Africa including southern Africa for treating coughs, colds, bronchitis, diabetes, sexually transmitted infections, hay fever, liver diseases, malaria, dysmenorrhea, asthma, worm and kidney infections in humans as well as skin problems in cattle (Soladoye et al., 2013; Rahman and Gulshana, 2014; Chauke et al., 2015). *Dolichandra*

unguis-cati is traditionally used to treat dysentery, rheumatism, inflammation, snakebite, malaria and venereal disease (Pio Correa, 1978; Houghton and Osibogun, 1993; Hilgert, 2001; Rahmatullah et al., 2010; Torres et al., 2013). Although there is no existing literature on the traditional use of the SAB biotype of *C. odorata*, our previous study has shown that the SAB has the same medicinal properties as the AWAB (Omokhua et al., 2017) which is used as a source of medicines in different parts of Africa, especially in the western region for treating skin infections, wounds, inflammation, diarrhoea, coughs and colds, malaria, abdominal and cervical pains, urinary retention, gonorrhoea, blood in urine, ulcers and skin eruptions (Ayensu, 1978; Inya-Agha et al., 1987; Omokhua et al., 2016).

Several phytoconstituents have been reported from different parts of these plants. Tannins, steroids and reducing sugars have been reported from *C. grandiflorum* (Olaoluwa and Olapeju, 2015). Alkaloids, flavonoids, tannins, saponins, amino acids, terpenoids, steroids, glycosides and reducing sugars have been identified from *G. celosioides* and two compounds; 3-(4-hydroxyphenyl) methyl propenoate and aurantiamide have been isolated (Botha and Gerritsma-Van der Vijver, 1986; Onocha et al., 2005; Dosumu et al., 2014). Vaccenic and palmitoleic acids, phenolic and flavonoid compounds have been reported to be present in *D. unguis-cati* (Aboutabl et al., 2008). Although a few studies (Mdee et al., 2009; Aderogba et al., 2014; Meela et al., 2017) have investigated the biological activities of some alien invasive plant species against fungi, studies on the pharmacological activities of alien invasive plants in South Africa are still scarce. Therefore a further understanding of the antimicrobial activities and pharmacological potential of some of these plants would not be trivial. Hence, the current study investigated and compared the antimicrobial activities, phytochemical constituents and safety levels of the selected South Africa alien invasive plants; *C. grandiflorum*, *C. odorata*, *D. unguis-cati* and *G. celosioides*.

2. Materials and Methods

2.1 Plant collection and sample preparation

The leaves of *C. grandiflorum*, *C. odorata, D. unguis-cati* and *G. celosioides* were collected from the wild in Pretoria, South Africa in the summer months. Voucher specimens (Coll. No. 2 PRU 123726 *Cardiospermum grandiflorum*, Coll. No. 5 PRU 123727 *Chromolaena odorata*, Coll. No. 3 PRU 123629 *Dolichandra unguis-cati* and Coll. No. 4 PRU 123630 *Gomphrena celosioides*) were prepared and deposited after being identified at the H.G.W.J. (Herold Georg Wilhelm Johannes) Schweickerdt Herbarium, University of Pretoria, South Africa. Leaves of plants collected were thoroughly cleaned and dried in a drying room at the Department of Paraclinical Sciences, University of Pretoria. The dried plants were ground to powder and stored in sealed glass jars before experiments.

2.2 Preparation of plant extracts for antimicrobial and toxicity assays

Powdered leaf plant material (4 g) of each plant was weighed into centrifuge tubes and 40 ml of 70% ethanol, hot distilled water, acetone or dichloromethane (DCM) was added to separate aliquots. The mixtures were centrifuged at 300 × g for 10 min and filtration was carried out using Whatman No. 1 filter paper. The resultant extracts were transferred into pre-weighed labelled glass vials and the procedure was repeated twice on the marc to exhaustively extract plant material. Resultant extracts were placed under a stream of air to dry completely and stored in the dark room at 4 ^{o}C while preparing for the experiment.

2.3 Extraction of plant material for phytochemical determination

Plant samples (0.1 g) were weighed into centrifuge tubes and 10 ml of 50% methanol (MeOH) was added and centrifuged at 300 × g for 10 min and filtered through Whatman No.

1 filter paper. The resultant extracts were immediately used for the phytochemical determination to prevent deterioration and decomposition of metabolites.

2.4 Antimicrobial screening

2.4.1 Microbial strains

Out of 13 microbial strains used in this study, eight were obtained from the American Type Culture collection (ATCC), one from the National Collection of Type Cultures (NCTC) and four were clinical isolates. The strains used were *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Salmonella* Dublin (ATCC 15480), *Salmonella* Typhimurium (ATCC 700720), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Mycobacterium aurum* (NCTC 10437), *M. fortuitum* (ATCC 6841) and *M. smegmatis* (ATCC 1441). Isolates were *Klebsiella pneumoniae* (from commercial chicken eggs (Elisha et al., 2017), *Candida albicans* (from a Gouldian finch), *Cryptococcus neoformans* (from a cheetah) and *Aspergillus fumigatus* (from a chicken with systemic mycosis).

2.4.2 Culturing microbial strains

All bacterial stocks were maintained on Müller-Hinton (MH) agar with the exception of *Mycobacterium* strains which were stored on Löwenstein–Jensen agar slants supplemented with glycerol (adopting the method of McGaw et al., 2008), while fungal strains were maintained on Sabouraud Dextrose (SD) agar. The agar was sterilized by autoclaving and poured into petri dishes and allowed to solidify. The plates were allowed to cool overnight and the stock bacterial and fungal strains were streaked on the plates. The inoculated bacterial plates were then incubated for 24 h at 37 ^{o}C, except for *M. aurum* which was incubated for 72

h, while fungal plates were incubated for 48 h and 72h at 30 ^{o}C to allow the colonies to develop. Plates were stored at 4 ^{o}C for maintenance.

2.4.3 *In vitro* antibacterial assay

Antibacterial assays were carried out using the serial microdilution method in 96-well microplates as described by Eloff (1998). *Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Typhimurium, *Salmonella* Dublin and *Klebsiella pneumoniae* were prepared by inoculating a single colony of each bacterial strain from an agar plate into sterilized MH broth in sterile McCartney bottles and incubated at 37 ^{o}C in an incubator with an orbital shaker for between 12 and 18 h. Following incubation, each bacterial strain was diluted in MH broth and the absorbance was measured at a wavelength of 560 nm using a microplate reader, compared to a McFarland standard No 1 (correlating to approximately 3×10^{8} cfu/ml). One hundred microliters of sterile water were added to each well of a sterilized 96-well microplate. Plant samples (100 µl) re-suspended to 10 mg/ml in distilled water for the water extracts and acetone for the organic solvent extracts were added to the first well of the microplates (row A) and then serially diluted two-fold downwards (column 1-12: A to H, discarding 100 µl from row H). Gentamicin (2 mg/ml) was used as a positive control while acetone, sterile distilled water and bacterial cultures were used as negative and untreated controls. Subsequently, 100 µl of each diluted bacterial culture were added to all wells of the microplates. The microplates were incubated at 37^{o}C for 24 h. To each well of the incubated microplates, 40 µl of 0.2 mg/ml p-iodonitrotetrazolium (INT) were added to indicate the minimum inhibitory concentration (MIC) and the plates were re-incubated at 37 ^{o}C for 1 h. The well with clear inhibition of bacterial growth was taken as the MIC. The experiment was repeated twice with three replicates in each assay.

2.4.4 *In vitro* antimycobacterial assay

Cultures of mycobacterial strains were prepared by inoculating each strain into fresh Middlebrook 7H9 broth supplemented with 10% oleic acid, albumin, dextrose, and catalase (OADC). The inoculated cultures were incubated at 37 ^{o}C (*M. fortuitum* and *M. smegmatis* for 24 h and *M. aurum* for 72 h). Innoculated cultures were diluted with the prepared broth and absorbance was read at 560 nm to correlate with a McFarland standard 1. Rifampicin, isoniazid and streptomycin were used as positive controls while acetone, sterile distilled water and cultures were used as negative and untreated controls. Middlebrook 7H9 broth (100 µl) was added to sterile 96-well plates followed by prepared extracts which were then two-fold serially diluted. The cultures (100 µl) were added to the wells and incubated for 24 h. After incubation, 40 µl of INT was added to obtain MIC values following the method described by Eloff (1998). The experiment was repeated twice with three replicates in each assay.

2.4.5 *In vitro* antifungal assay

Adopting the method of Eloff (1998) modified by Masoko et al. (2007), *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans* cultures were prepared by inoculating each fungal species in sterilized SD broth in sterile McCartney bottles. Cultures were incubated at 30^{o}C in an incubator with an orbital shaker for 24 h for *C. albicans* and 72 h for *A. fumigatus* and *C. neoformans*. Each fungal culture was diluted in SD broth and the absorbance was measured at a wavelength of 560 nm using a microplate reader, and adjusted to match a McFarland No 1 standard to correlate to approximately 3×10^{8} cfu/ml. Sterile water (100 µl) was added to each well of a 96 well microplate; 100 µl of each extract were added to the first well of the microplates (row A) and serially diluted two-fold. Following this, 100 µl of the prepared cultures were added to each well of the microplates. Similarly,

100 µl of 2 mg/ml amphotericin B (positive control) and acetone, sterile distilled water and cultures (negative and untreated controls) were also two-fold serially diluted. After incubating for 24 h at 30 ^{o}C, 50 µl of 0.2 mg/ml INT were added to the plates, and further incubated for 24 h, results were read and were further incubated for 24 h (results were read at 48 and 72 h respectively). The experiment was repeated twice with each sample prepared in triplicate.

2.5 Quantitative determination of phytochemicals

Standard methods were used to quantitatively determine total phenolics, total flavonoids, flavonol content and hydrolysable tannins using the freshly prepared 50% MeOH crude extracts. Total phenolic compositions of the plant extracts were evaluated using the Folin-Ciocalteu method (Makkar, 2003) with some modifications. Using gallic acid as the standard to determine total phenolic content, 50 µl of the 50% MeOH plant extracts were transferred into test tubes (5 test tubes replicates for each extract), 950 µl of sterile distilled water was added followed by the addition of 500 µl of 1 N Folin-C reagent and 2.5 ml of 2% sodium carbonate ($NaCO_3$) in the dark. Similarly, a blank containing 50% MeOH in place of the plant extracts and different concentrations of gallic acid were prepared (concentration between 0 and 150 mg/ml). The test tubes containing the mixtures were incubated at room temperature for 40 min, and 200 µl of the reacted mixtures were immediately transferred into 96 well plates and absorbance was measured at 725 nm using a microplate reader. Total phenolics were expressed as gallic acid equivalents (GAE) per gram dry weight.

Hydrolysable tannin content of the plant extracts was determined using the butanol-HCl assay as described by Makkar (2003). Five hundred microliters of plant extracts were dispensed into test tubes and diluted to 10 ml with 50% MeOH. Three mg of butanol/HCl (95%/5%)

reagent and 100 µl of 2% ferric ammonium sulphate in 2N HCl were added. The test tubes were loosely covered and heated in a boiling water bath for 50 min. After the tubes were allowed to cool at room temperature the absorbance was read at 550 nm. Absorbance of the unheated mixture was used as the blank.

Total flavonoid content of the plant extracts was determined using the aluminium chloride method as described by Abdel-Hameed et al. (2009) with some modification. One hundred microlitre of plant extract was mixed with 100 µl of 20% $AlCl_3$ and 2 drops of glacial acetic acid. The mixture was diluted with 50% MeOH to 3000 µl. Blank samples were prepared with plant extracts without $AlCl_3$, and standard curve was prepared using catechin (concentration between 0-150 mg/ml) in MeOH. After 40 min absorbance was read at 415 nm. The total flavonoid content was expressed as mg catechin equivalent/g of dry plant material.

Flavonol content of the plant extracts was determined by the aluminium chloride method as described by Abdel-Hameed et al. (2009) with some modification. One ml of each plant extract was mixed with 1 ml of 20 mg/ml of $AlCl_3$ and 3 ml of 50 mg/ml of sodium ethanoate (CH_3COONa). A standard curve was prepared using catechin in MeOH. The absorbance was read at 440 nm after incubation for 2.5 h. The amount of flavonol was expressed as mg catechin equivalent/g of dry plant material.

2.6 Toxicological assays

2.6.1 *In vitro* cytotoxicity assay

The cytotoxicity test was carried out by testing the plant extracts against Vero monkey kidney cells, using the tetrazolium-based colorimetric (MTT) assay as described by Mosmann (1983)

modified by McGaw et al. (2007). Vero cells were grown in Minimal Essential Medium (MEM) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). Cells of a subconfluent culture were harvested and centrifuged at 200 × g for 5 min, and resuspended in MEM to 5×10^4 cells/ml. Cell suspension (100 µl) was pipetted into each well of columns 2 to 11 of a tissue culture graded sterile 96 well microtitre plate and only MEM (200 µl) was pipetted in columns 1 and 12 to minimize the "edge effect" and maintain humidity. The plates were incubated for 24 h at 37 ºC in a 5% CO_2 incubator, to allow proper attachment of cells and to enable cells to reach exponential growth phase. Different concentrations were prepared in MEM from a stock of 100 mg/ml of the plant extract and 100 µl of each concentration were added to the plates in quadruplicate. The microtitre plates were incubated at 37 ºC in a 5% CO_2 incubator for 48 h with the plant samples. Doxorubicin was used as the positive control and untreated cells were also prepared. The MEM with plant extract was aspirated from the cells and washed with 150 µl phosphate buffered saline, and replaced with 200 µl of fresh MEM. After washing, 30 µl MTT (Sigma, stock solution of 5 mg/ml in PBS) was added to each well and the plates were incubated for a further 4 h at 37 ºC. The medium was aspirated and MTT formazan crystals were dissolved with 50 µl dimethyl sulphoxide (DMSO). The plates were shaken gently on an orbital shaker to allow the formazan to dissolve. The amount of MTT reduction was measured immediately by detecting absorbance in a microplate reader at a wavelength of 570 nm and a reference wavelength of 630 nm. The wells in columns 1 and 12, containing medium and MTT, without cells, were used to blank the plate reader. The lethal concentration (LC_{50}) value was calculated as the concentration of plant samples resulting in a 50% reduction of absorbance compared to untreated cells. Selectivity index (SI) values for antimicrobial activity were calculated using the formula $SI = LC_{50}/MIC$, with units for LC_{50} and MIC in mg/ml.

2.6.2 *In vitro* genotoxicity assay

The plate incorporation assay, also known as the Ames test (Maron and Ames, 1983), was used to determine the genotoxicity of the plant samples against *Salmonella typhimurium* strains TA98 and TA100. Acetone, 70% ethanol, DCM and hot water extracts were prepared at concentrations of 5, 0.5 and 0.05 mg/ml in DMSO under sterile conditions. The samples were tested against *S. typhimurium* strains TA98 and TA100 without an exogenous metabolic activation system. Briefly, 100 µl of each extract was added to the test tube followed by 500 µl of phosphate buffer, and 100 µl of a fresh overnight culture (prepared by inoculating 100 µl stock bacteria in 10 ml Oxoid nutrient broth), then 2 mL of top agar with biotin/histidine was added. The mixture was centrifuged and poured into minimal agar plates. A positive control, 4-nitroquinoline-N-oxide (4-NQO) at a concentration of 2 µg/ml, and negative controls of 10% DMSO and sterile distilled water were also prepared. The plates were incubated for 48 h at 37 °C and a colony counter was used to count the colonies.

2.7 Statistical analysis

A Student t-test using GENSTAT statistical software, version 14.0 (VSN International Ltd., UK), was used to compare the amount of total phenolics, total flavonoids, flavonol content and hydrolysable tannins of the plant extracts. When the results were significant, the differences among plant extracts were compared using Tukey's Honest Significant Difference (HSD) test because of the equality of sample size. Where the test statistics were not significant, no post hoc tests were done. Microsoft Excel and Student t-test were used to analyse cytotoxicity and genotoxicity tests.

3. Results

3.1. Antimicrobial activity

Results of the antibacterial and antimycobacterial activity of *C. grandiflorum, C. odorata, D. unguis-cati* and *G. celosioides* leaf extracts against selected strains are presented in Table 1. With reference to Eloff (2004) and Sánchez and Kouznetsov (2010), we refer to MIC activity between 0.313 mg/ml and 2.5 mg/ml as weak activity, 0.156 ≤ 0.313 mg/ml as moderate activity and below 0.156 mg/ml as good or very good activity. There was a broad spectrum of antibacterial/antimycobacterial activity ranging from 0.039 to 2.5 mg/ml, though the activity displayed by the different solvent extracts varied. For *C. grandiflorum*, good antibacterial activity was observed with the acetone extract against *K. pneumoniae* and *E. faecalis* and ethanol extract against *M. smegmatis* having MIC=0.078 mg/ml. The DCM and acetone extracts of *C. odorata* had moderate activity (MIC=0.156 mg/ml) against several bacterial species. Interestingly, the acetone extract of *C. odorata* had good activity (MIC=0.078 mg/ml) against *E. coli*. *Dolichandra unguis-cati* DCM and acetone extracts displayed very good activity (MIC=0.039 mg/ml) against *K. pneumoniae*, good activity (MIC=0.078 mg/ml) against *E. coli* and *E. faecalis*, and moderate activity (MIC=0.156 mg/ml) against *M. aurum* and *M. smegmatis*. The DCM extract of *G. celosioides* showed very good activity against *K. pneumoniae*, and moderate activity against *E. faecalis*, *S.* Typhymurium, *M. aurum*, *M. fortuitum* and *M. smegmatis*. The acetone extract showed good to very good activity (MIC=0.078 and 0.039 mg/ml) against *E. faecalis* and *K. pneumoniae* respectively, and moderate activity (0.156 mg/ml) against *S.* Typhimurium.

The antifungal activity of plant extracts tested against *C. neoformans*, *A. fumigatus* and *C. albicans* at two time intervals (48 h and 72 h) is presented in Table 2. *C. grandiflorum* DCM and acetone extracts had moderate activity (0.156 mg/ml) against *C. neoformans* at 48 h

Table 1. Antibacterial activity of solvent extracts of four alien invasive plants against selected bacterial strains

		MIC (mg/ml)									
Plant species	Extract	*Ec*	*Kp*	*Ef*	*SD*	*Pa*	*Sa*	*ST*	*Ma*	*Mf*	*Ms*
C. grandiflorum	Ethanol	>2.5	1.250	0.313	1.25	2.500	>2.5	>2.5	2.500	1.250	**0.078***
	DCM	>2.5	**0.156**	**0.156**	0.313	0.625	**0.156**	>2.5	0.313	**0.156**	0.625
	Acetone	>2.5	**0.078***	**0.078***	0.313	0.625	**0.156**	>2.5	0.313	**0.156**	**0.156**
	Hot water	>2.5	>2.5	>2.5	>2.5	>2.5	0.625	>2.5	1.250	1.250	>2.5
C. odorata	Ethanol	1.250	0.313	0.625	1.250	>2.5	0.625	>2.5	2.500	2.500	2.500
	DCM	0.313	**0.156**	0.625	**0.156**	**0.156**	0.625	**0.156**	0.313	0.313	**0.156**
	Acetone	**0.078***	**0.156**	**0.156**	**0.156**	**0.156**	0.313	**0.156**	0.313	0.313	0.313
	Hot water	1.250	0.313	0.625	1.250	>2.5	**0.156**	0.625	2.500	1.250	0.625
D. unguis-cati	Ethanol	1.250	0.625	**0.156**	0.625	1.250	1.250	0.625	0.313	1.250	0.625
	DCM	**0.078***	**0.039****	**0.078***	**0.156**	0.625	0.625	2.500	**0.156**	**0.156**	0.313
	Acetone	**0.078***	**0.039****	**0.078***	0.313	0.625	0.625	2.500	**0.156**	**0.156**	2.500
	Hot water	1.250	0.625	1.250	1.250	1.250	>2.5	>2.5	2.5	>2.5	>2.5
G. celosioides	Ethanol	2.500	0.625	0.625	1.250	1.250	0.313	0.625	0.625	1.25	0.625
	DCM	0.313	**0.039****	**0.156**	0.313	0.313	0.625	**0.156**	**0.156**	**0.156**	**0.156**
	Acetone	0.313	**0.039****	**0.078***	0.313	0.313	0.625	**0.156**	0.313	0.313	0.313
	Hot water	1.250	0.313	2.500	2.500	2.500	1.250	0.625	0.625	1.250	1.250
Gent (+ve con)		**0.016***	0.500	0.500	**0.040****	**0.008****	**0.080****	**0.008****	-	-	-
Strep (+ve con)		-	-	-	-	-	-	-	**>0.008**	**0.063**	>1
Rif (+ve con)		-	-	-	-	-	-	-	**>0.008**	>1	**0.063**
INH (+ve con)		-	-	-	-	-	-	-	>1	>1	>1

Ec = Escherichia coli, *Kp = Klebsiella pneumoniae*, *Ef = Enterococcus faecalis*, SD = *Salmonella* Dublin, *Pa = Pseudomonas aeruginosa*, *Sa = Staphylococcus aureus*, ST = *Salmonella* Typhimurium, *Ma = Mycobacterium aurum*, *Mf = Mycobacterium fortuitum*, *Ms = Mycobacterium smegmatis*, *C. grandiflorum = Cardiospermum grandiflorum*, *C. odorata = Chromolaena odorata*, *D. unguis-cati = Dolichandra unguis-cati*, *G. celosioides = Gomphrena celosioides*, Gent = Gentamicin, Strep = Streptomycin, Rif = Rifampicin, INH = isoniazid, +ve con = Positive control, DCM = Dichloromethane, MIC = Minimum inhibitory concentration. Values in bold indicate good activity, * = very good activity, ** = excellent activity.

Table 2. Antifungal activity of solvent extracts of four alien invasive plants against selected fungal strains

Plant species	Extract	*A. Fumigatus*		*C. neoformans*		*C. albicans*	
		48h	72h	48h	72h	48h	72h
C. grandiflorum	Ethanol	1.250	2.500	1.250	1.250	0.625	1.250
	DCM	**0.156**	0.625	**0.156**	0.313	>2.5	>2.5
	Acetone	0.313	0.625	**0.156**	0.313	>2.5	>2.5
	Hot water	0.625	1.250	0.625	>2.5	2.500	>2.5
C. odorata	Ethanol	0.625	1.250	**0.078***	0.313	2.500	2.500
	DCM	**0.019***	**0.039****	**0.019****	**0.019***	**0.156**	1.250
	Acetone	**0.156**	0.313	**0.039***	**0.156**	**0.156**	2.500
	Hot water	0.625	1.250	**0.078***	1.250	2.500	2.500
D. unguis-cati	Ethanol	0.625	0.625	0.313	1.250	0.313	1.250
	DCM	0.313	0.313	0.313	0.313	>2.5	>2.5
	Ace	0.313	0.313	**0.078***	0.313	2.5	>2.5
	HH_2O	0.313	0.625	2.500	>2.5	>2.5	>2.5
G. celosioides	Ethanol	1.25	1.25	0.313	0.625	1.25	2.500
	DCM	**0.019****	**0.019****	**0.078***	**0.156**	**0.156**	0.625
	Ace	**0.019****	**0.019****	**0.078***	**0.156**	**0.156**	2.500
	HH_2O	**0.019****	1.25	2.500	2.500	>2.5	>2.5
Amp B (+ve con)		**0.031**	0.063	**0.008****	0.125	**0.031**	0.500

C. neoformans = Cryptococcus neoformans, A. fumigatus = Aspergillus fumigatus, C. albicans = Candida albicans, *C. grandiflorum = Cardiospermum grandiflorum, C. odorata = Chromolaena odorata*, *D. unguis-cati = Dolichandra unguis cati, G. celosioides = Gomphrena celosioides*, DCM = Dichloromethane, Amp B = Amphotericin B, + ve con = positive control, MIC = Minimum inhibitory concentration. . Values in bold indicate good activity, * = very good activity, ** = excellent activity.

incubation. Ethanol and hot water extracts of *C. odorata* displayed good activity (MIC=0.078 mg/ml) against *C. neoformans* at 48 h. The *C. odorata* DCM extract had very good activity (MIC = 0.019 and 0.039 mg/ml respectively) against *C. neoformans* and *A. fumigatus* at 48 and 72 h and moderate activity (MIC=0.156) against *C. albicans* only at 48 h. The acetone extract displayed very good activity (MIC=0.039 mg/ml) against *C. neoformans* at 48 h. Among the five extracts of *Dolichandra unguis-cati* investigated, only the acetone extract exhibited good activity (MIC=0.078 mg/ml) and was against *C. neoformans* at 48 h. The DCM, acetone and hot water extracts of *G. celosioides* had very good (MIC=0.019 mg/ml) activity against *A. fumigatus* at 48 h but only DCM and acetone extracts were active after 72 h. Good and moderate activity (0.078 and 0.156 mg/ml) was observed with the DCM and acetone against *C. neoformans* and *C. albicans* only at 48 h respectively.

3.2. Cytotoxicity test of solvent extracts against Vero monkey kidney cells

3.2.1. Lethal Concentration 50% (LC_{50}) and selectivity index of bacterial strains tested

From the cytotoxicity results (Table 3) obtained with concentrations ranging from 0.025 to 1 mg/ml, the LC_{50} values of extracts of *C. grandiflorum* were 0.04 to 0.27 mg/ml with only the ethanol extract showing some level of toxicity to cells. The LC_{50} of *C. odorata* ranged from 0.23 to 0.69 mg/ml, values for *D. unguis-cati* extracts were from 0.25 to 0.81 mg/ml, while that of the extracts of *G. celosioides* were from 0.75 to 0.95 mg/ml. Selectivity index (SI) values calculated for the different extracts of the various plants tested are also presented in Table 3. In the case of *C. grandiflorum,* only the SI of DCM and acetone extracts were greater than 1, which were for *K. pneumoniae, E. faecalis, S. aureus, M. fortuitum* and only acetone extract for *M. smegmatis* in relation to their MICs. Although, the DCM, acetone and hot water extracts of *C. odorata* had SI greater than 1 for most of the strains, a higher SI was observed with the acetone extract for *E. coli*. The acetone extract of *D. unguis-cati* showed

Table 3. Lethal Concentration 50% (LC_{50}) of solvent extracts against Vero monkey kidney cells and the selectivity index of bacterial strains tested

			Selectivity index values (LC_{50}/MIC)									
Plant species	Extract	LC_{50}	*Ec*	*Kp*	*Ef*	*SD*	*Pa*	*Sa*	*ST*	*Ma*	*Mf*	*Ms*
C. grandiflorum	Ethanol	0.04 ± 0.19	NA	0.032	0.128	0.032	0.016	NA	NA	0.016	0.032	**0.513**
	DCM	0.27 ± 0.01	NA	**1.731**	**1.731**	**0.863**	0.432	**1.731**	NA	**0.863**	**1.731**	0.432
	Acetone	0.27 ± 0.04	NA	**3.462**	**3.462**	**0.863**	0.432	**1.731**	NA	**0.863**	**1.731**	**1.731**
	Hot water	0.25 ± 0.16	NA	NA	NA	NA	NA	0.400	NA	0.200	0.200	NA
C. odorata	Ethanol	0.23 ± 0.05	0.184	**0.735**	0.368	0.184	NA	0.368	NA	0.092	0.092	0.092
	DCM	0.32 ± 0.01	**1.022**	**2.051**	**0.512**	**2.051**	**2.051**	**0.512**	**2.051**	**1.022**	**1.022**	**2.051**
	Acetone	0.69 ± 0.09	**8.846**	**4.423**	**4.423**	**4.423**	**4.423**	**2.204**	**4.423**	**2.204**	**2.204**	**2.204**
	Hot water	0.62 ± 0.04	**0.496**	**1.981**	**0.992**	**0.496**	NA	**3.974**	**0.992**	0.248	**0.496**	**0.992**
D. unguis-cati	Ethanol	0.25 ± 0.12	0.200	0.400	**1.603**	0.400	0.200	0.200	0.400	**0.799**	0.200	0.400
	DCM	0.59 ± 0.38	**7.564**	**15.128***	**7.564**	**3.782**	**0.944**	**0.944**	0.236	**3.782**	**3.782**	**1.885**
	Acetone	0.81 ± 0.00	**10.385***	**20.769***	**10.385***	**2.588**	**1.296**	**1.296**	0.324	**5.192**	**5.192**	0.324
	Hot water	0.71 ± 0.03	**0.568**	**1.136**	**0.568**	**0.568**	**0.568**	NA	NA	0.284	NA	NA
G. celosioides	Ethanol	0.79 ± 0.05	0.316	**1.264**	**1.264**	**0.632**	**0.632**	**2.524**	**1.264**	**1.264**	**0.632**	**1.264**
	DCM	0.95 ± 0.05	**3.035**	**24.359***	**6.111**	**3.035**	**3.035**	**1.520**	**6.111**	**6.111**	**6.111**	**6.111**
	Acetone	0.76 ± 0.31	**2.426**	**19.487***	**9.744**	**2.426**	**2.426**	**1.216**	**4.872**	**2.428**	**2.428**	**2.482**
	Hot water	0.75 ± 0.23	**0.600**	**2.396**	0.300	0.300	0.300	**0.600**	**1.200**	**1.200**	**0.600**	**0.600**
Doxo (µM)		0.20 ± 0.00	-	-	-	-	-	-	-	-	-	-

Ec = Escherichia coli, *Kp = Klebsiella pneumoniae*, *Ef = Enterococcus faecalis*, SD = *Salmonella* Dublin, *Pa = Pseudomonas aeruginosa*, *Sa = Staphylococcus aureus*, ST = *Salmonella* Typhimurium, *Ma = Mycobacterium aurum*, *Mf = Mycobacterium fortitium*, *Ms = Mycobacterium smegmatis*, MIC = Minimum inhibitory concentration, Doxo = Doxorubicin, *C. grandiflorum = Cardiospermum grandiflorum*, *C. odorata = Chromolaena odorata*, *D. unguis-cati = Dolichandra unguis cati*, *G. celosioides = Gomphrena celosioides*, DCM = Dichloromethane. Values written in bold with asterisk are non-toxic extracts with a safe margin and good promising antibacterial agents, while those written in bold with no asterisk are extracts with a relatively safe margin.

the highest SI against *E. coli*, *K. pneumoniae* and *E. faecalis*. The DCM extract of *G. celosioides* showed the highest SI for all strains (up to 24.359 against *K.* pneumoniae) except for *E. faecalis* where the acetone extract had a higher SI value.

3.2.2. Lethal Concentration (LC_{50}) and selectivity index of fungal strains tested

Table 4 shows the LC_{50} values of extracts and their respective selectivity index values against fungal strains tested. The acetone and DCM extracts of *C. grandiflorum* both had high SI values for *C. neoformans* at 48 h and only DCM for *A. fumigatus* at 48 h. Among the extracts of *C. odorata* the DCM had the highest selectivity index (SI = 16.842 and 8.205) for *A. fumigatus*. at 48 and 72 h, but the acetone extract had the highest SI (17.692 and 4.423) for *C. neoformans* and *C. albicans*. Although, the DCM and acetone extracts of *D. unguis-cati* had SI greater than 1 against *A. fumigatus* and *C. neoformans*, a higher SI (10.382) was obtained with the acetone extract for *C. neoformans*. From the extracts of *G. celosioides*, DCM had the highest SI for all strains, with an SI value as high as 50.00 for *A. fumigatus.*

3.3 Genotoxicity test

The results of the Ames assay for genotoxicity carried out to determine if any of the plant extracts had the ability to mutate genes of humans or animals (Table 5) showed that none of the plant extracts had genotoxic effects in this assay.

3.4. Phytochemical determination

All four alien invasive plants investigated were rich in total phenolics (Fig. 1a) but *C. odorata* had the highest amount with a statistical difference (GLM ANOVA: $F_{3,19} = 55.05$; $P < 0.001$). Total flavonoid (Fig. 1b) and flavonol (Fig. 1c) contents were also higher in *C. odorata* and the difference were statistically significant (GLM ANOVA: $F_{3,19} = 83.85$; $P <$

Table 4. Lethal Concentration (LC_{50}) of solvent extracts against Vero monkey kidney cells and the selectivity index of fungal strains tested

			Selectivity Index Values (LC_{50}/MIC)					
Plant species	Extract		*A. fumigatus*		*C. neoformans*		*C. albicans*	
		LC_{50}	48 h	72 h	48 h	72 h	48 h	72 h
C. grandiflorum	Ethanol	0.04 ± 0.19	0.032	0.016	0.032	0.032	0.064	0.032
	DCM	0.27 ± 0.01	**1.731**	0.432	**1.731**	**0.863**	NA	NA
	Acetone	0.27 ± 0.04	**0.863**	0.432	**1.731**	**0.863**	NA	NA
	Hot water	0.25 ± 0.16	0.400	0.200	0.400	NA	0.100	NA
C. odorata	Ethanol	0.23 ± 0.05	0.368	0.184	**2.949**	**0.735**	0.092	0.092
	DCM	0.32 ± 0.01	**16.842***	**8.205**	**16.842***	**16.842***	**2.051**	0.256
	Acetone	0.69 ± 0.09	**4.243**	**2.204**	**17.692***	**4.423**	**4.423**	0.276
	Hot water	0.62 ± 0.04	**0.992**	**0.496**	**7.949**	**0.496**	0.248	0.248
D. unguis-cati	Ethanol	0.25 ± 0.12	0.400	0.400	**0.799**	0.200	**0.799**	0.200
	DCM	0.59 ± 0.38	**1.885**	**1.885**	**1.885**	**1.885**	NA	NA
	Acetone	0.81 ± 0.00	**2.588**	**2.588**	**10.382***	**2.588**	0.324	NA
	Hot water	0.71 ± 0.03	0.268	**1.136**	0.284	NA	NA	NA
G. celosioides	Ethanol	0.79 ± 0.05	**0.632**	**0.632**	**2.524**	**1.264**	**0.632**	0.316
	DCM	0.95 ± 0.05	**50.000***	**50.000***	**12.179***	**6.111**	**6.11**	**1.520**
	Acetone	0.76 ± 0.31	**40.000***	**40.000***	**9.711**	**4.872**	**4.872**	0.304
	Hot water	0.75 ± 0.23	**39.474***	**0.600**	0.373	0.373	NA	NA
Doxo (µg/ml)		0.20 ± 0.00	-	-	-	-	-	-

C. grandiflorum = Cardiospermum grandiflorum C. odorata = Chromolaena odorata, Doxo = Doxorubicin, DCM = Dichloromethane, *C. neoformans = Cryptococcus neoformans*, *A. fumigatus = Aspergillus fumigatus*, *C. albicans = Candida albicans*, NA = Not available. Values written in bold with asterisk are non-toxic extracts with a safe margin and good promising antifungal agents, while those written in bold with no asterisk are extracts with a relatively safe margin

Table 5. Genotoxicity of *T. diversifolia* and *T. rotundifolia* using *Salmonella typhimurium* strains TA98 and TA100 in the absence of exogenous metabolic activation

		Dose (µg/plate)	Histidine + revertant colonies	
Plant species	Extract		TA98	TA100
C. grandiflorum	Ethanol	5	54.52 ± 0.22	119.00 ± 0.00
		50	53.11 ± 0.71	123.14 ± 0.14
		500	52.12 ± 1.32	120. 10 ± 0.12
	DCM	5	56.33 ± 2.52	123.33 ± 0.58
		50	65.67 ± 1.53	121.67 ± 2.52
		500	69.00 ± 3.00	127.00 ± 3.00
	Acetone	5	73.00 ± 2.00	133.33 ± 3.06
		50	74.67 ± 2.52	138.00 ± 3.61
		500	57.67 ± 4.04	142.67 ± 5.13
	Hot water	5	57.31 ± 3.00	119.00 ± 0.00
		50	62.00 ± 1.33	129.23 ± 0.58
		500	63.55 ± 2.52	132.33 ± 2.52
C. odorata	Ethanol	5	35.21 ± 1.21	119.00 ± 0.32
		50	38.52 ± 0.12	121.22 ± 0.73
		500	37.92 ± 0.13	121.32 ± 0.15
	DCM	5	35.00 ± 2.00	122.33 ± 2.31
		50	45.67 ± 1.15	115.00 ± 1.73
		500	42.67 ± 0.08	110.33 ± 1.73
	Acetone	5	39.21 ± 2.05	107.67 ± 1.53
		50	44.33 ± 0.57	119.67 ± 1.53
		500	65.67 ± 1.52	120.00 ± 2.00
	Hot water	5	46.02 ± 1.21	121.22 ± 0.21
		50	39.53 ± 0.00	119.00 ± 0.11
		500	43.42 ± 2.12	127.00 ± 2.01

C. grandiflorum = Cardiospermum grandiflorum, *C. odorata = Chromolaena odorata*, DCM= Dichloromethane.

Table 5. (Cont)

		Dose (µg/plate)	Histidine + revertant colonies	
Plant species	Extract		TA98	TA100
D. unguis-cati	Ethanol	5	56.22 ± 3.11	138.92 ± 0.14
		50	58.00 ± 3.02	142.14 ± 2.44
		500	56.92 ± 1.77	140.00 ± 0.15
	DCM	5	56.67 ± 2.52	143.33 ± 3.06
		50	72.00 ± 1.73	142.00 ± 3.61
		500	61.00 ± 3.46	139.00 ± 3.46
	Acetone	5	87.33 ± 2.52	141.00 ± 2.65
		50	93.67 ± 2.08	133.00 ± 3.00
		500	56.33 ± 0.51	137.67 ± 3.22
	Hot water	5	55.00 ± 3.03	122.33 ± 3.04
		50	62.00 ± 3.00	123.00 ± 1.33
		500	57.22 ± 0.02	119.00 ± 0.00
G. celosioides	Ethanol	5	66.52 ± 2.90	136.33 ± 2.14
		50	59.23 ± 1.03	140.02 ± 0.34
		500	63.82 ± 0.03	139. 31 ± 3.62
	DCM	5	70.67 ± 1.16	139.00 ± 4.36
		50	72.33 ± 2.31	143.33 ± 3.06
		500	61.00 ± 1.00	140.67 ± 1.15
	Acetone	5	56.67 ± 2.08	135.67 ± 2.08
		50	49.00 ± 3.46	118.33 ± 0.58
		500	53.67 ± 2.31	129.67 ± 1.53
	Hot water	5	52.00 ± 0.03	112.22 ± 1.23
		50	51.67 ± 0.11	115.00 ± 0.00
		500	49.52 ± 0.00	114.31 ± 0.21
4NQO (+ve con)			212.70 ± 1.90	538.70 ± 5.50
H_2O (-ve con)			61.33 ± 1.45	130.00 ± 2.50
10% DMSO (-ve con)			57.00 ±1.15	125.70 ± 1.90

D. unguis-cati =*Dolichandra unguis-cati*, *G. celosioides* = *Gomphrena celosioides*, H_2O = Water, DMSO = Dimethylsulfoxide, DCM= Dichloromethane, +ve con = Positive control, -ve con = Negative control, 4-NQO = 4-nitroquinoline-N-oxide.

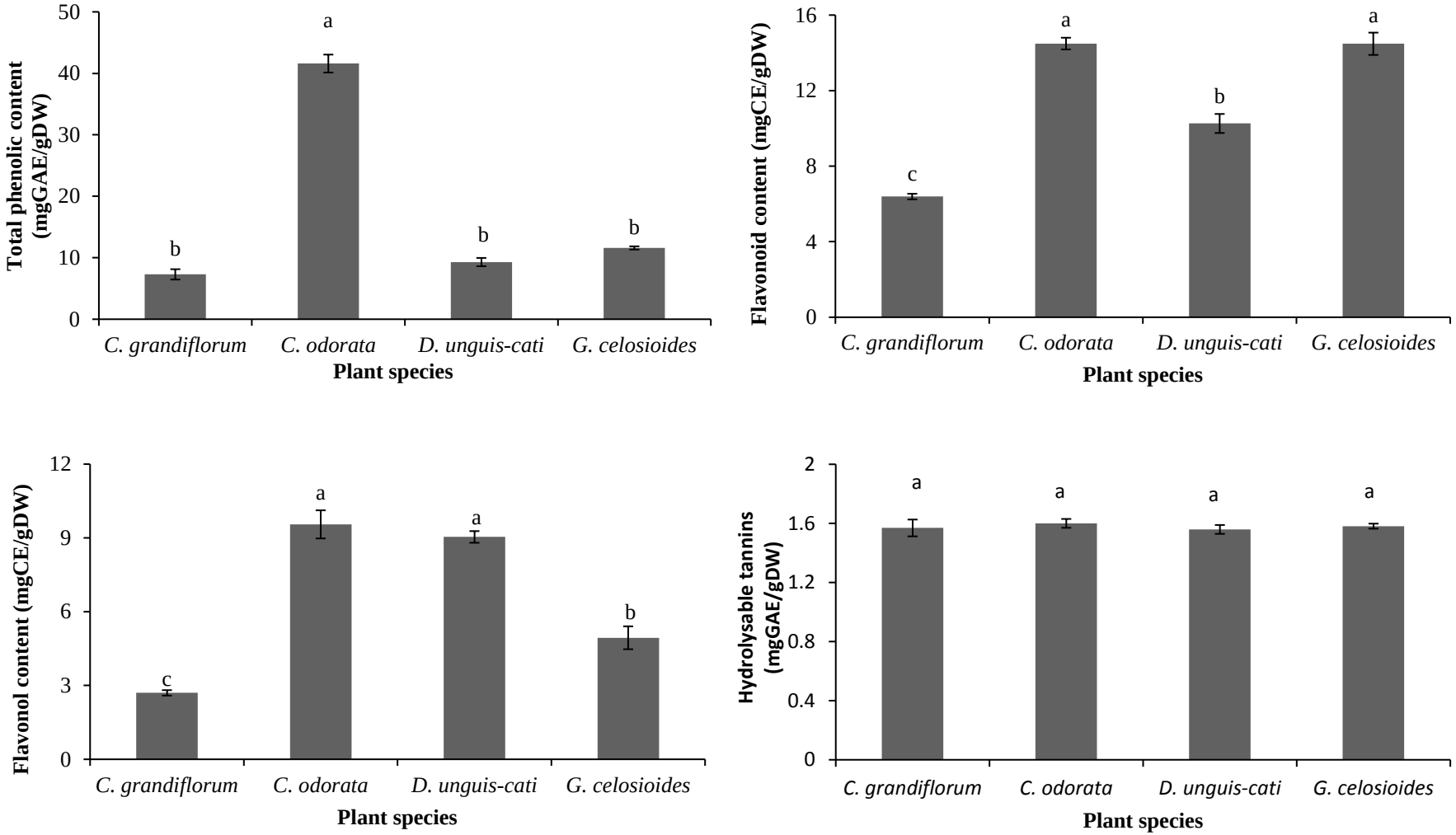

Fig 1 (a). Total phenolic content, as gallic acid equivalents, quantified in the leaves of *Cardiospermum grandiflorum, Chromolaena odorata, Gomphrena celosioides* and *Dolichandra unguis-cati* (b) flavonoid content as catechin equivalents quantified in the leaves of *Cardiospermum grandiflorum, Chromolaena odorata, Gomphrena celosioides* and *Dolichandra unguis-cati* (c) flavonol content as catechin equivalent quantified in the leaves of *Cardiospermum grandiflorum, Chromolaena odorata, Gomphrena celosioides* and *Dolichandra unguis-cati* (d) hydrolysable tannin content as gallic acid equivalents quantified in the leaves of *Cardiospermum grandiflorum, Chromolaena odorata, Gomphrena celosioides* and *Dolichandra unguis-cati.* Values in each bars are means ±SEM. Means capped with different letters are significantly different (Tukey HSD: $P<0.05$). W = dry weight; GAE = gallic acid equivalents; CE = catechin equivalents.

0.001) and (GLM ANOVA: $F_{3,19} = 83.85$; $P < 0.001$) . Although, *C. odorata* also had the highest amount of hydrolysable tannin content (Fig. 1d), there was no statistical difference (GLM ANOVA: $F_{3,19} = 2.56$; $P = 0.091$).

4. Discussion

From a critical point of view, analysis of the results of the antibacterial, antimycobacterial and antifungal activities shows that all the plants investigated in this study inhibited the growth of at least one pathogen. *Cardiospermum grandiflorum* had moderate to good antimicrobial activity against *K. pneumoniae*, *E. faecalis*, *S. aureus*, *M. smegmatis*, *C. neoformans* and *A. fumigatus*. Although reports on the antibacterial activity of this plant are scarce, antifungal activity was investigated by Olaoluwa and Olapeju (2015) against *C. albicans*. The authors recorded MIC values ranging from 50 to 200 mg/ml which was very high and not comparable to our findings of MIC=0.625 mg/ml. Apart from *S. aureus*, *M. smegmatis* and *M. fortuitum* which were weakly inhibited, *C. odorata* showed promising antimicrobial activity against *E. coli*, S. Typhimurium, *E. faecalis*, S. Dublin, *P. aeruginosa*, *K. pneumoniae*, *M. smegmatis*, *C. neoformans*, *A. fumigatus* and *C. albicans*. In our previous study (Omokhua et al., 2017), different growth stages of *C. odorata* were investigated using 70% ethanol, 50% methanol, petroleum ether and cold water extracts and MICs ranging from 0.39 to 6.25 mg/ml were recorded. The same range of MICs was recorded in this study for the 70% ethanol and 50% methanol which we refer to as weak. However, in the present study the DCM and acetone extracts displayed better results. The good activity observed might be due to the combined leaf growth stages of the young and mature leaves, unlike in our previous study. *Dolichandra unguis-cati* exhibited antimicrobial activity against *E. coli*, *K. pneumoniae*, *E. faecalis*, *S.* Dublin and *M. aurum*. This is the first study to report the antimicrobial activity of *D. unguis-cati*. However, the plant has been reported to possess anti-

inflammatory, antitumoral and antitrypanosomal properties (Duarte et al., 2000; Aboutabl et al., 2008).

The extracts of *G. celosioides* displayed moderate to very good activity against *K. pneumoniae*, *E. faecalis*, *S.* Typhimurium, *M. aurum*, *M. fortuitum*, *M. smegmatis*, *C. neoformans*, *A. fumigatus* and *C. albicans* showing outstanding antimicrobial potential. Our findings agree with those of Dosumu et al. (2010) for *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*, though the MIC (12.5 mg/ml) reported in their study was very high. This may explain why the plant is used traditionally for the treatment of various ailments in some of its invasive ranges (Rahman and Gulshana, 2014; Chauke et al., 2015). Earlier researchers (de Moura et al., 2004) reported the antibacterial activity of the ethanol extract of *G. celosioides* against *S. aureus* with a zone of inhibition of 7.6 mm using the Kirby-Bauer method. This led to the isolation of two antibacterial compounds with zones of inhibition reported as 7.3 and 7.6 mm respectively. Unfortunately MIC values were not given, leading to difficulties in comparison.

A number of bacterial, mycobacterial and fungal test organisms were included in this study. Many of these organisms are implicated in causing disease, or are commonly used as models for testing for activity against related pathogenic strains. An overall assessment of the antimicrobial qualities of the investigated alien invasive species compared to the positive controls showed that these weed plants may be exploited as antimicrobial agents. This may help in combating opportunistic infections and other infectious diseases such as diarrhoea, urinary tract and wound infections, meningitis, gastroenteritis, osteomyelitis, sinusitis, tuberculosis and disease outbreaks resulting from food poisoning and other infections.

Phytochemical determination results in this study indicated that *C. grandiflorum*, *C. odorata* (SAB), *D. unguis-cati* and *G. celosioides* contained reasonable amounts of total phenolics (Fig. 1a). Our previous analysis (Omokhua et al., 2017) showed that the SAB *C. odorata* is rich in total phenolic content, but when comparing the two biotypes, the AWAB contained higher amounts of these phytochemicals. Total flavonoid content (Fig. 1b) was also higher in the SAB *C. odorata* than the other plants, though there was no significant difference between *C. odorata* and *G. celosioides*. A previous report on the phytochemical determination of *G. celosioides* confirmed that the plant is rich in flavonoids conforming to our findings (Vieira et al., 1994). A significant difference was noticed between *G. celosioides* and *D. unguis-cati* and between *D. unguis-cati* and *C. grandiflorum*. Our result for *C. grandiflorum* conforms to the findings of Sofidiya et al. (2012) who also investigated the total phenolics of this plant. Flavonol content (Fig. 1c) was higher in *C. odorata* followed by *D. unguis-cati* with no statistical difference. Significant differences were noticed among *D. unguis-cati, G. celosioides* and *C. grandiflorum* which contained the least amount. *Chromolaena odorata* also contained the highest amount of hydrolysable tannins but there was no significant difference among all four weed species. *Dolichandra unguis-cati,* which had the least amounts of phytochemicals investigated in this study, has previously been reported to be rich in phenolic and flavonoid compounds (Aboutabl et al., 2008, Torres et al., 2013). Flavonoid compounds such as acacetin, 6-O apiosyl galactoside and cirsimarin B have been isolated (Aboutabl et al., 2008; Liu et al., 2015). A preliminary screening of *C. grandiflorum* showed that the plant contained tannins, leading to the isolation of a compound named L-pinitol (Olaoluwa and Olapeju, 2015). The antibacterial, antimycobacterial and antifungal activities displayed by the weedy species in this study are most likely connected to the presence of these quantified phytochemicals (Dosumu et al., 2010).

According to Meyer et al. (1982), crude extracts with LC_{50} values less than 0.1 mg/ml are cytotoxic while those greater than 0.1 mg/ml are non-cytotoxic. The cytotoxicity screening of different extracts of the alien invasive plants against Vero monkey kidney cells in this study showed different LC_{50} values ranging from relative cytotoxicity to non-cytotoxic. The LC_{50} values of the extracts of *C. grandiflorum* ranged from 0.04 to 0.27 mg/ml with only the ethanol extract showing toxicity. The antimicrobial activity exhibited by the ethanol extract against *M. smegmatis* may be related to the cytotoxicity. However, the DCM and acetone extracts, which displayed better antibacterial and antifungal activities, were not-toxic to cells. The LC_{50} values of *C. odorata* in this study ranged from 0.23 to 0.69 mg/ml with SI value as high as 17.692. From our previous study (Omokhua et al., 2017) where we investigated the DCM and 70% methanol extracts of the young, mature and flowering stages of the plant, we reported LC_{50} values ranging from 0.031 to 0.217 mg/ml with the DCM extract of the young leaf extract showing some level of cytotoxicity. The higher value obtained from this study may be due to the young and mature leaves not being separately investigated. There may be some explanation for the cytotoxicity of the young plant leaf extract, as studies have shown that the growth stage of a plant may influence the presence or absence of a phytochemical (Wouters et al., 2016).

The LC_{50} values of *D. unguis-cati* ranged from 0.25 to 0.81 mg/ml with highest SI value of 10.382. This shows that the antimicrobial activity displayed by the plant in some cases was not as a result of toxicity. Although no literature was available on cytotoxicity of *D. unguis-cati*, the plant has been reported to exhibit anti-proliferative and anticancer activity (Duarte et al., 2000; Aboutabl et al., 2008). Ursolic acid isolated from the chloroform fraction of ethanol extract of *D. unguis-cati,* which had the lowest LC_{50} value in our study, was reported to have strong cytotoxicity against liver cancer cell lines (Chen et al., 2017). There is a possibility

that the plant may have some selective cytotoxicity to cancerous cells. Values ranging from 0.75 to 0.95 mg/ml were recorded for *G. celosioides* with the plant showing the highest SI values, reaching up to 50.00 against *A. fumigatus*, among all plants tested. However, our findings are in contrast to that of Dosumu et al. (2010) who used the brine shrimp assay and recorded LC_{50} of 0.07 mg/ml for methanol and 0.052 mg/ml for hexane extracts which were not tested in this study.

Based on our findings, there was no indication of any genotoxicity of *C. grandiflorum*, *C. odorata*, *D. unguis-cati* and *G. celosioides*. There is no existing literature on the genotoxicity of these alien species, apart from our previous research on *C. odorata* which confirms results of this study (Omokhua et al., 2017). To the best of our knowledge this is the first study to evaluate the genotoxicity levels in the Ames test of *C. grandiflorum*, *D. unguis-cati* and *G. celosioides*.

5. Conclusions

The negative impact of alien invasive plants in their introduced range cannot be overemphasized and calls for concern. Some of these plants may not be an economic burden in their local origins, because there are natural enemies that feed on them, thereby controlling their spread. Also most of these plants are part of the lives of the people who have lived and grown up with them, passing the knowledge of use and medicinal values of the plants to their offspring from generation to generation. However, the same cannot be said of these plants in their introduced range (especially Africa) where only a few traditional healers have discovered them as a source of medicines. The antimicrobial activity and *in vitro* toxicity of extracts of four invasive species in South Africa, namely *C. grandiflorum*, *C. odorata*, *D. unguis-cati* and *G. celosioides* investigated in this study indicate their importance as

antimicrobial agents. Their potential development into sources of medicines for the treatment of infectious diseases may help control their spread and serve as alternatives to highly exploited endangered native species with similar medicinal potential. Further studies on these plants will involve investigating extracts and isolated bioactive compounds for mechanism of action, as well as other studies such as anti-biofilm activity as a way of overcoming antibiotic resistance. Based on the findings of the current study, we suggest that the ecological noxiousness of alien invasive plants should not preclude further research on elucidating their antimicrobial and pharmacological properties.

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Conflict of interest

The authors of this article declare no conflict of interest.

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