Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Assessment of the antimicrobial activity of the root extracts from *Chrysocoma ciliata* L.

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Accepted 30 July, 2013

***Chrysocoma ciliata* L. is a medicinal plant used in the management of pains, stomach and menstrual disorder in the Eastern Cape Province of South Africa. Studies were conducted to determine the antimicrobial efficacy of the root extracts using agar dilution method against 10 bacteria and 4 fungi species. The methanol and acetone extracts inhibited the majority of the Gram-positive and Gram-negative bacteria at minimum inhibitory concentration (MIC) of 1.0 mg/ml. Similarly, the acetone, methanol and water extracts exhibited 100% growth inhibitions on *Aspergillus Niger* and *A. flavus* at 0.5 mg/ml. All the extracts were able to inhibit the growth of *Candida albicans* at MIC ranging from 5.0 to 10 mg/ml. The results from this study have shown that extracts from the root of *C. ciliata* have strong antimicrobial activity against array of microorganisms. This may be a manifestation of the plant's broad spectrum potential for the treatment of microbial induced ailments. This herb could be a potential agent for antibiotic bioprospecting.**

Key words: *Chrysocoma ciliata,* medicinal plant, antibiotic bioprospecting, antimicrobial activity.

INTRODUCTION

During the past 50 years, there had been a great deal of interest in screening plants for therapeutic agents (Chang et al., 2001). Interest in medicinal plants as a re-emerging health-aid has been fuelled by the rising cost of orthodox/western medicines in the maintenance of personal health and well- being and the bioprospecting of new plant-derived drugs (Hoareau and Dasilva, 1999; Ojekale et al., 2007) . The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Prabuseenivasan et al., 2006). Bacteria for example, have shown a remarkable ability to endure and adapt to their environment including the development of different mechanisms of resistance to most old and new antimicrobial agents (Hersch-Martinez et al., 2005). Bacterial adaptation to antibiotics has been very successful, and over the years, the increase in antibiotic resistance has generated a considerable world-wide public health problem (De Esparza et al., 2007). From time immemorial, plants materials in different forms have been used in the management of ailments resulting from microbial infections. With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for bioactive compounds has become very important as potential sources of novel biomolecules (Meurer-Grimes et al., 1996; Rabe and Van Staden, 1997).

Chrysocoma ciliata L. otherwise known as bitterbos or bitter cowcurd is a dense, rounded shrub growing up to 50 cm in height. The yellowish green leaves are small and needle shaped, sticky to touch and with bitter taste. The plant is indigenous to Southern Africa, becoming invasive in overgrazed parts of the karoo and poorly managed velds (Van Wyk et al., 2002). Our preliminary investigations on the local uses of this herb revealed that the species is used in relieving menstrual pains and to reduce heavy blood flow during menstruations. In addition, it is used to boost fertility in women and in the management of stomach disorders. According to traditional healers, the aerial parts of the plant is crushed and boiled in water and the infusion taken orally 3 - 4 times daily for the treatment of pain, stomach disorders and other ailments. To the best of our knowledge, the antimicrobial activity of the crude extracts from the subterranean part of this species have not been reported in literature. This study was therefore undertaken to investigate the antimicrobial potential of the crude extracts from the root of *C. ciliata* against 10 bacteria and

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four fungal species. From our earlier studies on this herb, it was observed that extracts and essential oil from the aerial parts of this herb possess strong antibacterial and antifungal activities (Ashafa and Afolayan, 2009; Afolayan and Ashafa, 2009). The aqueous extract did not show any significant effect on the heamatology, liver and kidney function indices of Wistar rats (Ashafa et al., 2009). This study is part of our continuous validation of the medicinal potential of this species. According to Mathekga and Meyer (1998), *in vitro* antimicrobial screening could provide the preliminary observations necessary to select among plant materials, those with potentially useful properties for further chemical and pharmacological investigations. In this report, we present the antimicrobial activity of the acetone, methanol and water extracts from the root of *Chrysocoma ciliata*.

MATERIALS AND METHODS

Plant material and preparation of extracts

Plants material were collected in April 2009 from a single population of *C. ciliata* growing around Ntselamanzi township in Nkonkobe Municipality of the Eastern Cape Province (33º11.10'S and 7º 10.60'E; altitude 695 m). The mean annual rainfall of the area is about 700 mm and temperature range of 13 to 25ºC. The species was earlier authenticated by Mr. Tony Dold, Selmar Schonland Herbarium, Rhodes University, South Africa. Voucher specimen (AshMed.2008/1) had been deposited in the Giffen Herbarium of the University of Fort Hare.

The roots were separated, carefully rinsed under running tap, dried in the oven at 40°C to a constant weight before it was pulverized. 40 g each of the powdered material was extracted in acetone and methanol and distilled water. All extracts were filtered using number 1 Watman filter paper. The filtrates from acetone and methanol were concentrated under reduced pressure 40°C using (Laborota 4000-efficient, Heidolph, Germany) rotary evaporator. Acetone and methanol used were of high analytical grade (Merck Chemicals (PTY), Wadeville, South Africa). The water extract was freeze dried using "Virtis BenchTop 'K' Series, USA" freeze dryer. The yields were 2.2, 0.7 and 1.6 g for methanol, acetone and water respectively. Individual extract was reconstituted in their respective solvent to give a stock solution of 50 mg/ml (Taylor et al., 1996). This was diluted to the required concentrations of 0.1, 0.5, 1.0, 5.0 and 10 mg/ml for the bioassay analysis.

Test organisms

5 Gram-positive bacteria namely; *Staphylococcus aereus, Staphylococcus epidermidus, Bacillus cereus, Micrococcus kristinae, Streptococcus faecalis*, and 5 Gram-negative bacteria, *Escherichia coli, Pseudomonas aeruginosa, Shigelia flexneri, Klebsella pneumoniae* and *Serratia marcescens* were all laboratory isolates. They were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. The organisms were maintained on nutrient agar plates and were revived for bioassay by subculturing in fresh nutrient broth (Biolab, Johannesburg, South Africa) for 24 h before being used.

Antibacterial activity assay

Nutrient agar (Biolab, Johannesburg, South Africa) was prepared by autoclaving and allowed to cool to 55°C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 0.1, 0.5, 1.0, 5.0 and 10 mg/ml were poured into petri dishes, swirled gently until the agar began to set, and left over night for solvent evaporation (Afolayan and Meyer, 1997). Agar plates containing 1% acetone, methanol and water served as controls (Dulger and Ugurlu, 2005). Organisms were streaked in radial pattern on the agar plates (Meyer and Afolayan, 1995). The inoculum size of each test strain was standardized at 5×10^5 cfu/ml using McFarland Nephelometer standard according to the Clinical and Laboratory Standards Institute (CLSI). The plates were incubated under aerobic conditions at 37°C and examined after 24 h. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of an extract was required for it to be declared active (Sindambiwe et al., 1999; Mathekga et al., 2000). Chloramphenicol and streptomycin (standard antibiotics) were used as positive controls in the experiment.

Antifungal activity assay

Antifungal activity of *C. ciliata* root extracts was investigated using four fungal species (*Aspergillus niger, Aspergillus flavus, Penicillium notatum* and *Candida albicans*). All fungal cultures were maintained on potato dextrose agar (PDA) (Biolab, Johannesburg, South Africa) and were recovered for testing by subculturing on PDA for 3 days at 25°C prior to bioassay. PDA plates were prepared by autoclaving before the addition of the extracts. Each extract was vortexed with the molten agar at 45°C to final concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 mg/ml and poured into Petri dishes. Petri dishes containing only PDA or PDA with the respective solvent served as controls. The prepared plates containing the extracts were inoculated with plugs (5 mm in diameter) obtained from the actively growing portions of the mother fungal plates and incubated at 25°C for 3 and 5 days as required for fungal species. The diameter of fungal growth was measured and expressed as percentage growth inhibition of three replicates (Lewu et al., 2006; Ashafa et al., 2008). Due to the nature of *C. albicans,* the organism was streaked radially like the bacteria.

Statistical analysis

Significant differences within the means of treatments and controls were measured and calculated using the LSD statistical test (Steel and Torrie, 1960). LC_{50} (the concentration at which 50% of growth was obtained) was calculated by extrapolation.

RESULTS

Antibacterial activity

The minimum inhibitory concentrations of the extracts against each bacterium are presented in Table 1. All the extracts exhibited moderate to strong activity against both Gram-positive and Gram -negative bacteria. The methanol and acetone extracts inhibited the 10 bacteria strains with MIC ranging from 0.5 - 10.0 mg/ml. The water extract was not active against most of the bacteria tested in this study but was able to suppress the growth of *S. faecalis* and *E. coli* at 5.0 mg/ml.

Antifungal activity

The results of the antifungal activity of the acetone,

Table 1. Antibacterial activity of the extracts from the roots of *Chrysocoma ciliata* L.

Bacteria	Gram (+/-)	Minimum inhibitory concentration (MIC) mg/ml				
		Methanol	Acetone	Water	Clhoramphenicol (µg/ml)	Streptomycin (µg/ml)
S. aereus	+	1.0	0.5	Na	<2	<2
S. epidermidus	+	1.0	0.5	Na	<2	<2
B. cereus	+	1.0	0.5	Na	<2	<2
M. kristinae	+	0.5	10.0	Na	<0.5	<2
S. faecalis	+	5.0	10.0	5.0	<2	<4
E. coli	-	1.0	0.5	5.0	<2	<2
P. aeruginosa	-	0.1	5.0	Na	<10	<4
S. flexneri	-	1.0	1.0	Na	<2	<2
K. pneumoniae	-	0.5	5.0	Na	<2	<2
S. marcescens	-	1.0	1.0	Na	<2	<2

Na = not active at 10 mg/ml, which was the highest concentration tested.

methanol and water extracts from the root of *Chrysocoma ciliata* are presented in Table 2. All the extracts exhibited very strong antifungal activity against all the fungal species tested in this study. At 0.1 mg/ml, all the extracts had between 52.50 and 100% inhibition on all the fungi species tested in this study. Also, the water and methanol extracts inhibited the growth of *C. albicans* at 5.0 mg/ml.

DISCUSSION

Sexually transmitted microorganisms like bacteria (*Chlamydia trachomatis* , *Neisseria gonorrhoeae*) and fungus (*Candida* species) have been reported to interfere with human reproductive system leading to several disorders (CDCP, 2005; Patel et al., 2008; Shokeen et al., 2009). The results from our study have shown that crude extracts from the subterranean parts of *C. ciliata* could inhibit several bacteria and fungi species at relatively low concentrations. Majority of plant extracts have been reported to be more active against Gram-positive bacteria than the Gram -negative bacteria strains (Lewu et al., 2006; Ashafa et al., 2008; Ashafa and Afolayan, 2009). In this study, extracts from the root of *C. ciliata* were very active against both the Gram-negative and the Gram-positive bacteria strains. Generally the methanol extract was most active followed by the acetone and water extracts respectively. Recent research activities on antibacterial activities of crude extracts have implicated the methanol extract for being more active than the other solvents extracts (Rabe and van Staden, 1997; Grierson and Afolayan, 1999; Kelmanson et al., 2000; Ashafa et al., 2008; Ashafa and Afolayan, 2009). It will seem likely that the solvent is suitable for the extraction of antibacterial compounds from crude extracts.

The inhibitory effect of the root extracts from *C. ciliata* against arrays of bacteria and fungi strains could partly account for the use of the plant in the management of microbial infections including those responsible for com-

Table 2. Antifungal activity of extracts from the root of *Chrysocoma ciliata* L.

Concentration (mg/ml)	*A. niger*	*A. flavus*	*P. notatum*
Acetone			
10	100^{d}	100^{e}	100^{c}
5	100^{d}	100^{e}	100^{c}
1	83.33^{c}	95.56^{d}	86.67^{b}
0.5	81.67^{c}	89.17^{c}	0.00^{a}
0.1	52.50^{b}	74.72^{b}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.10	0.07	0.58
Methanol			
10	100^{c}	100^{c}	100^{c}
5	100^{c}	100^{c}	100^{c}
1	100^{c}	100^{c}	100^{c}
0.5	100^{c}	100^{c}	100^{c}
0.1	69.44^{b}	79.19^{b}	87.78^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.07	0.06	0.06
Water			
10	100^{c}	100^{c}	100^{b}
5	100^{c}	100^{c}	100^{b}
1	100^{c}	100^{c}	100^{b}
0.5	100^{c}	100^{c}	100^{b}
0.1	78.89^{b}	72.78^{b}	100^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.06	0.07	0.05

Values are means of percentage growth inhibition of three replicates. Values within a column followed by the same superscript are not significantly different at $p < 0.05$. LC_{50} values in mg/ml were calculated by extrapolation.

plications and disorders associated with the female reproductive and genital organs. This further validates the use of this species in the South African traditional medicine in the management of infections diseases. The results from our study showed that the root extracts were more active than the extracts from the aerial parts of this species (Ashafa and Afolayan, 2009). This suggests the use of the whole plant for a more effective treatment of ailments associated with microbial infections. We are progressing to isolating the bioactive compounds in this herb because the species appear to be a potential agent for antibiotic bioprospecting.

ACKNOWLEDGEMENT

We are grateful to the National Research Foundation and Govan Mbeki Research and Development Centre of the University of Fort Hare.

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