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

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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.

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

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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 Vijer, 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 °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 °C, except for *M. aurum* which was