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In vitro pharmacological evaluation and phenolic content of ten South African medicinal plants used as anthelmintics

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Abstract

Helminth infection is regarded as one of the neglected tropical diseases (NTDs). Although the disease is common in rural areas, information on the pharmacology of South African medicinal plants used against this disease is limited. We investigated the efficacy of ten South African medicinal plants against *Caenorhabditis elegans*. Because of the increased susceptibility of a host to microbial infections and other inflammatory responses associated with helminth infections, the antimicrobial and cyclooxygenase (COX) inhibitory activities of the plants were also investigated. Phenolics including flavonoids, condensed tannins and gallotannins have been linked to many pharmacological activities. Thus, the phenolic content of the plant extracts were quantitatively evaluated. In the three bioassays, organic solvent extracts from *Cyathea dregei* (roots and leaves), *Felicia erigeroides* (leaves and stems), *Hypoxis colchicifolia* (leaves) and *Senna petersiana* (leaves) exhibited noteworthy pharmacological activities while *Acokanthera oppositifolia* (leaves) had good COX inhibitory activity. The concentration of phenolics ranged from 56.7 to 1.7 mg GAE/g dry matter in *Ocimum basilicum* and *Cotyledon orbiculata* var. *dactylopsis*, respectively. Flavonoids, condensed tannin and gallotannin content also varied greatly among the plant extracts investigated.

Keywords: Anthelmintic; Antimicrobial; Cyclooxygenase inhibition; Phenolic content; South African medicinal plants; Tannin

1. Introduction

A large number of the South African population still depend on traditional medicine (TM) for their health care needs due to its affordability, accessibility and cultural importance (Light et al., 2005). Among the variety of diseases treated by traditional healers in TM are helminth infections. These infections are regarded as one of the neglected tropical diseases (NTDs) due to their high prevalence among the economically disadvantaged people in the rural areas in different regions of the world. In most developing countries, helminth infections are considered a major health concern because the factors that predispose humans to these infections abound in these regions (Ijagbone and Olagunju, 2006). Poor sanitation, poverty, unsafe water, malnutrition and ignorance are the factors that sustain the parasite life cycle and favour the proliferation of the disease vectors (Brooker et al., 2006). In South Africa, the prevalence of helminth infections has been reported in provinces such as KwaZulu-Natal and Mpumalanga. Helminth infections have remained relatively high over time, and the most vulnerable groups are women and children (Jinabhai et al., 2001).

Globally, a number of medicinal plants have been used for the treatment of helminth infections by the local people (Waller et al., 2001). South Africa is endowed with more than 24 000 indigenous plants, representing about 10% of all higher plants that occur globally. Approximately 3000 plant species are used as medicines by an estimated 200 000 traditional healers in South Africa (Van Wyk et al., 1997). Although the plant kingdom has remained poorly explored, natural products isolated from plants have been postulated to remain an essential part of the search for novel medicines against human diseases (Balunas and Kinghorn, 2005). There is a higher potential of discovery and development of more

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potent and efficient drugs from plants because a large number of medicinal plants have remained understudied for their pharmacological and phytochemical properties (Gurib-Fakim, 2006).

Although helminth infections do not always cause obvious diseases, the host's health integrity could be compromised due to the effect of the metabolic activities of the parasites on nutrition as well as the toxic products of excretion (Deacon, 2006). This probably accounts for the increased susceptibility and enhances the progression of bacterial diseases such as tuberculosis and other opportunistic infections among helminth hosts (Bentwich et al., 1995; Brooker et al., 2004). In addition, microorganisms such as bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) and fungi (*Candida albicans*) have been implicated in the increased infection rate in immune-compromised patients worldwide (Sleigh and Timburg, 1998). Globally, the increase in helminth infection and their growing resistance to most chemotherapeutics is a major problem facing human health (James and Davey, 2009). In addition, research on the discovery of new chemotherapies for helminth infections has been neglected by governments, researchers and the pharmaceutical industries due to the poor economic prospects and the presumed low priority of the diseases that go with it (Geary et al., 1999).

The importance of pharmacological screening of plant extracts for multiple biological activities has been extensively documented (Houghton et al., 2005). The importance has been demonstrated for diseases such as Alzheimer's and diabetes. The use of multiple bioassays gives a clearer indication of the effect of the extracts in relation to the disease state (Houghton et al., 2007). This study was aimed at screening extracts of medicinal plants to determine their efficacy as anthelmintics. Besides the anthelmintic investigation, antimicrobial activity and cyclooxygenase (COX) enzymes inhibition of the plant extracts were carried out as microbial infection and pain are often associated with helminth diseases. The plant extracts were also investigated for phenolic composition, to provide a clue on different phenolic compounds that could be responsible for any biological activity observed.

2. Materials and methods

2.1. Plant collection and extract preparation

Plant material of ten species (Table 1) was collected from the University of KwaZulu-Natal (UKZN) Botanical Garden, Pietermaritzburg and Mount Gilboa (29° 15.450′ S, 30° 20.289′ E) between February and April 2009. Voucher specimens of the plants were deposited in the UKZN Herbarium, Pietermaritzburg. For sustainable harvesting, twigs and leaves of *Acokanthera oppositifolia* were substituted for the roots. Plant material was oven dried at 50 °C, ground into powders through a 1-mm ring sieve using an Ultra Centrifugal Mill (ZM 200, Retsch®, Germany) and stored at room temperature in airtight containers in the dark.

Ground material (1 g) was extracted independently with 10 ml of petroleum ether (PE), dichloromethane (DCM), ethanol (EtOH) and water in an ultrasonic sonicator (Julabo GMBH, West Germany) with ice for 1 h. The extracts were filtered under vacuum through Whatman No. 1 filter paper. Organic solvent extracts were concentrated *in vacuo* using a rotary evaporator (Büchi, Germany) at 30 °C. The concentrates were transferred to pre-weighed glass vials and completely dried under a stream of air. Water extracts were collected into pre-weighed glass jars and freeze-dried. The percentage yield (Table 3) of each dried extract in terms of the starting plant material was determined after which it was stored in the dark at 10 °C until required for analysis.

Phenolic compounds were extracted from dry plant material as described by Makkar (2000). Ground plant samples (1 g) were extracted with 10 ml of 50% methanol (MeOH) by sonication in cold water for 20 min. The extracts were then filtered *in vacuo* through Whatman No.1 filter paper.

2.2. Pharmacological evaluation

2.2.1. Anthelmintic assay

An *in vitro* colourimetric assay used in the determination of free-living nematode larvae viability, as described by James and Davey (2007) with slight modifications, was used to evaluate the minimum lethal concentration (MLC) values of the extracts. A 3-day-old *Caenorhabditis elegans* var. Bristol (N2) culture was prepared by subculturing a stock culture seeded with autoclaved *E. coli*. The subcultured *C. elegans* was washed with 5 ml of M9 buffer (Brenner, 1974) into a sterile McCartney bottle and, using a UV–visible spectrophotometer (Varian Cary 50, Australia), the optical density (OD) at 530 nm was measured. Thereafter, 5 ml of M9 buffer (sufficient for inoculating one microtitre plate) were adjusted with appropriate volumes of the prepared stock of *C. elegans* culture to obtain a culture mixture in the OD_{530} range of 0.04–0.06 (approximately 100 worms per 50 µl). Organic solvent extracts were redissolved in DMSO (25 mg/ml), and water extracts in sterile water (50 mg/ml). Each redissolved extract (100 µl) was two-fold serially diluted with 100 µl sterile distilled water down a 96-well microtitre plate. A similar two-fold serial dilution of 100 µl of 1 mg/ml levamisole (Sigma-Aldrich, Germany) was used as a reference drug. The prepared *C. elegans* culture (50 µl) containing approximately 100 worms (*C. elegans*) were added to each well of the microtitre plate. *C. elegans* culture, DMSO and water were included as controls. The microtitre plates were covered with parafilm and incubated at 20 °C for 48 h in the dark. Thereafter, 50 µl of 1.25 mg/ml *p*-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, Germany) was added to the wells of the microtitre plate and further incubated at 20 °C for 24 h. Active organisms biologically reduce the colourless INT to a pink–red colour (McGaw et al., 2007). The concentration of the lowest clear well was recorded as the MLC value of the extract. Extracts were screened in duplicate and the assay was repeated twice.

2.2.2. Antimicrobial assay

2.2.2.1. Antibacterial assay. Minimum inhibitory concentration (MIC) values of extracts against four representative bacterial strains were determined using the microplate technique (Eloff, 1998a). Overnight cultures of two Gram-positive bacteria, (*Bacillus subtilis* ATCC 6051, 4.8×10^9 CFU/ml and *S. aureus* ATCC 12600, 2.7×10^9 CFU/ml) and two Gram-negative bacteria (*E. coli*

Table 1
South African plants used as anthelmintics based on ethnobotanical information from the literature.

Family	Plant species Voucher number[a] Plant part screened	Ethnopharmacological uses
Apocynaceae	*Acokanthera oppositifolia* (Lam.) Codd A.AREMU 1 NU Leaves, Twigs	Dried leaves or roots and wood are used as anthelmintics (Hutchings et al., 1996; Van Wyk et al., 1997).
Asteraceae	*Felicia erigeriodes* DC. A.AREMU 3 NU Leaves, stems	Hot leaf infusions are administered as enemas for intestinal parasites and abdominal pains and also as purgatives (Hutchings et al., 1996; Pooley, 1998).
Caesalpiniaceae (Fabaceae)	*Senna petersianna* (Bolle) Lock. A.AREMU 7 NU Leaves	*Senna* species are used pharmaceutically in laxative preparations. Root and leaf infusions are used as a purgative to treat stomach-ache and intestinal worms (Van Wyk et al., 1997).
Crassulaceae	*Cotyledon orbiculata* var. *dactylopsis* Tölken A.AREMU 12 NU Leaves, stems	Leaves are eaten as vermifuge and applied as a hot poultice to treat boils, earache and inflammation (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997).
Crassulaceae	*Cotyledon orbiculata* var. *orbiculata* A.AREMU 5 NU Leaves, stems	Leaves are eaten as vermifuge and applied as a hot poultice to treat boils, earache and inflammation (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997).
Cyatheaceae	*Cyathea dregei* Kunze A.AREMU 2 NU Leaves, roots	Dried roots are used as an anthelmintic (Hutchings et al., 1996).
Hypoxidaceae	*Hypoxis colchicifolia* Bak. A.AREMU 10 NU Leaves, corms	Used as purgatives and ascarifuges in unspecified parts of Africa (Watt and Breyer-Brandwijk, 1962).
Hypoxidaceae	*Hypoxis hemerocallidea* Fisch. and C.A. Mey. A.AREMU 11 NU Leaves, corms	Plant decoctions have purging effects (Watt and Breyer-Brandwijk, 1962).
Lamiaceae	*Ocimum basilicum* (L.) A.AREMU 6 NU Leaves	Juice of leaves is used to expel worms; leaves are used for respiratory disorders, dysentery, constipation (Okujagu et al., 2006).
Verbenaceae	*Clerodendrum myricoides* (Hochst.) Vatke. A.AREMU 8 NU Leaves, stems	Root and leaf infusions are taken as anthelmintics; they are also used for pains in the chest, colds, bleedings of the gums and indigestion (Hutchings et al., 1996).

[a] Voucher number: NU = Natal University Herbarium, Pietermaritzburg.

ATCC 11775, 7.0×10^{10} CFU/ml and *K. pneumoniae* ATCC 13883, 2.5×10^{9} CFU/ml) were diluted with sterile Mueller–Hinton (MH) broth. This resulted in a final inoculum of approximately 10^{6} CFU/ml. Each redissolved extract (100 µl) was two-fold serially diluted with 100 µl sterile distilled water down the wells in a 96-well microtitre plate for each of the four bacteria. Neomycin (100 µl; 0.4 mg/ml) (Sigma-Aldrich, Germany) was used as a reference drug against each bacterium. Each bacterial culture (100 µl) was then added to each well. MH broth, DMSO and water were included as controls. The plates were covered with parafilm and incubated at 37 °C for 24 h. After incubation, bacterial growth was indicated by adding 50 µl of 0.2 mg/ml INT to all the wells of the microtitre plate and further incubated at 37 °C for 1 h. Bacterial growth in the wells was indicated by a pink–red colour, whereas clear wells indicated growth inhibition. MIC values were recorded as the concentrations of the lowest clear wells of each extract. The assay was repeated twice in duplicate per test extract.

2.2.2.2. Antifungal assay. The antifungal activity of the extracts was evaluated against *C. albicans* (ATCC 10231, 5×10^{5} CFU/ml) using the microplate technique (Eloff, 1998a) with modifications (Masoko et al., 2007). Sterile saline (4 ml) was added to 400 µl of *Candida* overnight culture in Yeast Malt (YM) broth. The absorbance was measured at 530 nm and adjusted with sterile saline to match that of a 0.5 M McFarland standard solution. This resulted in a final inoculum of approximately 10^{6} CFU/ml. From this stock, a 1:1000 dilution with sterile YM broth was prepared. Amphotericin B (100 µl; 0.25 mg/ml) (Sigma-Aldrich, Germany) was used as a reference drug. The microtitre plates were covered with parafilm and incubated at 37 °C for 24 h after which 50 µl INT (0.2 mg/ml) was added to each well of the microtitre plate. MIC values were recorded as the concentrations of the lowest clear wells after 48 h. Fungicidal activity was determined by adding YM broth (50 µl) to all clear wells then incubating for a further 24 h. Minimum fungicidal concentration (MFC) values were recorded as the concentration of the lowest clear well. The assay was repeated twice in duplicate per test extract.

2.2.3. Cyclooxygenase (COX-1 and -2) inhibition assays

COX-1 and -2 enzyme assays were performed according to the methods by Jäger et al. (1996) as modified by Zschocke and Van Staden (2000) to determine the COX enzyme inhibitory activity of the plant extracts. The controls were a solvent blank (17.5 µl water+2.5 µl EtOH), and a background (17.5 µl water+2.5 µl EtOH) correction reaction in which the enzymes were inactivated

with HCl, and kept on ice before adding [^{14}C]arachidonic acid (16 Ci/mol; 30 μM). Indomethacin (Sigma-Aldrich, Germany) was used as a reference drug (5 μM for COX-1 and 200 μM for COX-2). Organic solvent and water extracts were tested at 250 μg/ml and 2 mg/ml, respectively. COX inhibition of the extracts was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank using the equation:

$$\text{COX Inhibition}\,(\%) = \left\{1-\left(\frac{\text{DPM}_{\text{extract}}-\text{DPM}_{\text{background}}}{\text{DPM}_{\text{solvent blank}}-\text{DPM}_{\text{background}}}\right)\right\} \times 100$$

where, DPM is the disintegrations per min of the extract, background and solvent blank. Results were presented as means of two experiments ($n=4$) and values were expressed as percentage mean ± standard error.

2.3. Phenolic content evaluation

The Folin-Ciocalteu (Folin-C) assay using gallic acid as a standard was used for the evaluation of total phenolics from the 50% MeOH extracts Makkar, (2007). Total phenolic concentrations were expressed as gallic acid equivalents (GAE) per gram of dry matter.

Gallotannin content was determined using the rhodanine assay as described by (Makkar 2007). The gallotannin concentrations in the extracts were expressed as GAE per gram of dry matter.

Condensed tannin content was evaluated using the butanol-HCl assay as described by Makkar (2007) with slight modifications by Ndhlala et al. (2007). The condensed tannin content (percentage dry matter) was calculated as equivalent amount of leucocyanidin (LCE) using the equation below:

$$\text{Condensed tannin}\,(\%) = \left(\frac{A_{550} \times 78.26 \times \text{dilution factor of extract}}{\%\,\text{dry matter}}\right) \times 100$$

where, A_{550} = absorbance of sample at 550 nm. The formula assumes the effective $E^{1\%,\,1\text{ cm},\,550\text{ nm}}$ of leucocyanidin to be 460.

The flavonoid content was evaluated as described by Hagerman (2002), with modifications. In triplicate, 50 μl of each 50% MeOH extract were diluted with 950 μl of glacial acetic acid, followed by the addition of 2.5 ml of 4% HCl in methanol (v/v) and 2.5 ml vanillin reagent (4% vanillin in glacial acetic acid, w/v), after which the reaction mixture was incubated for 20 min at room temperature. The presence of flavonoids was indicated by a pink colouration. After the incubation period, absorbance at 500 nm was measured using a UV–visible spectrophotometer against a blank consisting of water instead of extract. Flavonoid content was expressed as catechin equivalents (CTE) per gram of dry matter.

3. Results

3.1. Anthelmintic activity

The MLC values of the plant extracts against *C. elegans* are presented in Table 2. In this study, we defined MLC values less than 1 mg/ml, between 1 and 4 mg/ml and above 4 mg/ml as high, moderate and low anthelmintic activity, respectively. A total of 16 organic solvent extracts exhibited high anthelmintic activity and the DCM extract of *H. colchicifolia* leaves had the most noteworthy MLC value (0.13 mg/ml). Besides the eight water extracts that exhibited moderate anthelmintic activity, other water extracts showed low or poor anthelmintic activity. Of the four extracting solvents, EtOH extracts yielded the highest number of plant extracts with high anthelmintic activity.

3.2. Antimicrobial activity

The MIC values of plant extracts against the test bacteria as well as the MIC and MFC values against *C. albicans* are presented in Table 3. Plant extracts with MIC or MFC values less than 1.0 mg/ml were considered as having good antimicrobial activity (Aligiannis et al., 2001). The most noteworthy MIC values of 0.39 mg/ml were displayed by the EtOH and PE extracts of *C. dregei* roots against *S.aureua* and *B. subtilis, and S. petersiana* leaves against three bacterial strains (*B. subtilis*, *S. aureus* and *K. pneumoniae*). Gram-positive bacteria were more susceptible than the Gram-negative ones and *S. aureus* was the most sensitive bacterium to the plant extracts. Although the antifungal activity of the extracts investigated was generally poor, the leaf extracts of *H. colchicifolia* (PE) and *O. basilicum* (water) exhibited noteworthy antifungal activity against *C. albicans*. Generally, the organic solvent extracts showed better antimicrobial activity than water extracts.

Table 2
Anthelmintic activity of plant extracts expressed as minimum lethal concentration (MLC) values (mg/ml) against *Caenorhabditis elegans*. Values in bold indicate high anthelmintic activity.

Plant species	Plant part	MLC (mg/ml)			
		PE	DCM	EtOH	Water
Acokanthera oppositifolia	Leaves	**0.52**	1.04	**0.52**	4.17
	Twigs	2.08	4.17	2.08	2.08
Clerodendrum myricoides	Leaves	2.08	1.04	**0.26**	1.04
	Stems	2.08	2.08	2.08	2.08
Cotyledon orbiculata var. *dactylopsis*	Leaves	**0.52**	**0.52**	**0.26**	>16.67
	Stems	4.17	4.17	4.17	>16.67
Cotyledon orbiculata var. *orbiculata*	Leaves	**0.26**	1.04	**0.26**	>16.67
	Stems	4.17	4.17	8.33	8.33
Cyathea dregei	Leaves	1.04	**0.52**	**0.52**	1.04
	Roots	8.33	4.17	2.08	4.17
Felicia erigeroides	Leaves	1.04	**0.52**	4.17	16.67
	Stems	4.17	2.08	4.17	>16.67
Hypoxis colchicifolia	Leaves	**0.52**	**0.13**	**0.26**	4.17
	Corms	4.17	2.08	2.08	2.08
Hypoxis hemerocallidea	Leaves	1.04	1.04	2.08	2.08
	Corms	2.08	4.17	2.08	1.04
Ocimum basilicum	Leaves	2.08	**0.26**	1.04	1.04
Senna petersiana	Leaves	1.04	1.04	**0.52**	8.33

Extracting solvent: PE = petroleum ether, DCM = dichloromethane, and EtOH = ethanol.
MLC of levamisole was 40 μg/ml.

Table 3
Antimicrobial activity (MIC) of plant extracts determined using the microtitre plate dilution technique method. Values in bold (MIC < 1 mg/ml) are considered noteworthy antimicrobial activity. Dry weight yield (%) from plant parts of investigated species.

Plant species	Plant part	Extract [a]	Extract yield (%)	Antibacterial activity MIC [b] (mg/ml)				Antifungal activity	
				Bacteria [c]				*Candida albicans*	
				B.s.	*S.a.*	*E.c.*	*K.p.*	MIC [b] (mg/ml)	MFC [d] (mg/ml)
Acokanthera oppositifolia	Leaves	PE	3.6	3.13	3.13	3.13	3.13	6.25	6.25
		DCM	5.4	3.13	3.13	3.13	1.56	3.13	6.25
		EtOH	11.8	6.25	3.13	3.13	1.56	6.25	6.25
		Water	22.7	12.50	6.25	6.25	6.25	12.50	>12.50
	Twigs	PE	5.9	6.25	6.25	3.13	3.13	6.25	6.25
		DCM	7.0	3.13	3.13	3.13	3.13	6.25	6.25
		EtOH	10.0	6.25	6.25	6.25	3.13	6.25	6.26
		Water	20.3	12.50	6.25	6.25	6.25	12.50	12.50
Clerodendrum myricoides	Leaves	PE	3.2	3.13	6.25	3.13	3.13	3.13	3.13
		DCM	3.5	1.56	3.13	3.13	**0.78**	3.13	3.13
		EtOH	4.4	1.56	1.56	3.13	**0.78**	3.13	6.25
		Water	30.3	>12.50	12.50	6.25	>12.50	>12.50	>12.50
	Stems	PE	0.4	3.13	1.56	3.13	3.13	3.13	3.13
		DCM	0.6	6.25	6.25	6.25	3.13	3.13	3.13
		EtOH	3.6	**0.78**	1.56	3.13	3.13	3.13	3.13
		Water	13.8	>12.50	1.56	6.25	>12.50	12.50	>12.50
Cotyledon orbiculata var. *dactylopsis*	Leaves	PE	2.6	3.13	3.13	3.13	3.13	6.25	6.25
		DCM	3.3	3.13	1.56	3.13	3.13	6.25	6.25
		EtOH	1.8	1.56	1.56	1.56	3.13	6.25	6.25
		Water	30.8	>12.50	>12.50	>12.50	>12.50	>12.50	>12.50
	Stems	PE	0.6	6.25	3.13	3.13	3.13	6.25	6.25
		DCM	1.0	6.25	3.13	3.13	3.13	3.13	6.25
		EtOH	0.8	6.25	3.13	3.13	3.13	6.25	6.25
		Water	17.5	>12.50	>12.50	>12.50	>12.50	>12.50	>12.50
Cotyledon orbiculata var. *orbiculata*	Leaves	PE	1.7	3.13	3.13	3.13	3.13	6.25	6.25
		DCM	2.1	3.13	1.56	3.13	3.13	6.25	6.25
		EtOH	1.5	1.56	1.56	1.56	3.13	6.25	6.25
		Water	33.8	>12.50	>12.50	>12.50	>12.50	>12.50	>12.50
	Stems	PE	0.5	6.25	3.13	3.13	3.13	6.25	6.25
		DCM	0.7	6.25	3.13	3.13	3.13	3.13	6.25
		EtOH	3.0	6.25	3.13	3.13	3.13	6.25	6.25
		Water	15.6	>12.50	>12.50	>12.50	>12.50	>12.50	>12.50
Cyathea dregei	Leaves	PE	4.0	6.25	3.13	6.25	6.25	6.25	6.25
		DCM	4.4	3.13	3.13	3.13	3.13	3.13	6.25
		EtOH	6.4	3.13	3.13	3.13	3.13	6.25	6.25
		Water	6.8	12.50	3.13	3.13	6.25	6.25	>12.50
	Roots	PE	0.2	**0.39**	**0.78**	3.13	3.13	3.13	6.25
		DCM	0.3	1.56	1.56	3.13	3.13	1.56	3.13
		EtOH	1.0	1.56	**0.39**	3.13	3.13	6.25	6.25
		Water	2.3	3.13	3.13	6.25	6.25	12.50	>12.50
Felicia erigeroides	Leaves	PE	1.5	1.56	3.13	3.13	3.13	6.25	6.25
		DCM	4.5	1.56	1.56	1.56	1.56	6.25	6.25
		EtOH	6.4	**0.78**	**0.78**	3.13	3.13	12.50	>12.50
		Water	20.2	>12.50	12.50	>12.50	>12.50	1.56	3.13
	Stems	PE	0.3	**0.78**	**0.78**	3.13	3.13	6.25	6.25
		DCM	0.6	**0.78**	1.56	1.56	1.56	6.25	6.25
		EtOH	1.2	1.56	1.56	3.13	3.13	12.50	>12.50
		Water	10.2	>12.50	12.50	12.50	12.50	12.50	>12.50
Hypoxis colchicifolia	Leaves	PE	2.4	**0.78**	**0.78**	3.13	3.13	**0.78**	**0.78**
		DCM	3.0	**0.78**	**0.78**	1.56	1.56	1.56	6.25
		EtOH	5.6	**0.78**	**0.78**	1.56	**0.78**	6.25	12.50
		Water	7.1	>12.50	12.50	6.25	12.50	12.50	>12.50
	Corms	PE	0.3	6.25	6.25	6.25	6.25	6.25	>12.50
		DCM	0.5	6.25	3.13	6.25	3.13	6.25	6.25
		EtOH	10.6	3.13	3.13	6.25	3.13	6.25	6.25
		Water	24.4	1.56	3.13	6.25	3.13	12.50	>12.50
Hypoxis hemerocallidea	Leaves	PE	0.8	1.56	1.56	3.13	3.13	6.25	12.50
		DCM	1.1	**0.78**	**0.78**	1.56	3.13	1.56	6.25
		EtOH	2.0	3.13	1.56	3.13	1.56	1.56	3.13
Hypoxis hemerocallidea	Leaves	Water	6.2	>12.50	3.13	3.13	3.13	>12.50	>12.50

Table 3 (*continued*)

Plant species	Plant part	Extract [a]	Extract yield (%)	Antibacterial activity MIC [b] (mg/ml)				Antifungal activity	
				Bacteria [c]				*Candida albicans*	
				B.s.	*S.a.*	*E.c.*	*K.p.*	MIC [b] (mg/ml)	MFC [d] (mg/ml)
	Corms	PE	0.2	3.13	3.13	6.25	3.13	6.25	12.50
		DCM	0.4	3.13	1.56	6.25	3.13	6.25	12.50
		EtOH	33.4	3.13	3.13	6.25	6.25	6.25	6.25
		Water	35.0	>12.50	3.13	3.13	3.13	>12.50	>12.50
Ocimum basilicum	Leaves	PE	3.2	1.56	**0.78**	3.13	3.13	3.13	6.25
		DCM	11.4	1.56	**0.78**	1.56	**0.78**	3.13	6.25
		EtOH	7.8	3.13	1.56	**0.78**	**0.78**	3.13	6.25
		Water	12.0	>12.50	3.13	>12.50	3.13	**0.78**	1.56
Senna petersiana	Leaves	PE	3.5	3.13	3.13	3.13	3.13	3.13	3.13
		DCM	3.4	3.13	**0.78**	3.13	1.56	1.56	3.13
		EtOH	8.4	**0.39**	**0.39**	**0.78**	**0.39**	1.56	1.56
		Water	35.2	3.13	3.13	6.25	6.25	>12.50	>12.50
Neomycin (μg/ml)	–	–		1.56	1.56	0.39	0.78	–	–
Amphotericin B (μg/ml)	–	–		–	–	–	–	0.15	9.80

– = not tested.
[a] Extract: PE = petroleum ether, DCM = dichloromethane, and EtOH = ethanol.
[b] MIC = minimum inhibitory concentration.
[c] Bacteria: *B.s.* = *Bacillus subtilis*, *S.a.* = *Staphylococcus aureus*, *E.c.* = *Escherichia coli* and *K.p.* = *Klebsiella pneumoniae*.
[d] MFC = minimum fungicidal concentration.

3.3. COX-1 and -2 inhibitions

The COX-1 and -2 inhibitions of the plant extracts are presented in Table 4. Based on the scheme devised by Tunón et al. (1995), four levels of inhibitory activity were defined. Percentage activity of above 70% was regarded as 'high' inhibition, from 40 to 70% indicates 'moderate' inhibition, 20 to 40% was regarded as 'low' inhibition, below 20% was considered 'insignificant' inhibition at the extract concentration tested.

The highest COX-1 inhibition was exhibited by the DCM extract of *A. oppositifolia* leaves with 99.98% inhibition, DCM extracts of *F. erigeroides* and *H. hemerocallidea* also had a comparably high COX-1 inhibition. In COX-2 bioassay, the most active extract was DCM extract of *F. erigeroides* stems with 99% inhibition. High COX-2 inhibition was exhibited by 10 PE and DCM extracts while only six EtOH extracts and one water extract displayed similar high COX-2 inhibition. In general, non-polar solvent extracts showed higher COX

Table 4
COX-1 and COX-2 inhibitions (%) of the plant extracts in the COX bioassay which was tested at a final concentration of 250 μg/ml and 2 mg/ml for the organic solvent and water extracts, respectively. Values are presented as mean±standard error, where $n=4$.

Plant species	Plant part	COX-1 inhibition (%)				COX-2 inhibition (%)			
		PE	DCM	EtOH	Water	PE	DCM	EtOH	Water
Acokanthera oppositifolia	Leaves	98.2±4.6	99.8±1.8	97.8±2.9	48.1±0.7	84.7±0.4	81.0±0.3	68.1±2.4	0.0
	Twigs	0.0	31.5±2.7	0.0	37.7±1.3	38.5±3.4	72.6±1.1	29.6±3.1	16.9±0.7
Clerodendrum myricoides	Leaves	51.5±2.7	76.7±2.1	0.0	0.0	6.8±1.1	32. 8±2.4	42.5±0.8	0.0
	Stems	68.9±0.8	84.4±2.2	10.4±2.7	16.3±0.9	74.5±0.7	70.9±0.6	54.2±1.4	0.0
Cotyledon orbiculata var. *dactylosis*	Leaves	70.1±4.0	98.0±1.5	98.0±1.3	27.3±1.6	58.8±2.8	72.6±4.0	73.1±3.8	10. 5±4.3
	Stems	68.5±2.5	58.9±1.1	26.5±2.7	44.3±0.9	53.0±0.9	58.5±2.1	32.4±5.2	29.2±1.9
Cotyledon orbiculata var. *orbiculata*	Leaves	97.6±3.4	93.3±3.5	67.4 ±4.0	15.1±0.7	85.2 ±2.3	80. 8±0.7	66. 8±1.2	0.0
	Stems	0.0	62.6±5.0	97.1±4.2	42.1±0.8	32.5±3.2	46.7±3.1	71.8±2.0	25.1±1.0
Cyathea dregei	Leaves	98.0±0.5	73.8±2.9	99.2±0.8	68.0±1.0	55.9±1.1	61.1±1.4	71.7±1.0	55.1±0.7
	Roots	91.8±2.7	96.1±0.9	77.9±0. 2	84.2±1.2	70.1±4.3	74.3±1.7	48. 6±3.5	70.2±2.4
Felicia erigeroides	Leaves	93.6±1.8	99.4±1.1	87.6±3.2	61.1±2.1	85.4±1.5	87.3±1.7	69.4±1.7	0.5±0.1
	Stems	88.0±1.7	71.6±2.3	36.1±4.8	33.0±1.3	98.3±2.7	99.8±2.1	78.7±1.9	3.4±1.0
Hypoxis colchicifolia	Leaves	99.3±0.9	35.4±2.5	98.4±1.2	68.1±1.1	90.1±2.0	49.9±1.1	83.5±0.1	51.5±3.8
	Corms	93.6±1.8	96.4±1.2	0.0	63.4±1.2	81.0±2.6	76.2±3.8	0.0	7. 9±0.6
Hypoxis hemerocallidea	Leaves	60.7±1.3	78.4±2.9	0.0	59.6±1.2	37.4±2.1	55.2±1.3	19.9±0.3	2.7±0.9
	Corms	97.1±3.0	98.4±1.3	0.0	83.0±1.3	74.2±2.5	69.1±1.3	0.0	25.7±1.2
Ocimum basilicum	Leaves	62.0±5.9	76.7±2.2	94.1±2.4	83.6±0.6	94.4±0.8	67.1±2.3	85.7±2.4	30.2±1.0
Senna petersiana	Leaves	77.6±1.2	85.5±1.9	99.3±1.2	92.5±0.4	65.2±2.3	77.7±0.1	47.8±3.8	56.8±1.6

Extracting solvents: PE = petroleum ether, DCM = dichloromethane and EtOH = ethanol. Inhibition (%) by indomethacin for COX-1 at final bioassay concentration of 5 μM was 75.50±2.34 and COX-2 at final bioassay concentration of 200 μM was 69.10±2.44.

inhibition than the polar solvent extracts. For instance, each of the extracting solvents (PE, DCM, EtOH and water) yielded 18 extracts out of which a total of 14 DCM and 11 PE extracts compared to nine EtOH and four water extracts exhibited high COX-1 inhibition. The inhibitory activity against COX-2 enzyme was lower than COX-1.

3.4. Phenolic composition

The total phenolics, gallotannin, condensed tannin and flavonoid contents in the 50% MeOH extracts are depicted in Fig. 1A, B, C and D, respectively. Besides the *C. orbiculata* var. *dactylopsis* leaf and *F. erigeroides* stem extract that lacked condensed tannin, the other extracts contained various phenolics at varying concentrations. This disparity was especially evident between the different plant parts, as well as among the plant species investigated. The extract with the highest amount (56.70 mg GAE/g dry matter) of total phenolics was *O. basilium* leaves while the lowest quantity (1.70 mg GAE/g dry matter) was found in *C. orbiculata* var. *dactylopsis* (leaves). The gallotannin concentration in the 50% MeOH extracts ranged from 45.36 µg GAE/g dry matter determined in *O. basilicum* leaves to 1.36 µg GAE/g dry matter in *C. orbiculata* var. *dactylopsis* leaves. The condensed tannin content in the 50% MeOH extracts of plant material ranged from 0.009% LCE/g dry matter in *C. orbiculata* var. *orbiculata* leaves to 0.48% LCE/g dry matter in *C. myricoides* leaves. The corm extract of *H. hemerocallidea* had the highest flavonoid concentration of 0.462 µg CTE/g dry matter while the lowest flavonoid concentration of 0.008 µg CTE/g dry matter was observed in the leaf extract of *C. orbiculata* var. *orbiculata*.

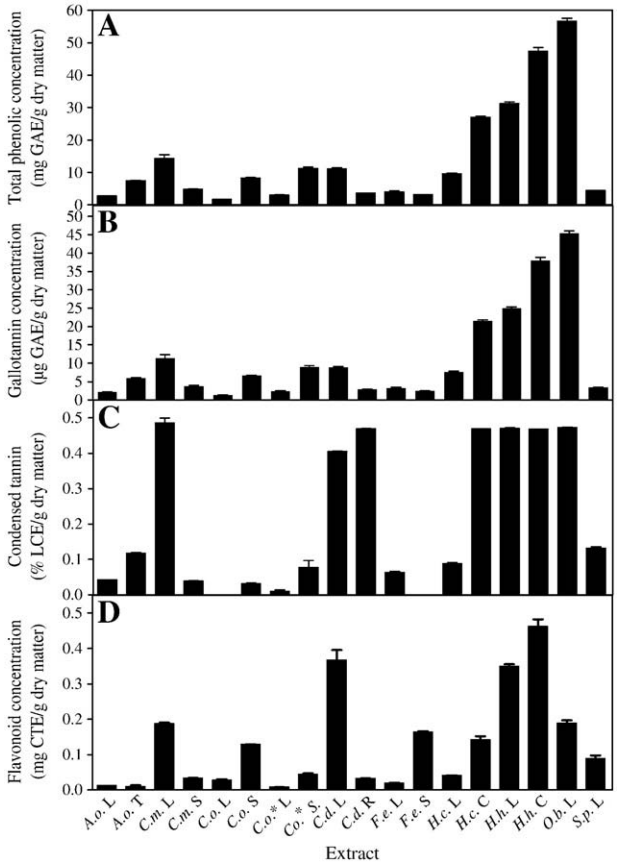

Fig. 1. Phenolic content of South African medicinal plants used as anthelmintics. (A) Total phenolic content; (B) gallotannin content; (C) condensed tannin content; (D) flavonoid content. *A.o.* L — *Acokanthera oppositifolia* (leaves), *A.o.* T — *A. oppositifolia* (twigs), *C.m.* L — *Clerodendrum myricoides* (leaves), *C.m.* S — *C. myricoides* (stems), *C.o.* L — *Cotyledon orbiculata* var. *dactylopsis* (leaves), *C.o.* S — *C. orbiculata* var. *dactylopsis* (stems), *C.o.** L — *C. orbiculata* var. *orbiculata* (leaves), *C.o.** S — *C. orbiculata* var. *orbiculata* (stems), *C.d.* L — *Cyathea dregei* (leaves), *C.d.* R — *C. dregei* (roots), *F.e.* L — *Felicia erigeroides* (leaves), *F.e.* S — *F. erigeroides* (stems), *H.c.* L — *Hypoxis colchicifolia* (leaves), *H.c.* C — *H. colchicifolia* (corms), *H.h.* L — *H. hemerocallidea* (leaves), *H.h.* C — *H. hemerocallidea* (corms), *O.b.* L — *Ocimum basilicum* (leaves), *S.p.* L — *Senna petersiana* (leaves).

4. Discussion and conclusions

Apart from *H. hemerocallidea*, all the plant species had at least one extract from the screened parts showing high anthelmintic activity. The potential anthelmintic activity shown by these plant extracts confirms the importance of using an ethnopharmacological approach in screening plants as a source of novel pharmaceuticals. This approach provides a valuable short-cut by taking advantage of information available from traditional healers and ethnopharmacological records (Elgorashi and Van Staden, 2004). The extracts of *C. dregei* leaves exhibited better anthelmintic activity than the root extracts, although traditional healers are known to use the dried roots as an anthelmintic (Hutchings et al., 1996). The destructive harvesting of the roots for medicinal uses is probably responsible for this plant being listed as a threatened species in southern Africa (Talukdar, 2002). This study has shown that the leaves could be substituted for the roots as anthelmintic medication by traditional healers. However, further investigation will be necessary to support this plant part substitution.

Although it is ideal to test plant extracts against specific target microorganisms, representative microbes were used in this preliminary antimicrobial screening to avoid handling numerous pathogenic microorganisms. Activity against these selected microorganisms will give an indication of possible similar action against more pathogenic strains (Taylor et al., 2001). The most susceptible bacterium was *S. aureus* and the most resistant *E. coli*. The presence of unique lipopolysaccharides on the outer membrane of Gram-negative bacteria makes them impermeable to most antibacterial compounds (Clements et al., 2002). This can explain the low number of active plant extracts against *E. coli* and *K. pneumoniae*. The observed trend is in agreement with previous research findings by various workers (Lin et al., 1999; Tshikalange et al., 2005). *C. albicans* was not sensitive to the majority of extracts screened. Pathogenic fungi are more resistant to most therapies used against other infections such as bacteria (Sleigh and Timburg, 1998). Antifungal activity screening of plant extracts by other workers also showed that pathogenic fungi are resistant to most plant extracts (Buwa and Van Staden, 2006; Fawole et al., 2009).

Although a high number of extracts inhibited both the COX-1 and -2 enzymes, many extracts showed better COX-1 inhibition

than COX-2 which could be attributed to the COX-1 active site being smaller than that of COX-2 (Botting, 2006). The high to moderate inhibitory activity exhibited by most leaf extracts against COX-2 inhibition is important for conservation of medicinal plant species. For example, extracts from the leaves of plant species such as *H. colchicifolia* and *C. dregei* exhibited better COX-2 inhibition activity than the underground parts. Leaves can be harvested sustainably while utilizing these plants as medicine without the inherent survival threat associated with destructive harvesting of underground parts such as corms and roots. The observed high COX-2 inhibitory activity of the leaf extracts was in line with the suggestion for the need to investigate non-destructive medicinal plant parts for possible noteworthy similar pharmacological activity as present in the underground parts (Zschocke et al., 2000).

In the three bioassays used in this study, water extracts generally exhibited poor pharmacological activities. Although traditional healers often use water or alcohol for extracting medicinal plants, studies have shown that the use of water for plant material extraction naturally limits the type and amount of compounds extracted (Eloff, 1998b). This probably indicates that dosage is important with regard to which solvent is being used. A number of extracts exhibited poor pharmacological activity, especially antimicrobial activity. The absence of significant activity could be due to factors such as dilution of active principle(s) and antagonistic effects by other compounds against the active principle(s) and not necessarily the inactivity of the plant extracts (Taylor et al., 2001). Generally, extracts from plants such as *C. dregei*, *F. erigeroides* and *H. colchicifolia* showed noteworthy pharmacological activity in the three bioassays, supporting the recommendation by Houghton et al. (2005) for the need to investigate plant extracts in multiple *in vitro* tests associated with the disease state. In this study, many of the extracts inhibited both COX-1 and -2. This denotes the potential of these extracts to reduce inflammation. They are possibly used in TM to reduce pain which is associated with helminth infections. Besides, TM lacks scientific investigations to determine the cause of diseases and common symptoms that are observed provide a clue on plant material used and other treatment regimes (Hewson, 1998).

The phenolics as well as gallotannin, condensed tannin and flavonoid content, varied in the plant extracts investigated. Plants with high phenolic content have been popularly used as anthelmintics, antimicrobials (Polya, 2003) and anti-inflammatories (Erdèlyi et al., 2005). The results obtained from the investigation of phenolic content provide preliminary information on some important groups of phenolics that are present in the plants investigated. This also suggests the types of bioactive compounds that may be responsible for the biological activities exhibited by the plant extracts.

Although remarkable biological activity identified from these *in vitro* tests does not necessarily confirm that the active plant extracts are effective medicines, nor suitable candidates for drug development, it does provide basic knowledge of the efficacy of the plant material. Preliminary *in vitro* screening techniques are a crucial and important step in the long and expensive process of drug discovery and development from plants. Further investigation of these extracts against more pathogenic strains of test organisms as well as the use of *in vivo* test systems will also be necessary to further ascertain their pharmacological effectiveness. Studies on the toxicology of active extracts as well as isolation of active compounds from the extracts of plants such as *C. dregei*, and *F. erigeroides* are ongoing in our laboratories.

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