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Full Length Research Paper

Screening of *Chromolaeana odorata* (L.) King and Robinson for antibacterial and antifungal properties

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***Chromolaeana odorata* (L.) is classified as an invasive weed in Southern Africa and is a major contributing species towards reduction of land masses for indigenous vegetation. The species have been noted to have some medicinal values abroad. The current investigation on the South African has indicated potential use of the species in traditional medicines. This could assist in alternative use of *C. ordorata* for traditional usage rather than an invasive species. The methanol leaf extracts was effective against all Gram positive bacteria (*Bacillus subtilis, Bacillus cereus, Staphylococcus aereus, Staphylococcus epidermidis*) as well as one Gram negative bacteria, *Escherichia coli*. The stem extract was not as effective as the leaf extract. However, some activity was noted for stem extracts in both the ethyl acetate and methanol extracts for all Gram-positive bacteria tested.**

Key words: *Chromolaena odorata*, antimicrobial, traditional medicines.

INTRODUCTION

Chromolaena odorata (L.) R. M. King and Robinson is a perennial shrub belonging to the family Asteraceae. It is mainly a perennial weed of plantation crops and pastures of Southern Asia and Western Africa (Phan et al., 2001). A decoction of the leaf is used as a cough remedy and as an ingredient with lemongrass and guava leaves for the treatment of malaria (Vital and Rivera, 2009). A formulation prepared from the aqueous extract of the leaves of *C. odorata*, in Vietnam, has been licensed for clinical use (Phan et al., 2001). Traditionally, fresh leaves or decoction have been used throughout Vietnam as well as other tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Truong, 1989; Nghiem, 1992). Clinical studies using aqueous extracts from *Chomolaena* leaves have shown antimicrobial and anticoagulation effects as well as promotion of tissue re-modelling in the wound healing process (Phan et al., 1998). Other medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Iwu et al., 1999). Previous investigations of the leaves and stems of *C. odorata* revealed the presence of essential oils (Lamaty et al., 1992; Chowdhury, 2002), steroids (Ahmad and Nabi, 1967), triterpenes (Talapatra et al., 1977), flavonoids (Hai et al., 1995; Wollenweber et al., 1995; Wollenweber and Roitman, 1996).

Flowers of the weed have also been subjected to investigation for essential oils (Baruah and Leclercq, 1993); fats (Baruah and Pathnak, 1993); and alkaloids (Biller et al., 1994; Suksamrarn et al., 2004). A decoction of flowers is used as tonic, antipyretic and heart tonic (Bunyapraphatsara and Chokechaijaroenporn, 2000). Studies on another species of *Chromolaena* found that that species possessed antiprotozoal activity (Taleb-Contini et al., 2004). Work on *Chromolaena moritziana*, based on interviews with herbalists, indicated that leaves and flowers of the plant are taken orally as an anti-catarrhal and depurative (Baez et al., 1998). Furthermore, external compresses and washes are used to treat skin diseases such as ulcers and skin eruptions (Baez et al., 1998).

There is a continuous and urgent need to discover plants with antimicrobial properties, especially in rural Africa, where most dwellers are too impoverished to afford specialised health care. To this end, the low socio-economic standing of the great majority of people in KwaZulu-Natal, especially in rural areas, suggests that many use traditional methods of healthcare (Hirst, 1990). Traditional healers therefore play a crucial role in

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providing health care to the majority of the population. The role of natural products, herbal medicine, tribal and traditional medicines is increasingly appreciated for the prevention and treatment of many human ailments (Janardhanan and George, 2006). There is a need to develop safe and alternative antimicrobial agents such as the use of medicinal plants is escalating due to the development of resistance to antibiotics by microorganisms. The wide acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases bring about the necessity to investigate medicinal plants (Vital and Rivera, 2009).

The aim of this investigation was to evaluate the potential antimicrobial benefit of *C. odorata* occurring in Southern Africa against selected bacteria and fungi, thereby providing an alternative source for traditionally healers that currently exploit highly endangered indigenous species. The detrimental effects of the weed are great especially in sensitive ecosystems where the weed smothers indigenous vegetation and changes landscapes and pristine ecosystems. However, if the weed can be used successfully as an antimicrobial agent, it may alleviate stress on other overexploited traditionally collected species and help to curtail the spread of the weed.

MATERIALS AND METHODS

Fresh plant material was collected by Mr. S. Zulu, research assistant, Medicinal Plant project, Mangosuthu University of Technology. Additional fresh plant material was obtained from Silverglen Nature Reserve, Silverglen, Durban. A voucher specimen (Naidoo 2003/2010) is in the Medicinal plant laboratory, Faculty of Natural Sciences, Mangosuthu University of Technology, South Africa.

Antibacterial assay

Methods used in extraction of plant extract by traditional healers were performed according to Coopoosamy et al. (2010). Briefly, of *C. odorata* leaf material collected into three separate potions of 500 g and subjected to drying in an oven at 60°C until sufficiently dried (approximately 72 h). The stems of *C. odorata* were subjected to similar collection and drying procedure. Each portion of dried material was then weight before crushing using a warring industrial blender and placed into separate conical flasks containing one of three mediums, that is, water, ethyl acetate and methanol, for extraction. The media were left for 72 h in an orbital shaker at 20 shakes per minute. After 72 h the extracts were filtered and concentrated using a rotovapor. The extracts were then used for further tests.

The plant extracts were then tested for antibacterial properties against four strains of Gram-positive (*Bacillus subtilis* (ATCC 11744), *Bacillus cereus* (ATCC 11778), *Staphylococcus aereus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228)) and four strains of Gram-negative bacteria (*Escherichia coli* (ATCC 13706), *Proteus vulgaris* (ATCC 49132), *Enterobacter aerogenes* (ATCC 35029), and *Shigella sonnei* (ATCC 9290)) for antibacterial activity. Each organism was prepared by diluting in 24 h old broth cultures with sterile nutrient broth. The cultures were then diluted 100 fold to give approximately 10^6 bacteria ml^1.

Antifungal assay

C. odorata leaves (approximately 1 kg) and stem material (approximately 1 kg) were cut into small pieces and crushed separately in a homogenizer. The plant materials were soaked in ethanol (95% v/v) and in distilled water in 2 L conical flasks for 3 weeks. The extracts (water and ethanol) obtained were evaporated at reduced pressure (45°C) to a residue. Extracts for testing methanol and aqueous extracts were prepared in three different concentrations. The stock solutions were prepared by dissolving 100 mg of dry extract in 1 ml of ethanol and water separately in order to obtain a concentration of 100 mg/ml dilutions (1:10, 1:100, and 1:500). These stock solutions were then used in phosphate buffer at pH 6.0 to evaluate the antifungal activity (Champion et al., 1992). The solutions were then tested for antifungal activity using the following fungal cultures: *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, and Trichophyton rubrum.* Plates containing potato dextrose agar were used to serve as controls.

RESULTS AND DISCUSSION

Scientifically, it is known that Gram-positive bacteria often cause human diseases such as colds, wounds and sores (Waihenya et al., 2002). In the current study, all Gram positive bacteria were inhibited by the ethyl acetate extracts obtained from the leaves, except for *S. aereus* (Table 1). However, the methanol extracts derived from the leaves inhibited all Gram positive bacteria and including one Gram-negative bacteria, *E. coli*. In contrast, there was no inhibitory effect both in Gram-positive and Gram negative bacteria in aqueous extracts. However, if traditional healers' wishes to use the extracts obtained from *C. odorata* leaves or stems, it is assumed that they would have to boil the fresh leaves to obtain antimicrobial results. Coopoosamy (2010) indicate that boiling of the leaves would unlock the active compounds and in the process provide the necessary ingredients for traditional cures.

The antifungal assay indicates a possibility of activity on higher concentration of extracts. Previous screening of both antibacterial and antifungal activities of plants has shown similar results against Gram-positive bacteria (Coopoosamy and Magwa, 2007; Grierson and Afolayan, 1999; Kelmanson et al., 2000; Rabe and van Staden, 1997; Shamim et al., 2004; Vlietinck et al., 1995).

The minimum inhibitory concentration (MIC) values obtained in the current study are relatively high. High MIC was obtained for the Gram negative *E. coli* for the methanol extract derived from the leaves when compared to the control.

The antifungal activity (Tables 2 and 3) of the ethanol extracts was found to be more effective than aqueous extracts in the leaf and stem extracts. However, the stem extract indicated a greater range of antifungal activity than the stem against the test organisms. Growth inhibition (zone of inhibition) was recorded as very high

Table 1. Minimal inhibitory concentration (MIC) of *C. odorata* antibacterial assay on crude extract (Controls: Chloramphenicol and streptomycin sulfate) (n = 3).

Bacteria (10^6 Bacteria/ml)	Gram +/-	Medium (MIC) (mg/ml)						Control (µg/ml)	
		Water		Ethyl acetate		Methanol		Chlor[a]	Strept[b]
		Leaves	Stem	Leaves	Stem	Leaves	Stem		
Bacillus subtilis	+	Na	Na	7.0	9.0	8.0	9.0	<2.0	<2.0
Bacillus cereus	+	Na	Na	8.0	8.5	7.5	8.5	<2.0	<2.0
Staphylococcus aereus	+	Na	Na	8.0	Na	8.0	Na	<2.0	<2.0
Staphylococcus. epidermis	+	Na	Na	7.0	8.0	8.0	8.5	<2.0	<2.0
Escherichia coli	-	Na	Na	Na	Na	8.5	Na	<2.0	<2.0
Proteus vulgaris	-	Na	Na	Na	Na	Na	Na	<2.0	<2.0
Shigella sonnei	-	Na	Na	Na	Na	Na	Na	<2.0	<2.0
Enterobacter aerogene	-	Na	Na	Na	Na	Na	Na	<2.0	<2.0

All tests were done in triplicates and the averages are indicated. Na, no activity. Chlor[a], chloramphenicol Strept [b]; streptomycin sulfate.

Table 2. Effect of ethanol and aqueous extracts obtained from *C. ordorata* on different fungal species.

Fungal species	Ethanol extract						Aqueous extract					
	Leave	Stem	Leave	Stem	Leave	Stem	Leave	Stem	Leave	Stem	Leave	Stem
	1:10	1:10	1:100	1:100	1:500	1:500	1:10	1:10	1:100	1:100	1:500	1:500
Aspergillus flavus	++	+	+	-	-	-	+	-	-	-	-	-
Aspergillus glaucus	++	+	++	-	-	-	++	+	-	-	-	-
Candida albicans	+++	+	++	-	-	-	+	+	-	-	-	-
Candida tropicalis	++	++	+	+	-	-	+	+	-	-	-	-
Trichophyton rubrum	+	+	+	-	-	-	+	-	-	-	-	-

-, Negative antifungal activity, S, stem, L, leaves; +, positive antifungal activity (low inhibition); ++, positive antifungal activity (medium inhibition); +++, positive antifungal activity (high inhibition); ++++, positive antifungal activity (very high inhibition); Plates containing potato dextrose agar only served as controls. Control did not show any inhibition of any of the test fungal species.

Table 3. Minimal inhibitory concentration observed in different concentrations prepared from stock solution of 100 mg/ml of aqueous and ethanol extracts of *C. ordorata* (n = 3).

Fungal species	Ethanol extract		Aqueous extract	
	Stems	Leaves	Stems	Leaves
A. flavus	1:10	1:100	-	1:10
A. glaucus	1:10	1:100	1:10	1:10
C. albicans	1:10	1:100	1:10	1:10
C. tropicalis	1:100	1:100	1:10	1:10
T. rubrum	1:10	1:100	1:10	-

Number of replications = 3.

(++++), high (+++), medium (++), and low (+), which indicated zones of inhibition between 41 to 50, 31 to 40, 21 to 30, and 11 to 20 mm, respectively (Coopoosamy et al., 2010). The ethanol extract of both the leaves and stem indicate a greater antimicrobial effect when compared to the aqueous extracts. The high zones of inhibition noted in the ethanol extracts (using a 1:10 concentration) suggest further investigation of the possibility of using this plant in alternative uses against diseases caused by the aforementioned fungal organisms.

Conclusion

C. ordorata, is a known invasive in Southern Africa and readily spreads with ease inhabiting any available space making it impossible for indigenous species to grow. The

ability for extracts of *C. odorata* exhibiting anti-microbial activities in the current investigation indicates a potential of alternative use rather than removal by destruction.

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