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A comparison of the antimicrobial activity and *in vitro* toxicity of a medicinally useful biotype of invasive *Chromolaena odorata* (Asteraceae) with a biotype not used in traditional medicine

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ABSTRACT

Two biotypes of the invasive species, *Chromolaena odorata* are known to be present in Africa, *viz.* the Asian/West African biotype (AWAB) and the southern African biotype (SAB). Although the phytochemistry, ethnomedicinal and ethnopharmacological relevance of the AWAB has been elucidated, the SAB plants have received little or no attention. This study investigated and compared the phytochemistry and pharmacological activities of two biotypes of *C. odorata* (AWAB and SAB). Antimicrobial activities of leaf extracts of the two biotypes and three different growth stages of the SAB were evaluated against several bacterial and fungal strains using a serial microdilution assay. Phytochemicals were determined through standard methods of analysis. Toxicity of the extracts of the different growth stages of the SAB was determined using the colorimetric MTT assay, while the mutagenicity assay was performed using the Ames test. The AWAB had the overall best antibacterial activity, while the SAB showed better antifungal activity. Results showed that young and mature non-flowering extracts of the SAB were the most active. AWAB contained the highest amount of phenolics and flavonoids while SAB contained the highest amount of tannins. Extracts of young SAB plants showed a low level of cytotoxicity and none of the extracts of the three growth stages were mutagenic. This is the first report suggesting that the SAB of *C. odorata* can be exploited as a source of medicine similar to the AWAB, in combating antimicrobial infections and other health problems.

1. Introduction

Chromolaena odorata (L.) R.M. King & H. Rob. (Asteraceae) is an invasive perennial shrub native to the Americas (McFadyen, 1989). Since its introduction into West Africa in 1937 (Ivens, 1974; Uyi et al., 2014) and into southern Africa in 1947 (Zachariades et al., 2011), the plant has spread into different parts of the continent. Two biotypes are known to be invasive in Africa, *viz.* the widespread Asian/West African biotype (AWAB) which originated from Trinidad and Tobago, and the southern African biotype (SAB) traced to be of Cuban or Jamaican origin (Paterson and Zachariades, 2013). *Chromolaena odorata* has become an economic burden especially because of the negative effects it has on agriculture, biodiversity and livelihoods. The plant competes effectively with native plants and becomes dominant because of its ability to compete for water, nutrients, light and space forming shade over other species. This may lead to extinction of local plant species, thereby reducing plant biodiversity. Other species around it are often eliminated and this may be due to the allelopathic properties and novel biochemical "weapons" possessed by this plant (Callaway and Ridenour, 2004).

Despite its negative effects, the AWAB of *C. odorata* is seen by locals in West Africa and in some parts in Central Africa and south Asia as a source of medicine. The locals exploit the plant for the treatment of coughs and colds, skin infections, dysentery, wounds, toothache, malaria, stomach problems, diarrhoea, stomach ulcers, and also bacterial and fungal infections, possibly because of the presence of phenolics, flavonoids, tannins and saponins (Akinmoladun et al., 2007; Panda et al., 2010; Anyasor et al., 2011; Vijayaraghavan et al., 2013). The species is said to have anthelmintic, antioxidant, analgesic, antipyretic, antispasmodic, anti-inflammatory, antimicrobial, antimalarial, and wound healing properties (Omokhua et al., 2016 and references therein).

Due to the morphological and genetic differences between the two biotypes (Paterson and Zachariades, 2013), it is possible that their phytochemistry and pharmacological potentials may differ. To our knowledge, no studies exist on the phytochemistry of the SAB plants. Although Naidoo et al. (2011) screened SAB plants for antibacterial and antifungal properties, no comparison was made with AWAB plants to decipher which of the biotypes possesses better medicinal potential. Therefore the objectives of this study were several fold: (i) to determine

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and compare the antimicrobial activities of both biotypes of *C. odorata* using the microdilution assay against bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*) and a yeast fungus (*Candida albicans*); and (ii) to investigate possible phytochemical content and to ascertain if phytochemicals present in the AWAB are also present in the SAB. Due to the fact that phytochemicals may differ between growth stages of plants (Hol, 2011), phytochemical investigation and antimicrobial screening were done using different growth stages of SAB *C. odorata*. A further objective of this study was to conduct cytotoxicity and mutagenicity tests on leaf extracts of different growth stages of the SAB plants.

2. Materials and methods

2.1. Study species

Chromolaena odorata is a scrambling perennial shrub native to the Americas from southern USA to northern Argentina (Gautier, 1992). In its invasive range, *C. odorata* grows in a wide range of vegetation types such as forest margins, grasslands, roadsides, agricultural lands, and disturbed forests posing a significant threat to agriculture, biodiversity, and livelihoods (see reviews in Zachariades et al., 2009; Omokhua et al., 2016). The weed is intolerant of deep shade, but performs well in partial shade and full-sun conditions (Zachariades et al., 2009). In an open land situation, the shrub can grow 2–3 m in height, but it can reach up to 5–10 m when supported by other vegetation. Flowering, which is often prolific, peaks in December to January in the northern hemisphere and in June to July in the southern hemisphere (Zachariades et al., 2009). The southern African biotype of *C. odorata*, which originated from Cuba or Jamaica, is morphologically and genetically distinct from the more widespread biotype (Asian/West African biotype) invading Asia, Oceania, and West-, East-, and Central Africa (Paterson and Zachariades, 2013).

2.2. Plant collection and sample preparation

Stem cuttings of the AWAB *C. odorata* were collected from the Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI), Cedara (22° 38′ 36.25“ N, 120° 36′ 12.36 E), near Pietermaritzburg on 19 February 2014. The stem cuttings of the SAB were collected from an open field within the vicinity of the South African Sugarcane Research Institute (SASRI), Mount Edgecombe (29° 70′ S, 31° 05′ E), near Durban, South Africa on the same day. All cuttings were initially planted in a mist bed in vermiculite with rooting hormone (Seradix™ No. 1) for four weeks before they were later planted in nursery pots (25 cm diameter). All plants benefited from the same potting medium (Umgeni sand: Gromor Potting Medium™ 1:1), fertilizer (Plantacote™) and watering regimes. The plants were maintained in a shade house at the Botanical Gardens of the University of KwaZulu-Natal, Pietermaritzburg and plants were watered daily using automatic drip irrigation. During the growth of the plants, 40 pots from the AWAB plants tagged mature non-flowering (AMNF) were set to be used for the experiment. While for the SAB, 40 potted plants were tagged as “young plants (SY)”, another 40 plants were tagged as “mature flowering plants (SMF)” and a final 40 were tagged as “mature non-flowering plants (SMNF)”. Each plant category was used for antimicrobial screening at their appropriate stage of development. The SY leaves were harvested in May, the SMF in July while the SMNF and AMNF were harvested in September 2014. Two voucher specimens (Omokhua 01 and Omokhua 02) were prepared for the AWAB and the SAB and were deposited in the Bews Herbarium (NU), University of KwaZulu-Natal. The leaves of all plants were carefully harvested at their allocated stages of growth and dried in an oven at 55 °C for 72 h, ground, and the powders stored in dark airtight containers at room temperature.

2.3. Preparation of plant extracts for antimicrobial, cytotoxicity and mutagenicity assays

Two grams of the powdered samples of the AWAB and SAB biotypes (AMNF and SMNF) were weighed into 50 ml conical flasks and extracted using 20 ml of redistilled 70% ethanol (EtOH). Two gram aliquots from SY, SMF and SMNF of the SAB were also weighed into 50 ml conical flasks and extracted separately using redistilled 70% EtOH, 50%, 70% methanol (MeOH), petroleum ether (PE), dichloromethane (DCM) and distilled water. All mixtures were sonicated in a sonication bath on ice for 25 min. The extracts were filtered under vacuum through filter paper (Whatman No. 1) and the filtrates were poured into weighed glass pill vials. The organic extracts were placed under a stream of air at room temperature and allowed to dry, while the water extracts were placed in glass jars and freeze-dried. The dried extracts were kept in the dark at 10 °C until required for the experiments.

2.4. Preparation of extracts for phytochemical analyses

From the ground plant material, 0.1 g was weighed into 50 ml conical flasks, 10 ml of 50% methanol were added and the flasks were sonicated in a sonication bath for 25 min. The mixtures were filtered through filter paper (Whatman No. 1) under a vacuum pressure pump, poured into pill vials and immediately used for the assays. This was done to prevent deterioration and decomposition of the metabolites in the plant samples.

2.5. Antimicrobial screening

2.5.1. Preparation of microbial stock cultures

Bacterial and fungal stock strains used for the assay were cultured in Mueller-Hinton (MH) agar (Merck, Germany) and Yeast Malt (YM) agar (Becton Dickson, USA) respectively, sterilized by autoclaving and poured into petri dishes and allowed to gel. The plates were allowed to cool at 4 °C overnight and the stock bacterial and fungal strains were streaked and sub-cultured on the plates. The inoculated plates were incubated for 24 h at 37 °C to allow the colonies to develop. Bacterial and fungal growth was controlled by storing the plates at 4 °C until required for bioassays.

2.5.2. In vitro antibacterial bioassay

All leaf extracts of the two biotypes (AMNF, SMNF) and that of the three different growth stages (SY, SMF and SMNF) of the SAB *C. odorata*, were tested for antibacterial activity through determination of the minimum inhibitory concentration (MIC) using a serial microdilution bioassay in 96-well microplates (Eloff, 1998). *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883) and *Salmonella typhimurium* (ATCC 700720) cultured overnight (20 h) were prepared by inoculating a single colony of each bacterial species in 5 ml sterilized Mueller-Hilton (MH) broth in sterile McCartney bottles and incubated at 37 °C in a water bath with an orbital shaker. The absorbance of each overnight culture was measured at a wavelength of 600 nm using a UV–visible spectrophotometer with a starting absorbance of 0.001. The overnight bacterial cultures were diluted with 19.8 ml of sterile MH broth in McCartney bottles and used in the screening. One hundred microliters of sterile water were added to each well of a 96-well microplate. From the re-suspended plant extracts (25 mg/ml in 70% EtOH for the organic extracts and water for the water extracts), 100 μl were added to the first well of the microplates (row A) and serially diluted two-fold downwards (column 1–12: A to H). Subsequently, 100 μl of the bacterial culture were added to each well of the microplates. Similarly, 100 μl of neomycin used as the positive control were two-fold serially diluted for use in the assay. Sterile water, 70% EtOH and bacteria-free MH broth were used as the negative controls. The final concentration of