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Full Length Research Paper

In vitro antimicrobial activity of the extracts from the leaves of *Chrysocoma ciliata* L.

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***Chrysocoma ciliata* L. is a medicinal plant used in the management of pains, stomach and menstrual disorders in the Eastern Cape province of South Africa. Studies were conducted to determine the antimicrobial activity of the extracts from this herb. All the extracts except water extract, inhibited *Staphylococcus aereus*, *Staphylococcus epidermidus, Bacillus cereus* and *Streptococcus faecalis* at minimum inhibitory concentration (MIC) less than 0.1 mg/ml. The extracts also inhibited the gram-negative bacteria tested including the popularly known antibiotic resistant *Pseudomonas aeruginosa* with MIC ranging from 1.56 to 12.5 mg/ml. All the extracts at the concentration of 0.5 mg/ml, suppressed the growth of *Aspergillus niger, Aspergillus flavus* and *Penicilium notatum* with percentage inhibition ranging from 50.83 to 88.33%. The ethanol and methanol extracts were able to inhibit the growth of *Candida albicans* at 5.0 mg/ml. Results from this study have shown that extracts from *C. ciliata* displayed strong antimicrobial activity, which is a manifestation of the plant's broad spectrum potential for the treatment of microbial induced ailments including complications and disorders associated with the female reproductive and genital organs. This herb could be a potential agent for antibiotic bioprospecting.**

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

INTRODUCTION

Over the years, plants have been used in medicine for both preventive and curative therapies. The records of indigenous knowledge from various parts of the world illustrate an age long tradition of plants being a major bioresource base for health care (Stepp and Moerman, 2001; Yesilada, 2005). Despite the availability of different approaches to drug discovery, plants still remain the main reservoirs of natural medicines (Mahomed and Ojewole, 2006). It is estimated that a considerable percentage of the drugs in the modern pharmacopeias were derived from plants (Farnsworth et al., 1985; De Silva, 2005; Kim, 2005).

South Africa is rich in flora biodiversity, which has provided herbal health practitioners and other traditional healers with an immense pool of natural pharmacy from which ingredients are selected for the preparation of health remedies (Mahomed and Ojewole, 2006). Approximately 80% of the black population is engaged in the use of more than 4000 plant taxa as herbal preparation and formulations countrywide for the treatment of various ailments in man and animals (Kelmanson et al., 2000; Mulholand, 2005; Lewu et al., 2006). With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important as potential sources of novel antibiotic (Meurer-Grimes et al., 1996; Rabe and Van Staden, 1997).

Chrysocoma ciliata, otherwise known as bitterbos or bitter cowcurd is a dense, rounded shrub that grows up to 50 cm in height. The yellowish green leaves which are small and needle shaped are sticky to touch and have bitter taste (Van Wyk et al., 2002). The genus *Chrysocoma* with about 18 species is indigenous to South Africa (Bohlmann and Ahmed, 1982; Zdero and Bohlmann, 1991), becoming invasive in overgrazed parts of the karoo and poorly managed velds (Van Wyk et al., 2002). The plant has been reported to be responsible for *kaalsiekte* (alopecia) in lambs and *lakseersiekte* (purging disease or diarrhoea) in adult animals (Van Wyk et al., 2002). Our preliminary investigations on the local uses of the plant revealed its medicinal importance in relieving menstrual pains and reducing heavy blood flow during menstruations. It is also used to manage stomach disorders. In

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all the cases, the aerial parts of the plant is crushed and boiled and the infusion is taken orally 3 times daily.

A substantial number of South African women seek treatment from traditional healers for a variety of complications and disorders associated with the female reproductive and genital organs (Steenkamp, 2003). Menstrual disorder is classified as painful menstruation (dysmenorrhoea), excessive or prolonged uterine bleeding (menorrhagia), absence of menstruation (amenorrhoea) and /or infertility (sterility), (Steenkamp, 2003). Sexually transmitted microorganisms like bacteria (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*) and fungus (*Candida* species) have been implicated with interference in human reproductive system leading to several disorders (CDCP, 2005; Patel et al., 2008; Shokeen et al., 2009). *C. trachomatis* infection is the most common bacterial sexually transmitted disease (STD) throughout the world, causing major medical, social and economic problems (Eggert-Kruse et al., 2003). Past *Chlamydia* infection was found to be associated with secondary infertility in women (De Barbeyrac and Bébéar, 2005; Malik et al., 2009).

To the best of our knowledge, the antimicrobial activity of the crude extracts from this species has not been reported in literature. According to Mathekga and Meyer (1998), *in vitro* antimicrobial screening could provide the preliminary observations necessary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigations. This preliminary study was therefore conducted to investigate the antimicrobial potential of the extracts of *C. ciliata* against some selected bacteria and fungi species with the aim that if the crude extracts could inhibit an array of bacteria and fungi, the herb could be a potential agent for antibiotic bioprospecting. While in the process of validating the folkloric use of this species, we present the antimicrobial activity of ethanol, methanol, acetone and water extracts of *C. ciliata*.

MATERIALS AND METHODS

Plant material and extract preparation

Plants material were collected in April 2008 from a single population of *C. ciliata* growing around Ntselamanzi township in Nkokobe municipality of the Eastern Cape Province (33 11.10'S and 7 10.60'E; altitude 695 m). The mean annual rainfall of the area is about 700 mm and temperature range of 13 to 25 °C. The species was authenticated by Mr. Tony Dold, Selmar Schonland herbarium, Rhodes University, South Africa. A voucher specimen (AshafaMed .2008/1) was prepared and deposited in the Griffen herbarium of the university of Fort Hare.

Due to the tiny needled shaped nature of the leaves, they were allowed to dry on the stem in the laboratory at room temperature (25 °C). The leaves were shaken off from the stem and pulverized before extraction. Powdered plant material (40 g each) was separately extracted in ethanol, methanol, acetone and water for 48 h at 30 °C, on an orbital shaker (Stuart Scientific Orbital Shaker, UK). Acetone and methanol used were of high analytical grade. The extracts were filtered separately through Whatman no. 1 filter paper and each filtrate was evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator (Laborota 4000-efficient, Heidolph, Germany). The water extract was freeze-dried using Savant refrigerated vapor Trap, (RVT4104, USA). The freeze-dried extract was stored at 4 °C before bioassay.

Antibacterial activity assay

Since *C. trachomatis* and *N. gonorrhoeae* were not available for this study, 5 gram-positive bacteria namely; *Staphylococcus aereus, Staphylococcus epidermidus, Bacillus cereus, Micrococcus Kristinae* and *Streptococcus faecalis* and 5 gram-negative bacteria; *E. coli, Pseudomonas aeruginosa, Shigelia flexneri, Klebsella pneumoniae* and *Serratia marcescens* used in this study 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. Organisms were chosen based on reports of their human pathogenicity.

The minimum inhibitory concentration (MIC) values of each extract on each organism were determined using microplate dilution method (Ellof, 1998) with slight modifications. Briefly, bacterial strains were cultured overnight on nutrient broth (Biolab, Johannesburg, South Africa) and were adjusted to a final density of 10^6 cfu/ml. Under aseptic condition, this was used to inoculate 96-well microtitre plates containing serial twofold dilutions of the extracts (12.50 - 0.09 mg/ml). The plates were incubated under aerobic conditions at 37 °C and examined after 24 h. As an indicator of bacterial growth, 40µL of 0.2 mg/mL p-iodonitrotetrazolium (97% purity, Fluka Chemie) solution was added to each well and incubated for 30 min at 37 °C. The colourless tetrazolium salt was reduced to a red-coloured product by the biological activity of the organisms. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of the extract was required for it to be declared active (Ellof, 1998). Streptomycin and chloramphenicol were used as positive control in the experiment with pure solvent and sample free solutions as blank controls.

Antifungal activity assay

Antimycotic activity of *C. ciliata* 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. Blank plates 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 5 days. The diameter of fungal growth was measured and expressed as percentage growth inhibition of 3 replicates (Lewu et al., 2006; Koduru et al., 2006). 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 AND DISCUSSION

Antibacterial activity

The minimum inhibitory concentrations (MICs) of the

Table 1. Antibacterial activity of extracts from the leaves of *C. ciliata* L.

Bacteria	Gram +/-	Ethanol	Methanol	Acetone	Water	Chloramphenicol (μgmL^{-1})	Streptomycin (μgmL^{-1})
S. aereu	+	0.09	0.09	0.09	na*	<2	<2
S. epidermidus	+	0.09	0.09	0.09	na	<2	<2
Bacillus cereus	+	0.09	0.09	0.09	na	<2	<2
M. kristinae	+	1.56	0.78	0.78	na	<0.5	<2
S. faecalis	+	0.09	0.09	0.09	na	<2	<4
E. coli	-	12.50	6.25	6.25	na	<2	<2
P. aeruginosa	-	3.13	3.13	6.25	na	<10	<4
S. flexneri	-	1.56	0.09	1.56	na	<2	<2
K. pneumoniae	-	3.13	6.25	6.25	na	<2	<2
S. marcescens	-	1.56	1.56	1.56	na	<2	<2

* = not active.

extracts from *C. ciliata* on the tested organisms are presented in Table 1. Apart from the water extract that was not active against any of the bacteria at 12.50 mg/ml, all other extracts inhibited both the gram-positive and gram-negative bacteria at MICs ranging from less than 0.1 mg/ml to 12.5 mg/ml. The ethanol, methanol and acetone extracts inhibited *S. aereus*, *S. epidermidus, B. cereus* and *S. faecalis* at less than 0.1 mg/ml and *M. Kristinae* at 1.56 mg/ml. Similarly, these extracts inhibited all gram-negative bacteria at MIC ranging from less than 0.1 mg/ml in *Shigelia flexneri* to 12.50 mg/ml in. *E.coli*. Generally, all the extractants with exception of water were able to extract the active antibacterial compounds in *C. ciliata*'s leaf. The activity was more pronounced on the gram-positive bacteria than the gram-negative strains. The inability of the water extract to inhibit any of the tested organisms at 12.50 mg/ml is not surprising. Water is known to extract almost all the active ingredients from plant samples. Although, its activity at low concentration is very rare (Lewu et al., 2006; Koduru et al., 2006; Ashafa et al., 2008). Seemingly, it becomes efficacious due to dosage administration by traditional healers. The susceptibility of these nosocomial opportunistic pathogens to the extracts from *C. ciliata* is interesting, as some of them have been implicated in cases of immuno-compromised patients (Hoffman and Roggenkamp, 2003).

Antifungal activity

The results of the antifungal activity of the extracts from *C. ciliata* against four human pathogenic fungal species with the exception of *Candida albicans* are presented in Table 2. All the extracts showed appreciable dose dependent inhibition against all the fungal strains tested in this study. The acetone, methanol, ethanol and water extracts exhibited good percentage inhibition against *A. niger, A. flavus* and *P. notatum* at 0.1 mg/ml. The acetone and water extracts did not show any activity against *C. albicans* at 10 mg/ml which was the highest dose in this study but the ethanol and methanol extracts were able to inhibit the growth of the organism at 5.0 mg/ml.

Generally, all the extracts showed very good activity against all the fungal species, giving an LC_{50} (lowest con-centration at which 50% inhibition is obtained) range of 0.06 to 0.46. Noteworthy is the ability of the extracts from *C. ciliata* to suppress the growth of the 2 *Aspergillus* spe-cies at concentration less than 1 mg/ml. The genus *As-pergillus* is known to cause a large spectrum of diseases called aspergillosis, though the manifestation and severity of the disease depend upon the immunologic state of the patient (Bennett, 1995). These species have recently been implicated in cases of immuno-compromised patients that frequently develop opportunistic and superficial mycosis (Ngane et al., 2000; Portillo et al., 2001; Silva et al., 2001). Also, the capability of the extracts to suppress *C. albicans* at 5 mg/ml is encouraging as the organism is rarely susceptible to plant extracts action at low concentrations, this could partly explain why the plant used in folkloric medicine.

The results from this study have shown that extracts from this plant could inhibit the growth of several human pathogenic bacteria and fungi species at relatively low concentrations. This is an indication of the broad spectrum antimicrobial potential of *C. ciliata* that could make the species a candidate for antibiotic bioprospecting. The ability of the extracts from this herb to effectively suppress the growth of *C. albicans* at 5 mg/ml further validates the folkloric use of this plant for the treatment of various ailments including complications and disorders associated with the female reproductive and genital organs. Further study is ongoing on the isolation, purification and structural elucidation of the bioactive compounds in this plant.

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Table 2. Antifungal activity of extracts from the leaves of *C. ciliata* L.

Concentration (mg/ml)	*A. niger*	*A. flavus*	*P. notatum*
Acetone			
10	75.09^{e}	90.65^{e}	100^{e}
5	73.33^{e}	78.89^{u}	95.83^{u}
1	61.11^{u}	70.00^{c}	89.17^{c}
0.5	50.83^{c}	64.72^{b}	88.33^{c}
0.1	42.50^{b}	64.17^{b}	81.39^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.46	0.08	0.06
Methanol			
10	100^{f}	100^{e}	100^{u}
5	89.72^{e}	100^{e}	100^{u}
1	72.22^{u}	88.89^{u}	92.78^{c}
0.5	62.78^{c}	83.33^{c}	89.17^{c}
0.1	37.78^{b}	76.67^{b}	79.17^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.30	0.07	0.06
Ethanol			
10	100^{f}	100^{e}	100^{e}
5	88.61^{e}	95.28^{f}	96.39^{u}
1	73.89^{u}	90.56^{u}	86.11^{c}
0.5	55.28^{c}	82.22^{c}	82.78^{c}
0.1	45.56^{b}	78.89^{b}	76.11^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.28	0.06	0.07
Water			
10	80.09^{f}	75.56^{e}	90.46^{e}
5	69.17^{e}	67.78^{u}	90.28^{e}
1	63.89^{u}	63.33^{c}	86.11^{u}
0.5	58.33^{c}	62.22^{c}	78.33^{c}
0.1	48.06^{b}	49.72^{b}	74.72^{b}
Control	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.18	0.11	0.07

Values are means of percentage growth inhibition of 3 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.

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