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J.R. Appidi, D.S. Grierson & A.J. Afolayan

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RESEARCH ARTICLE

Antimicrobial activity of *Hermannia incana*

J.R. Appidi, D.S. Grierson, and A.J. Afolayan

Department of Botany, University of Fort Hare, Alice, South Africa

Abstract

Hermannia incana Cav. (Sterculiaceae) is a prostrate herb used to treat stomachache and diarrhea, and as an emetic by the people of Eastern Cape Province, South Africa. Acetone, methanol, and water extracts from the leaves and roots of the plant were investigated for antibacterial and antimycotic activities. The methanol extracts of the plant showed appreciable activity against Gram-positive and Gram-negative bacteria at concentrations ranging from 0.5 to 7 mg/mL. The acetone and water extracts of both the leaves and the roots showed moderate activity against Gram-positive bacteria and less activity against Gram-negative bacteria. All the extracts inhibited the growth of the fungi *Aspergillus flavus, Aspergillus niger,* and *Mucor hiemalis* with growth inhibition based on MIC ranging from 54% to 96% at 0.1- 10 mg/mL. None of the extracts suppressed the growth of *Candida albicans* at the maximum concentration (10 mg/mL) tested. This study has pointed to the potential application of *Hermannia incana* as a bacteriocide and fungicide.

Keywords: Hermannia incana*; antibacterial; antimycotic; bacteriocide; fungicide*

Introduction

Diarrhea still remains one of the leading causes of morbidity and mortality throughout the world, especially among children living in developing countries. It is the second largest cause of premature mortality and disability (Murray & Lopez, 1997). Despite the availability of a vast spectrum of approaches for its management, an estimated 4.6 million people, including 2.5 million children, die from diarrhea every year (Thapar & Sanderson, 2004). This disease is very common in those countries where living conditions are crowded and hygiene is poor. Food and water contamination by bacterial enteropathogens are among the main causes of infectious diarrhea (WHO, 1998). *Shigella* spp. are the most important causes of acute bloody diarrhea and account for about 15% of all deaths attributable to diarrhea in children younger than five years. *Vibrio cholerae* remains a major cause of epidemic diarrhea (Thapar & Sanderson, 2004), while *E. coli* accounts for up to 70% of cases of travelers' diarrhea (Ramzan, 2001). Diarrhea occurs when these organisms disrupt intestinal function, causing malabsorption or diarrhea by microbial attachment and localized effacement of the epithelium, production of toxins and direct epithelial cell invasion (Guerrant et al., 1999). During the past three decades there has been major improvement in the treatment of infectious diarrhea, with a number of new antibiotics which pharmacological industries have produced. However, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to acquire resistance to drugs (Cohen, 1992; Gislene et al., 2000). Oral rehydration therapy has contributed greatly to the reduction of diarrheal mortality rates in children and the elderly. However, the attack rate of the disease has remained unchanged and this treatment often fails in the high stool output state (Brijesh et al., 2006).

In developing countries, the majority of people still depend on traditional medicines for the treatment of many diseases including diarrhea. The evidence provided by recent studies of medicinal plant-based therapies encourages further investigation in the anticipation that alternative treatments for diarrheal diseases will be developed. For this reason WHO has encouraged scientific studies for the treatment and prevention of diarrheal diseases based on traditional medical practices (Atta & Mouneir, 2004). A range of medicinal plants

Address for Correspondence: AJ Afolayan, Department of Botany, University of Fort Hare, Alice 5700, South Africa. Tel: +27 866282295; Fax: +27 866282295; E-mail: Aafolayan@ufh.ac.za

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with anti-diarrheal properties has been widely used by the traditional healers of different tribes in South Africa (Lin et al., 2002).

Hermannia incana Cav. (Sterculiaceae), known as sweet yellow bells, is a medicinal plant used by the people of the Eastern Cape for the treatment of diarrhea. It is a prostrate herb with yellow flowers. The leaves are sparsely hairy and slightly glandular, occurring in grassland and marshes in the Eastern Cape Province of South Africa. From discussion with traditional healers, herbalists and local people it was found that the plant is used as an emetic and the leaf sap extracted in cold water is used to treat stomach ache and diarrhea, having a purgative and diaphoretic effects. Decoctions of the whole plant are taken to soothe coughs. No studies relating to the chemical composition or antimicrobial activities of this species have previously been reported. This study investigated the antimicrobial activity of *Hermannia incana* by preliminary bioassay screening of its extracts against 10 selected bacterial and four fungal strains. Among these organisms are *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Shigella flexneri, Vibrio cholerae,* and *Candida albicans* which have been implicated in causing diarrhea in humans (Anne & Geboes, 2002; Robert et al., 2001; McGaw et al., 2000).

Materials and methods

Plant material

The leaves and roots of *H. incana* were collected in August 2007 from a natural population growing near the University of Fort Hare in the Eastern Cape Province of South Africa. The plant was identified by D.S. Grierson at the Department of Botany, University of Fort Hare, and a voucher specimen (Jaipal Med 001) was deposited in the Griffen Herbarium.

Extract preparation

The leaves and roots were air-dried separately at room temperature. Dried plant materials (200 g each) were shaken separately in acetone, methanol, and water for 48 h at room temperature on an orbital shaker (Stuart Scientific Orbital Shaker, Greater Manchester, UK). Extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper, and each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany). The water extracts were freeze-dried (Savant Refrigerated Vapor Trap, RVT4104, Farmingdale, USA). Each extract was resuspended in its respective solvent to make a 50 mg/mL stock solution.

Bioassays

The bacterial cultures used in this study were laboratory isolates obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. They consisted of five Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Micrococcus kristinae,* and *Streptococcus faecalis*) and five Gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Klebsella pneumoniae,* and *Vibrio cholerae*). Each organism was maintained on nutrient agar plates and was recovered for testing by growth in nutrient broth for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan & Meyer, 1997). Using the agar dilution method of Meyer & Afolayan (1995), test organisms were streaked in radial patterns on sterile nutrient agar plates containing filtered extracts at final concentrations of 0.1, 0.5, 1, 5, 7, and 10 mg/mL. Plates containing only nutrient agar and another set containing nutrient agar and the respective solvents served as controls. Chloramphenicol and streptomycin were used as standard controls in the experiment at concentrations 1, 2, 4, 6, 8, and10 µg/mL. After inoculation the plates were incubated at 37°C for 24-48 h. Each treatment was performed in triplicate, and complete inhibition of bacterial growth was required for an extract to be declared bioactive. This concentration was regarded as the minimum inhibitory concentration (MIC).

Four species of fungi (*Aspergillus flavus, Aspergillus niger*, *Mucor hiemalis,* and *Candia albicans*) were used for the antimycotic investigation. The cultures were maintained on potato dextrose agar (PDA) and were recovered for testing by subculturing on fresh PDA for three days. PDA plates were prepared in the usual fashion by autoclaving before the addition of the filtered extracts. Each extract was mixed with the molten agar (at 45°C) to final concentrations of 0.1, 0.5, 1, 5, and 10 mg/mL, poured into Petri dishes and left overnight for the solvent to evaporate (Koduru et al., 2006). Control plates containing only PDA or PDA with the respective solvents served as controls. The prepared plates containing the extracts were inoculated with plugs (5 mm in diameter) obtained from the actively growing margins of the recovered fungal cultures and were incubated at 25°C for 5 days. Due to the nature of *Candida albicans,* the organism was streaked radially like the bacteria. The diameter of fungi growth was measured and expressed as percentage growth inhibition of three replicates (Afolayan & Meyer, 1997; Barreto et al., 1997; Quiroga et al., 2001). Significant differences within the means of the treatments and the controls were calculated using the LSD statistical test (Koduru et al., 2006). LC_{50} (the concentration at which there was 50% inhibition of the growth of the test fungi) was calculated by extrapolation.

Results and discussion

Antibacterial property

Minimal inhibitory concentration (MIC) values of ethanol, acetone, and water extracts from the leaves and roots of *Hermannia incana* against the tested bacteria are given in Table 1. The methanol extracts of leaves and roots inhibited the growth of both the Gram-positive and Gram-negative bacteria at MIC values ranging between 0.5 and 7 mg/mL. There was, however, more inhibition of Gram-positive strains. The acetone extracts of the leaves and roots showed moderate activity at concentrations between 5 and 10 mg/mL, while there was no activity against *Pseudomonas aeruginosa* and *Klebsella pneumoniae*. The water extract of leaves and roots showed activity against Gram-positive bacteria at concentrations between 5 and 10 mg/mL. There was no activity against the Gram-negative bacteria at the highest concentration tested with the exception of *Escherichia coli* and *Shigella flexneri*. The methanol extract of this plant's leaves and roots thus showed appreciable activity against all 10 organisms. The action of *H. incana* against *Escherichia coli, Shigella flexneri, Bacillus cereus, Staphylococcus aureus,* and *Vibrio cholerae* is noteworthy, because all these bacteria have been implicated as causal agents of diarrhea. *Shigella flexneri* causes bacillary dysentery, an invasive disease of the human colonic mucosa (Perdomo et al., 1994). Over the past several decades, strains of *Shigella* have progressively become resistant to most of the widely used and inexpensive antimicrobials (Bennish et al., 1992). It is interesting to note that the methanol extract showed appreciable activity against *Shigella flexneri*. The results also showed *S. aureus*, a common food-poisoning organism (Accoa et al., 2003), to be the most susceptible of all the bacteria studied. Diarrhea caused by *Escherichia coli* infection is an emergent problem in both developing and developed world and is responsible for high rates of mortality in newborn children and animals (Radu et al., 2001). The significant antibacterial activities of *H. incana* suggest that it could be useful for treating diarrhea caused by enteropathogenic strains of *Escherichia coli*. The study also showed that *Vibrio cholerae* is highly susceptible to the effects of the extract. Such an inhibitory property would prevent the organism from producing the vibrio toxin. Species of the Sterculiaceae family (*Cola greenwayi* Brenan, *Cola natalensis* Oliv., *Dombeya burgessiae* Gerr. ex Harv., *Dombeya cymosa* Harv., and *Hermannia depressa* N.E.Br), to which *H. incana* belongs, were screened for antibacterial activity in KwaZulu-Natal. The ethanol and ethyl acetate extracts of these plants have shown moderate activity against some diarrhea-causing organisms (Reid et al., 2005). This claim is supported by the current bioassay results.

Antifungal property

The results of the antifungal assay of *Hermannia incana* leaf and root extracts are presented in Table 2. The majority of the extracts (71.22%) showed antimycotic activity against the test organisms at concentrations of 10 mg/mL or lower. The methanol, acetone, and water extracts of both leaf and root inhibited the growth of *Aspergillus flavus, Aspergillus niger* and *Mucor hiemalis* with inhibitory percentage ranging from 54% to 96%. Table 2 does not include the column for *Candida albicans* because the extracts did not show activity against this fungus at the

Table 1. Antibacterial activity of the methanol acetone and water extracts of the leaves and roots of *Hermannia incana*.

		[*]MIC (mg/mL)							
		MeOH extract		Acetone extract		Water extract		Chloramphenicol	Streptomycin
Bacteria	Gram +/-	Leaf	Root	Leaf	Root	Leaf	Root	(µg/mL)	(µg/mL)
Staphylococcus aereus	+	1.0	0.5	5.0	5.0	7.0	7.0	<2	<2
Staphylococcus epidermidis	+	0.5	0.5	5.0	5.0	7.0	10.0	<2	<2
Bacillus cereus	+	1.0	1.0	5.0	5.0	10.0	10.0	<2	<2
Micrococcus kristinae	+	0.5	1.0	5.0	5.0	7.0	7.0	<0.5	<2
Streptococcus faecalis	+	5.0	5.0	7.0	5.0	7.0	10.0	<2	<4
Escherichia coli	-	5.0	7.0	7.0	7.0	10.0	10.0	<2	<2
Pseudomonas aeruginosa	-	5.0	1.0	na[b]	na	na	na	<10	<4
Shigella flexneri	-	7.0	1.0	7.0	10.0	10.0	10.0	<2	<2
Klebsella pneumoniae	-	7.0	7.0	na	na	na	na	<2	<2
Vibrio cholerae	-	1.0	1.0	7.0	7.0	na	na	<2	<2

[*]Minimum inhibitory concentration, [b]na = not active.

Table 2. Antifungal activity of the methanol acetone and water extracts of the leaves and roots of *Hermannia incana.*

	Growth inhibition (%)					
	Leaf			Root		
Concentrations(mg/mL)	*A. flavus*	*A. niger*	*M. hiemalis*	*A. flavus*	*A. niger*	*M. hiemalis*
Acetone extracts						
10	77.31^{e}	61.48^{e}	62.50^{d}	72.22^{c}	70.74^{c}	88.61^{c}
5	68.06^{d}	48.33^{d}	46.11^{c}	55.00^{b}	58.61^{b}	73.33^{b}
1	47.50^{c}	41.11^{c}	29.44^{b}	0.00^{a}	0.00^{a}	0.00^{a}
0.5	33.33^{b}	28.61^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
0.1	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	1.49	5.63	6.19	4.64	4.41	3.59
Methanol extracts						
10	91.11^{e}	91.02^{f}	92.87^{e}	85.19^{d}	78.98^{d}	96.67^{d}
5	76.67^{d}	86.94^{e}	86.11^{d}	77.50^{c}	73.61^{c}	87.78^{c}
1	46.94^{c}	64.72^{d}	73.61^{c}	64.44^{b}	65.28^{b}	60.00^{b}
0.5	34.72^{b}	49.17^{c}	46.94^{b}	0.00^{a}	0.00^{a}	0.00^{a}
0.1	0.00^{a}	40.56^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	1.41	0.53	0.56	0.89	0.88	0.92
Water extracts						
10	64.81^{c}	67.31^{d}	54.31^{b}	55.00^{b}	61.67^{c}	71.94^{b}
5	42.78^{b}	48.33^{c}	0.00^{a}	0.00^{a}	36.94^{b}	0.00^{a}
1	0.00^{a}	26.67^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
0.5	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
0.1	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	6.64	5.44	9.60	9.55	7.64	8.48

Values are means of percentage growth inhibition of three replicates: values within a column followed by the same superscript of the same species are not significantly different at $p < 0.05$ according to the LSD test. LC_{50} values in mg/mL.

tested concentrations. In this study, the methanol extracts were found to have broad-spectrum activity against *Aspergillus flavus, Aspergillus niger* and *Mucor hiemalis*. Generally, the methanol extracts were more active than the acetone and water extracts. *Aspergillus flavus* is an imperfect filamentous fungus which is an opportunistic pathogen causing invasive and non-invasive aspergillosis in humans and animals (Jiujiang, 2005).

Both organic and aqueous extracts of the leaf and roots of *H. incana* have indicated varied levels of antibacterial and antifungal activity. The traditional use of *H. incana* extracts to treat diarrhea, stomach-ache, and other infections has been supported by laboratory results from this study, suggesting a need to isolate and evaluate the most active compounds responsible for the exhibited biological activities. Work is in progress on the isolation, purification, and structural identification of the bioactive compounds in this plant.

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