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Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants

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Abstract

Hexane, ethanol and water extracts of plants used by South African traditional healers for treating stomach ailments were screened for antibacterial, anthelmintic and anti-amoebic activities. To evaluate antibacterial activity, the disc-diffusion assay was used against several Gram-positive and Gram-negative species. Minimal inhibitory concentration values were determined with a microdilution assay. Ethanolic extracts showed the greatest activity, and Gram-positive bacteria were the most susceptible microorganisms. The free-living nematode *Caenorhabditis elegans* was used in two different assays to evaluate anthelmintic activity. A microdilution technique was employed to investigate anti-amoebic activity against the enteropathogenic *Entamoeba histolytica*. These assays were suitable for the screening of a large number of extracts at one time. Several plants exhibited significant activity against these test organisms.

Keywords: Antibacterial; Anthelmintic; Anti-amoebic; South Africa; Traditional medicine

1. Introduction

Traditional South African medicine makes use of a wide variety of plants to treat gastrointestinal disorders such as diarrhoea and intestinal parasites, which are particularly prevalent in rural areas of the country. Developed and developing countries show a great interest in indigenous medicine, and many developing countries use traditional medicines at the primary health care level. Many currently used drugs are expensive or not readily available, and a major setback to their continued usage is the development of resistance. There is thus an urgent need for new, inexpensive drugs that will be able to act for longer periods before resistance sets in.

There are numerous causes of diarrhoea. Acute diarrhoea results from bacterial or viral enteritis, food and toxin poisoning, chemical poisoning and gastrointestinal allergy. Chronic diarrhoea may be produced by parasite infestations among other causes (Lewis and Elvin-Lewis, 1977). They are rarely associated with mortality, but they cause significant morbidity such as impaired physical and mental development (Taylor et al., 1995).

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The purpose of this study was to investigate South African plants for potential antibacterial, anthelmintic or anti-amoebic activity by preliminary bioassay screening. Plants used to treat stomach ailments such as diarrhoea, dysentery or intestinal worm infestations were selected and submitted to bioassays according to their traditional uses. In total, 138 extracts were tested for antibacterial activity, 72 for anthelmintic activity, and 42 for anti-amoebic activity. Antibacterial activity was evaluated by using the disc-diffusion assay, and minimal inhibitory concentration (MIC) values were determined using a microdilution assay. The extracts were tested against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, and the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*, since *Bacillus* species and *S. aureus* strains may cause diarrhoea and an enteropathogenic form of *E. coli*, and *Klebsiella* species may cause food poisoning.

Extracts were screened for anthelmintic activity against the free-living nematode *Caenorhabditis elegans*. The effect of the plant extracts on the mortality and reproductive ability of the nematodes was evaluated. Anti-amoebic activity was investigated using a microdilution technique with the enteropathogenic *Entamoeba histolytica*.

2. Materials and methods

2.1. Plant collection

A sampling of South African ethnobotanical literature (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Van Wyk et al., 1997) was surveyed to compile a representative list of those plants used medicinally by traditional healers of the Zulu, Xhosa and Sotho. From this list, plants were collected, predominantly from the KwaZulu-Natal region of South Africa from May to July 1998. The traditional usage and preparation of these plants is recorded in Table 1. Voucher specimens (Table 1) for each plant were deposited at the herbarium of the University of Natal, Pietermaritzburg.

2.2. Plant extract preparation

The plant material for use in the screening procedures was dried in a 50°C oven and stored at room temperature until extraction. Dried plant material was ground to a powder. Two separate samples of 1 g were extracted with 10 ml water and ethanol, respectively, and 4 g samples of plant material were extracted with 40 ml hexane. Extraction was performed by sonication for 30 min in a Julabo ultrasound bath. The plant extracts were filtered through Whatman No. 1 filter paper into pill vials. The filtrates were air-dried and the residues stored at -15°C.

2.3. Antibacterial screening

The test organisms in the investigations of antibacterial activity, *B. subtilis*, *E. coli*, *K. pneumoniae* and *S. aureus*, were obtained from the bacterial collection of the Department of Microbiology, University of Natal, Pietermaritzburg. The number of cells in Mueller–Hinton (MH) broth cultures of each bacterial species was estimated using a serial dilution technique (Lech and Brent, 1987). Tenfold serial dilutions of overnight MH broth cultures were prepared, and 100 μl of each dilution were spread onto MH agar plates using a glass spreader. The plates were incubated overnight at 37°C and colonies were counted using an Anderman Colony Counter. Following the assumption that each living bacterial cell will grow into a separate colony on the plate, the number of cells present per millilitre of the original overnight cultures was calculated. The optical density (OD) at 600 nm for each dilution was determined using a Varian Cary 50 Spectrophotometer, and used to indicate numbers of bacterial cells in cultures for the antibacterial screening and MIC determination.

The disc-diffusion assay (Rasoanaivo and Ratsimamanga-Urverg, 1993) was used in the antibacterial screening procedure. Residues of plant extracts were resuspended in their extracting solvents at a concentration of 100 mg ml^{-1}. MH agar (Biolab) base plates were prepared using sterile 90 mm Petri dishes. MH agar at 48°C was inoculated with a MH broth culture (10^6–10^8

Table 1
South African medicinal plants investigated for anthelmintic, anti-amoebic and antibacterial activity

Family and botanical name (voucher specimen[a])	Plant part[b] used in traditional medicine	Traditional uses and administration
ALLIACEAE (LILIACEAE)		
Tulbaghia violacea Harv. (*McGaw*50NU)	TB	Decoctions as enemas for stomach ailments (Hulme, 1954). Tubers as anthelmintics (Watt and Breyer-Brandwijk, 1962)
ANACARDIACEAE		
Harpephyllum caffrum Bernh. ex Krauss (*McGaw*38NU)	BK	Decoctions as emetics (Pujol, 1990)
Sclerocarya birrea (A. Rich.) Hochst. (*McGaw*44NU)	BK	Decoctions as enemas for diarrhoea (Gerstner, 1938; Pujol, 1990) and dysentery (Watt and Breyer-Brandwijk, 1962)
APIACEAE (UMBELLIFERAE)		
Heteromorpha trifoliata (Wendl.) Eckl. & Zeyh. (*Zschocke*2NU)	LF	Infusions as enemas for abdominal disorders; decoctions for intestinal worms in children. (Bryant, 1966; Watt and Breyer-Brandwijk, 1962)
	RT	Dysentery (Watt and Breyer-Brandwijk, 1962)
Pimpinella caffra (Eckl. & Zeyh.) D. Dietr. (*McGaw*67NU)	WH	Intestinal worms (Watt and Breyer-Brandwijk, 1962)
APOCYNACEAE		
Acokanthera oblongifolia (Hochst.) Codd (*McGaw*49NU)	WH	Anthelmintic (Hutchings et al., 1996)
Rauvolfia caffra Sond. (*McGaw*42NU)	LX	Emetic for abdominal complaints (Watt and Breyer-Brandwijk, 1962)
ARACEAE		
Acorus calamus L. (*McGaw*47NU)	RH	Carminatives, stomachics, for dysentery (Watt and Breyer-Brandwijk, 1962) and diarrhoea (Van Wyk et al., 1997)
ARALIACEAE		
Cussonia spicata Thunb. (*McGaw*56NU)	FR/ST/RT	Nausea (Watt and Breyer-Brandwijk, 1962)
ASCLEPIADACEAE		
Asclepias fruticosa L. (*McGaw*36NU)	LF	Infusions for diarrhoea and stomach pain in children (Hulme, 1954; Watt and Breyer-Brandwijk, 1962)
	RT	Decoctions for stomach ailments (Mabogo, 1990)
ASPHODELACEAE (LILIACEAE)		
Aloe arborescens Mill. (*McGaw*48NU)	LF	Infusions for stomach ache (Hutchings and Johnson, 1986)
Aloe marlothii Berger (*McGaw*62NU)	LF/RT	Decoctions administered orally or as enemas against roundworms and for stomach ailments (Watt and Breyer-Brandwijk, 1962)
Bulbine latifolia (L. f.) Roem. & Schult. (*McGaw*73NU)	TB	Decoctions for dysentery and diarrhoea (Pujol, 1990)
ASTERACEAE		
Artemisia afra Jacq. ex Willd. (*McGaw*30NU)	WH	Enemas for constipation and intestinal worms (Roberts, 1990). Plants widely used in southern Africa as anthelmintics and emetics (Hutchings et al., 1996)
Bidens pilosa L. (*McGaw*74NU)	LF/RT	Infusions as enemas for stomach complaints (Bryant, 1966)

Table 1 (*Continued*)

Family and botanical name (voucher specimen[a])	Plant part[b] used in traditional medicine	Traditional uses and administration
	FL	Diarrhoea (Hutchings et al., 1996)
Brachylaena discolor DC. (*McGaw*84NU)	LF	Infusions as purgatives against intestinal parasites (Bryant, 1966; Mabogo, 1990)
Tarchonanthus camphoratus L. (*McGaw*55NU)	LF	Infusions for abdominal pain (Hutchings et al., 1996)
BIGNONIACEAE		
Kigelia africana (Lam.) Benth. (*McGaw*57NU)	AP	Dysentery (Hutchings et al., 1996)
	RT	Constipation and tapeworm (Hutchings et al., 1996)
	FR/BK	Decoctions as enemas to children with stomach ailments (Hutchings et al., 1996)
Tecomaria capensis (Thunb.) Spach (*McGaw*52NU)	BK	Infusions for diarrhoea, dysentery and stomach pains (Roberts, 1990)
CAESALPINACEAE		
Senna didymobotrya (Fresn.) Irwin + Barneby (*McGaw*64NU)	LF	*Senna* spp. are used pharmaceutically in laxative preparations (Hutchings et al., 1996)
CELASTRACEAE		
Cassine transvaalensis (Burtt Davy) Codd (*McGaw*70NU)	BK	Infusions as emetics or enemas for stomach ache (Gerstner, 1939). Decoctions for diarrhoea and intestinal cramps (Pujol, 1990) and as an anthelmintic (Mabogo, 1990)
	RT	Diarrhoea (Hutchings et al., 1996)
Catha edulis (Vahl) Forssk. Ex Endl. (*McGaw*63NU)	RT	Stomach ailments (Hutchings et al., 1996)
COMBRETACEAE		
Combretum apiculatum Sond. subsp. *apiculatum* (*McGaw*78NU)	LF	Decoctions as steam or as enemas for abdominal disorders (Watt and Breyer-Brandwijk, 1962)
CORNACEAE		
Curtisia dentata (Burm. f.) C.A. Sm. (*McGaw*53NU)	BK	Stomach ailments including diarrhoea (Pujol, 1990)
DRACAENACEAE (LILIACEAE)		
Sansevieria hyacinthoides (L.) Druce (*McGaw*51NU)	LF	Intestinal worms (Watt and Breyer-Brandwijk, 1962), stomach disorders and diarrhoea (Pujol, 1990)
EBENACEAE		
Euclea divinorum Hiern (*McGaw*60NU)	BK/RT	Anthelmintics, tonics and purgatives (Kokwaro, 1976)
EUPHORBIACEAE		
Clutia pulchella L. (*McGaw*61NU)	LF	Infusions for stomach ache (Bryant, 1966), diarrhoea and dysentery (Hutchings et al., 1996)
Croton sylvaticus Hochst. (*Zschocke*1NU)	BK	Abdominal disorders (Bryant, 1966)
Ricinus communis L. (*McGaw*28NU)	LF	Infusions administered orally or as enemas for stomach ache (Gerstner, 1939)
	RT	Chewed as anthelmintics, decoctions for abdominal complaints (Kokwaro, 1976)
	AP	Stomach ache and diarrhoea (Kokwaro, 1976)
Spirostachys africana Sond. (*McGaw*45NU)	RT/ST	Diarrhoea, dysentery and stomach pains (Mabogo, 1990)
FABACEAE		
Albizia adianthifolia (Schumach.) W.F. Wight (*McGaw*34NU)	WH	Stomach ailments (Gerstner, 1939)
	LF/RT	Stomach ache and dysentery (Mabogo, 1990)

Table 1 (*Continued*)

Family and botanical name (voucher specimen[a])	Plant part[b] used in traditional medicine	Traditional uses and administration
Erythrophleum lasianthum Corbishley (*Zschocke*5NU)	BK	Abdominal pains (Watt and Breyer-Brandwijk, 1962) and anthelmintic (Palmer and Pitman, 1972)
Schotia brachypetala Sond. (*McGaw*58NU)	RT	Dysentery and diarrhoea (Bryant, 1966)
GUNNERACEAE		
Gunnera perpensa L. (*McGaw*39NU)	RT	Decoctions for stomach ailments (Watt and Breyer-Brandwijk, 1962)
ICACINACEAE		
Apodytes dimidiata E. Mey. ex Arn. (*McGaw*32NU)	RT BK	Decoctions as enemas for intestinal parasites (Bryant, 1966)
IRIDACEAE		
Crocosmia paniculata (Klatt) Goldbl. (*McGaw*83NU)	CM	Decoctions for dysentery and diarrhoea, followed by enemas of the same decoctions (Gerstner, 1941; Watt and Breyer-Brandwijk, 1962)
LAMIACEAE		
Leonotis leonurus (L.) R. Br. (*McGaw*35NU)	AP	Infusions for dysentery (Gerstner, 1941; Watt and Breyer-Brandwijk, 1962)
	LF/FL	Tapeworm (Hutchings et al., 1996)
Tetradenia riparia (Hochst.) Codd (*McGaw*31NU)	LF	Diarrhoea (Watt and Breyer-Brandwijk, 1962)
LAURACEAE		
Cinnamomum camphora (L.) J. Presl. (*McGaw*65NU)	LF	Antibacterial and to treat diarrhoea (Watt and Breyer-Brandwijk, 1962)
Ocotea bullata (Burch.) Baill. (*Jäger*16NU)	BK	Infusion for infantile diarrhoea (Van Wyk et al., 1997) and stomach trouble (Pujol, 1990)
LOGANIACEAE		
Buddleja salviifolia (L.) Lam. (*McGaw*43NU)	RT	Decoctions for stomach upsets and diarrhoea (Roberts, 1990)
Strychnos spinosa Lam. (*McGaw*79NU)	WH	Dysentery (Watt and Breyer-Brandwijk, 1962)
MELIACEAE		
Ekebergia capensis Sparrm. (*McGaw*69NU)	LF	Infusion as purgative parasiticide (Hutchings et al., 1996)
	RT	Dysentery (Pooley, 1993)
Melia azedarach L. (*McGaw*77NU)	LF	Infusions for abdominal pains and anthelmintics (Hutchings et al., 1996)
MYRSINACEAE		
Maesa lanceolata Forssk. (*McGaw*75NU)	FR/SD	Anthelmintic (Watt and Breyer-Brandwijk, 1962)
OLEACEAE		
Olea europaea L. (*McGaw*59NU)	FR	Astringents against diarrhoea (Iwu, 1993)
PEDALIACEAE		
Ceratotheca triloba (Bernh.) Hook. f. (*McGaw*81NU)	LF	Infusions administered for diarrhoea and gastro-intestinal cramps (Watt and Breyer-Brandwijk, 1962; Roberts, 1990)
PERIPLOCACEAE		
Mondia whitei (Hook. f.) Skeels (*McGaw*82NU)	RT	Chewed for stomach ache (Bryant, 1966; Gerstner, 1941)
PITTOSPORACEAE		
Pittosporum viridiflorum Sims (*McGaw*37NU)	BK	Decoctions as emetics or enemas for stomach troubles (Watt and Breyer-Brandwijk, 1962). Roasted bark used for dysentery (Hutchings et al., 1996)

Table 1 (*Continued*)

Family and botanical name (voucher specimen[a])	Plant part[b] used in traditional medicine	Traditional uses and administration
POLYGONACEAE		
Rumex lanceolatus (*McGaw*29NU)	RT	Infusions for tapeworm (Watt and Breyer-Brandwijk, 1962)
RHAMNACEAE		
Ziziphus mucronata Willd. (*McGaw*80NU)	RT	Infusions for dysentery (Hutchings et al., 1996)
RUBIACEAE		
Canthium inerme (L. f.) Kuntze (*McGaw*68NU)	LF	Stomach complaints (Bryant, 1966); infusions in milk for dysentery and diarrhoea (Hutchings et al., 1996)
Psychotria capensis (Eckl.) Vatke (*McGaw*72NU)	RT	Infusions as emetics (Watt and Breyer-Brandwijk, 1962) and gastric complaints (Pooley, 1993)
RUTACEAE		
Clausena anisata (Willd.) Hook. f. ex. Benth. (*McGaw*66NU)	RT	Tapeworm remedy (Bryant, 1966)
	LF	Infusions as parasiticides and purgatives (Hutchings et al., 1996)
Zanthoxylum capense (Thunb.) Harv. (*McGaw*41NU)	LF	Ingredient in infusions used as purgative parasiticides and for stomach complaints (Hutchings et al., 1996)
SAPINDACEAE		
Deinbollia oblongifolia (E. Mey. ex Arn.) Radlk. (*McGaw*33NU)	RT	Dysentery and diarrhoea (Bryant, 1966)
STANGERIACEAE		
Stangeria eriopus (Kunze) Baill. (*Zschocke*7NU)	TB	Emetics and purgatives (Osborne and Grove, 1992; Watt and Breyer-Brandwijk, 1962)
STERCULIACEAE		
Dombeya rotundifolia (Hochst.) Planch. (*McGaw*54NU)	WH	Decoctions for diarrhoea and intestinal upsets (Hutchings et al., 1996)
TYPHACEAE		
Typha capensis (Rohrb.) N.E. Br. (*McGaw*46NU)	RH	Decoctions for dysentery and diarrhoea (Hutchings et al., 1996)
ULMACEAE		
Trema orientalis (L.) Blume (*McGaw*40NU)	BK	Anthelmintic against hookworm and roundworm (Hutchings et al., 1996)
	WD	Dysentery (Hutchings et al., 1996)
VERBENACEAE		
Clerodendrum glabrum E. Mey. (*McGaw*71NU)	LF	Ingredients in infusions against intestinal parasites (Hutchings et al., 1996)
Lippia javanica (Burm. f.) Spreng. (*McGaw*76NU)	LF	Infusions as anthelmintics and prophylactics against dysentery and diarrhoea (Mabogo, 1990)

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

[b] Plant part: AP, aerial parts; BK, bark; BL, bulb; LF, leaf; RT, root; RT BK, root bark; RH, rhizome; TB, tuber; CM, corm; WH, whole plant; ST, stem; FR, fruit; LX, latex; WD, wood.

bacteria ml^{-1}) of each bacterial species and poured over the base plates to form a homogeneous layer. Filter paper discs (Whatman No. 3, 6 mm diameter) were sterilized by autoclaving. One milligram of plant residue (10 μl of 100 mg ml^{-1} suspension) was applied to each filter paper disc and allowed to air-dry. The dry discs were placed on the seeded MH agar plates; extracts were tested in quadruplicate, with four discs of each extract on one plate. Neomycin (5 μg) as a positive control and hexane, ethanol and water as negative solvent controls were used. The plates

were incubated at 37°C overnight, after which the zones of inhibition around each disc were measured. The ratio between the diameter of the inhibition zones (mm) produced by plant extracts and the inhibition zone around the disc with neomycin (mm) was used to express antibacterial activity (Vlietinck et al., 1995).

The microplate method of Eloff (1998a) was used with slight modifications to determine the MIC values for plant extracts with antibacterial activity. Residues of the plant extracts that were active in the disc-diffusion assay were dissolved at 50 mg ml^{-1} with the extracting solvent in the case of ethanol and water. Hexane extracts were resuspended in acetone. All extracts were initially tested at 12.5 mg ml^{-1} in 96-well microtitre plates and serially diluted twofold to 0.38 μg ml^{-1}, after which 100 μl bacterial culture (approximately 10^6 bacteria ml^{-1}) were added to each well.

In an effort to determine the effect of culture age on MIC determination, Eloff (1998a) showed that *S. aureus* culture age from 1 to 6 h, and storing cultures up to 10 days in a cold room, had little or no effect on the MIC values. This contrasts with larger volume serial dilution assays where the number of cells inoculated has an effect on the MIC (Hewitt and Vincent, 1989; cited by Eloff, 1998a). Eloff (1998a) suggested that the 50% inoculum size used in the microdilution assay compared with the approximately 50 times lower inoculum used in the standard tube MIC method (Eloff, 1998b) may explain the difference.

The antibiotic neomycin was included as standard in each assay. Extract-free solution was used as blank control. The microplates were incubated overnight at 37°C. As an indicator of bacterial growth, 40 μl *p*-iodonitrotetrazolium violet (INT) (SIGMA) dissolved in water were added to the microplate wells and incubated at 37°C for 30 min. MIC values were recorded as the lowest concentration of extract that completely inhibited bacterial growth. Since the colourless tetrazolium salt is reduced to a red-coloured product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT.

2.4. Anthelmintic screening

C. elegans var. Bristol (N2) nematodes were cultured on nematode growth agar seeded with *E. coli* according to the method of Brenner (1974). The extracts of plants used by traditional healers for treating intestinal worm infestations were tested at concentrations of 1 and 2 mg ml^{-1}. Ethanol and water extracts were resuspended in their extracting solvents, while hexane extracts were resuspended in acetone or dimethylsulfoxide (DMSO).

Two types of anthelmintic assay were carried out. The first was a simple bioassay described by Rasoanaivo and Ratsimamanga-Urverg (1993) where nematode mortality after addition of nematocidal compound or plant extract was assessed. In this assay, 500–1000 nematodes (7–10-day-old cultures) in M9 buffer (Brenner, 1974) were incubated with 1 and 2 mg ml^{-1} plant extract for 2 h at 25°C in the dark. The assay was first standardized using the anthelmintic drug levamisole in place of plant extract. A standard concentration of 5 μg ml^{-1} levamisole was used as a control in all subsequent experiments. A second control consisted of nematodes incubated with no plant extract or levamisole. Using a dissecting microscope, the percentage of living worms was estimated, and their movement recorded and compared with the controls.

The second anthelmintic assay was performed following the protocol of Simpkin and Coles (1981) which is useful to determine the effect of plant extracts on reproductive ability of nematodes. Nematodes for the test were washed from 4–8-day-old cultures with M9 buffer and held in M9 supplemented with 1 μg ml^{-1} chlorhexidine digluconate for 1 h. This treatment does not harm the worms and helps to avoid contaminating bacteria overgrowing the test medium. The test medium was made by adding 10 ml of 3–5-day-old *E. coli* (grown at 20°C) to 100 ml M9 buffer along with 5 mg ampicillin and 10 000 U nystatin. The ampicillin inhibits the *E. coli* so that multiplication is reduced and the bacteria are less likely to metabolize the added compounds. After 2 h at room temperature, 2 ml test medium was placed in each well of a sterile repli-dish (Sterilin) together with 10 μl extract or levamisole solution. A drop

of the worm suspension containing about 20 nematodes was added to the wells after a few minutes so the worms did not drop into a region of high concentration of extract or levamisole. Control wells containing test medium, nematodes and water, ethanol, DMSO or acetone as well as blank wells containing test medium only were included in each repli-dish. In a second control, 5 μg ml^{-1} levamisole was added in place of plant extract. Each concentration of plant extract or levamisole was tested in duplicate. The plates were incubated in the dark at 20°C for 7 days. After incubation, the number and movement of nematodes in each well compared with the controls was assessed using a dissecting microscope.

2.5. Anti-amoebic screening

A rapid and sensitive procedure for evaluating the in vitro activity of plant extracts against the enteropathogenic amoeba *E. histolytica* has been developed by Wright et al. (1988). This test was validated with the standard amoebicidal drug metronidazole. Amoebae were cultured at 35.5°C in liquid Diamond's TYI-S-33 medium (Diamond et al., 1978).

Ethanolic and aqueous extracts of plants used in traditional medicine for treating dysentery were tested for anti-amoebic activity against *E. histolytica*. Approximately 10 mg extract was placed in a pill vial and allowed to evaporate. Ethanol or water (50 μl) was added to the ethanol and water extracts, respectively, followed by culture medium to result in concentrations of 10 mg ml^{-1}. Plant extracts were dissolved or suspended for 5–10 min in a Julabo ultrasound bath. Twofold serial dilutions were made in wells of 96-well microtitre plates (Nunc) in 170 μl culture medium. Each plate included metronidazole as a standard amoebicidal drug, control wells (culture medium with amoebae), and a blank (culture medium only).

The tubes of amoebal culture were placed in ice-cold water for 5 min to allow detachment of amoebae from the glass culture tube wall. The tubes were centrifuged for 5 min at 1800 rpm to form a pellet. The supernatant was discarded and fresh culture medium was added in order to have 10^5 amoebae ml^{-1} in each tube. An amoebal suspension (170 μl) was added to each of the control and test wells. Plates were covered with expanded polystyrene (approximately 5 mm thick), partially sealed with tape and gassed for 10 min with nitrogen before being sealed and incubated at 35.5°C for 72 h.

After incubation, the growth of amoebae was checked with a microscope. Numbers of amoebae in each well were estimated using a haemocytometer and compared with the metronidazole standard and control wells. The 50% inhibitory concentration (IC_{50}), or concentration of plant extract killing approximately 50% of amoebae in the test wells as compared with those in the control, for each extract was recorded.

3. Results and discussion

3.1. Antibacterial activity

The antibacterial activities of crude hexanic, ethanolic and aqueous extracts are presented in Table 2. In total, 138 extracts belonging to 46 species (45 genera in 31 families) were tested. Plants exhibiting no antibacterial activity were *Albizia adianthifolia*, *Aloe arborescens*, *Aloe marlothii*, *Buddleja salviifolia*, *Bulbine latifolia*, *Canthium inerme*, *Ceratotheca triloba*, *Cinnamomum camphora*, *Clutia pulchella*, *Croton sylvaticus*, *Deinbollia oblongifolia*, *Kigelia africana*, *Lippia javanica*, *Melia azedarach*, *Mondia whitei*, *Ocotea bullata*, *Olea europaea*, *Ricinus communis*, *Stangeria eriopus*, *Strychnos spinosa*, *Tarchonanthus camphoratus*, *Tetradenia riparia*, *Tulbaghia violacea* and *Zanthoxylum capense*.

According to Vlietinck et al. (1995), Gram-positive bacteria (*B. subtilis* and *S. aureus*) were significantly more susceptible to the extracts tested than the Gram-negative ones (*E. coli* and *K. pneumoniae*). Only four extracts showed activity against *E. coli*, while two extracts were active against *K. pneumoniae*. Concerning results against Gram-positive bacteria, 27 extracts were active against *B. subtilis* and 24 of them against *S. aureus*. The ethanolic extracts displayed the greatest antibacterial activity, with 20 active extracts. Ten aqueous extracts showed activity while only seven hexane extracts were active.

Table 2
Determination of the antibacterial activity of South African medicinal plants with the disc-diffusion and microdilution assays (MIC recorded in mg ml^{-1}).

Botanical name	Plant part[a]	Extract[b]	Bacteria tested[c]							
			B.s.		E.c.		K.p.		S.a.	
			Dif[d]	MIC[e]	Dif	MIC	Dif	MIC	Dif	MIC
Acorus calamus	RH	H	0.29	0.39	0	–	0	–	0	–
		E	0.33	0.78	0	–	0	–	0	–
		W	0	–	0	–	0	–	0	–
Artemisia afra	LF	H	0	–	0	–	0	–	0	–
		E	0.2	0.39	0	–	0	–	0.33	0.39
		W	0	–	0	–	0	–	0	–
Asclepias fruticosa	LF	H	0	–	0.22	6.25	0	–	0	–
		E	0	–	0	–	0	–	0	–
		W	0	–	0	–	0	–	0	–
Cassine transvaalensis	BK	H	0	–	0	–	0	–	0	–
		E	0.23	0.195	0	–	0	–	0.58	0.098
		W	0.18	0.78	0	–	0	–	0.33	0.195
Catha edulis	RT	H	0	–	0	–	0	–	0	–
		E	0.21	0.012	0	–	0	–	0.50	0.012
		W	0	–	0	–	0	–	0	–
Combretum apiculatum	LF	H	0	–	0	–	0	–	0	–
		E	0.17	0.049	0	–	0	–	0.29	0.049
		W	0.15	0.39	0	–	0	–	0.43	0.39
Curtisia dentata	BK	H	0	–	0	–	0	–	0	–
		E	0.28	0.78	0	–	0	–	0	–
		W	0.13	3.13	0	–	0	–	0	–
Cussonia spicata	LF	H	0.14	12.5	0.29	6.25	0	–	0.14	12.5
		E	0.29	3.13	0.29	3.13	0.125	12.5	0	–
		W	0	–	0	–	0	–	0	–
Dombeya rotundifolia	LF	H	0	–	0	–	0	–	0	–
		E	0.40	1.56	0	–	0	–	0.33	1.56
		W	0.40	1.56	0	–	0	–	0.36	1.56
Erythrophleum lasianthum	LF	H	0	–	0	–	0	–	0	–
		E	0.17	0.78	0	–	0	–	0	–
		W	0	–	0	–	0	–	0	–
Gunnera perpensa	RT/RH	H	0	–	0	–	0	–	0	–
		E	0	–	0	–	0	–	0.23	3.13
		W	0	–	0	–	0	–	0.23	0.78
Harpephyllum caffrum	BK	H	0.33	0.78	0	–	0	–	0	–
		E	0.50	0.098	0.17	1.56	0.17	3.13	0.17	3.13
		W	0.25	1.56	0	–	0	–	0	–

Table 2 (*Continued*)

Botanical name	Plant part[a]	Extract[b]	Bacteria tested[c]							
			B.s.		E.c.		K.p.		S.a.	
			Dif[d]	MIC[e]	Dif	MIC	Dif	MIC	Dif	MIC
Heteromorpha trifoliata	LF	H	0	–	0	–	0	–	0	–
		E	0.25	0.78	0	–	0	–	0	–
		W	0	–	0	–	0	–	0	–
Pittosporum viridiflorum BK	H	0.17	3.13	0	–	0	–	0	–	
		E	0.50	1.56	0	–	0	–	0.29	1.56
		W	0	–	0	–	0	–	0	–
Psychotria capensis	RT	H	0	–	0	–	0	–	0.43	12.5
		E	0	–	0	–	0	–	0.71	0.39
		W	0	–	0	–	0	–	0.43	3.13
Rauvolfia caffra	LF	H	0	–	0	–	0	–	0.13	0.78
		E	0	–	0	–	0	–	0.13	1.56
		W	0	–	0	–	0	–	0	–
Sansevieria hyacinthoides LF	H	0	–	0	–	0	–	0	–	
		E	0	–	0	–	0	–	0	–
		W	0.42	6.25	0	–	0	–	0	–
Schotia brachypetala	LF	H	0	–	0	–	0	–	0	–
		E	0.29	0.024	0	–	0	–	0.29	0.195
		W	0.25	1.56	0	–	0	–	0.14	6.25
Sclerocarya birrea	BK	H	0	–	0	–	0	–	0	–
		E	0.33	0.012	0	–	0	–	0.29	0.049
		W	0.33	0.78	0	–	0	–	0.43	0.78
Spirostachys africana	RT/ST	H	0	–	0	–	0	–	0	–
		E	0	–	0	–	0	–	0.36	3.13
		W	0	–	0	–	0	–	0	–
Tecomaria capensis	BK	H	0	–	0	–	0	–	0	–
		E	0	–	0	–	0	–	0.17	3.13
		W	0	–	0	–	0	–	0	–
Typha capensis	RH	H	0	–	0	–	0	–	0	–
		E	0.17	3.13	0	–	0	–	0	–
		W	0	–	0	–	0	–	0	–
Neomycin				2.0×10^{-4}		1.3×10^{-2}		7.8×10^{-4}		3.9×10^{-4}

[a] Plant part: BK, bark; LF, leaf; RH, rhizome; RT, root; ST, stem; TB, tuber; WH, whole plant.

[b] Extract: H, hexane; E, ethanol; W, water.

[c] Bacteria: B.s., *Bacillus subtilis*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; S.a., *Staphylococcus aureus*.

[d] Dif, Results obtained in the disc-diffusion assays. Antibacterial activity is expressed as the ratio of the inhibition diameter around the extract to the inhibition zone around the reference neomycin antibiotic. The symbol 0 indicates no activity, i.e. no inhibition zone around the extract discs.

[e] MIC, Results obtained in the dilution assays. Antibacterial activity is expressed as the minimum inhibitory concentration (mg ml^{-1}). –, MIC not determined.

MIC values of active extracts are shown in Table 2. Among plants tested, *Cassine transvaalensis*, *Catha edulis*, *Combretum apiculatum*, *Harpephyllum caffrum*, *Heteromorpha trifoliata*, *Schotia brachypetala* and *Sclerocarya birrea* showed the best activities with MIC < 200 μg ml^{-1} and could provide useful leads for the discovery of antibacterial compounds.

C. transvaalensis bark contains tannins, known antidiarrhoeic compounds (Bruneton, 1995). No antibacterial compounds have been reported from *C. edulis*. Martini and Eloff (1998) isolated several unidentified antibacterial compounds from *Combretum erythrophyllum*. Breytenbach and Malan (1989) isolated three unidentified antibacterial compounds from *Combretum zeyheri*. These researchers ascribed the ethnopharmacological use of this plant against diarrhoea to its antibacterial properties, particularly towards Gram-positive species. Antimicrobial components have been found in several other *Combretum* species (Alexander et al., 1992; Eloff, 1999). *H. caffrum* is reported to contain phenolic compounds that may be responsible for its biological activity (El Sherbeiny and El Ansari, 1976).

Chemical compounds of *Schotia* species have not been well investigated. Polyhydroxystilbenes have been isolated from the heartwood of *S. brachypetala*. Many stilbenes have antibiotic properties but the biological activity of the *Schotia* stilbenes is not known (Van Wyk et al., 1997). Astringent tannins are present in the bark (Bruneton, 1995), and activity may be partly attributed to these compounds. In preliminary studies, the antidiarrhoeal effects of *S. birrea* bark have been linked to procyanidins (Galvez et al., 1993). Extracts from stem bark have shown antibacterial activity against several species of bacteria (Hussein and Deeni, 1991).

It is interesting to note that although we have not found antibacterial activity in extracts of *K. africana* and *T. riparia*, both species had previously shown antimicrobial properties. Antimicrobial activity of the aqueous extracts of stem bark of *K. africana* (synonym *K. pinnata*) (Akunyili et al., 1991) has been partially attributed to the presence of iridoids (Akunyili et al., 1991). Fruit and bark extracts of *K. africana* have also displayed antibacterial activity (O. Grace, personal communication, 1999). *T. riparia* leaves have been reported to inhibit several mycobacteria (Van Puyvelde et al., 1994). A diterpene diol from the same species exhibited significant antimicrobial activity against several bacteria and fungi (Van Puyvelde et al., 1986).

3.2. Anthelmintic activity

The anthelmintic test systems using *C. elegans* are cheap and easy to use. The IC_{50} values of the standard anthelmintic drug levamisole were calculated to be 4.674 and 6.901 μg ml^{-1} for the 2-h and 7-day assays, respectively. Simpkin and Coles (1981) reported the minimum detectable dose of levamisole on *C. elegans* as 0.5 μg ml^{-1}. No literature available reports IC_{50} values. Inhibition of *C. elegans* by plant extracts in both anthelmintic assays is recorded in Table 3. The number of extracts tested was 72, derived from 24 genera (18 families).

Several crude extracts of plants used by traditional healers for treating intestinal worms displayed activity against *C. elegans*. The results of the two anthelmintic assays correlated well, with the 7-day incubation assay appearing to be more sensitive than the shorter assay. Many extracts exhibited activity at a concentration of 2 mg ml^{-1} but a more selective effect was noted at 1 mg ml^{-1}. In general, water extracts were more active than ethanol extracts, and very little anthelmintic activity was shown by hexane extracts.

Acokanthera oblongifolia was the only plant displaying no anthelmintic activity. Anthelmintic activity has been reported in ethanol extracts of root bark of *Albizia anthelmintica*, and the active principle was identified as musennin (Tschesche and Forstman, 1957). However, *A. adianthifolia* did not display significant anthelmintic activity.

Extracts of *Acorus calamus* exhibited a high level of anthelmintic activity. There are possibly carcinogenic and toxic compounds in *A. calamus*, with toxic effects owing to the presence of β-asarone, a phenylpropanoid (Lander and Schreier, 1990; Bruneton, 1995). This compound may cause liver and duodenal cancer (Bruneton, 1995). *A. marlothii* displayed some activity against the ne-

Table 3
Inhibition of nematodes by plant extracts (2-h and 7-day anthelmintic assays)

Plant name	Plant part[a]	Extract[b]	Concentration (mg ml^{-1})	Activity[c]	
				2 h	7 day
Acokanthera oblongifolia	LF	H	2	–	–
			1	–	–
		E	2	–	–
			1	–	–
		W	2	–	–
			1	–	–
Acorus calamus	RH/RT	H	2	+	++
			1	+	+
		E	2	++	+++
			1	+	++
		W	2	–+	+
			1	–+	+
Albizia adianthifolia	LF	H	2	–	+
			1	–	–
		E	2	+	+
			1	–+	–
		W	2	+	+++
			1	+	+++
Aloe marlothii	LF	H	2	–+	–
			1	–+	–
		E	2	++	++
			1	+	++
		W	2	+	++
			1	–+	–
Apodytes dimidiata	LF	H	2	–	–
			1	–	–
		E	2	+	++
			1	+	+
		W	2	–+	–
			1	–+	–
Artemisia afra	LF	H	2	–	–
			1	–	–
		E	2	+	++
			1	+	++
		W	2	++	+++
			1	+	++
Brachylaena discolor	LF	H	2	+	+
			1	–+	–
		E	2	+	++
			1	–	++
		W	2	–+	++
			1	–	–
Clausena anisata	LF	H	2	–	–
			1	–	–
		E	2	+	++
			1	+	++
		W	2	+	+++
			1	+	+
Clerodendrum glabrum	LF	H	2	–	–
			1	–	–

Table 3 (*Continued*)

Plant name	Plant part[a]	Extract[b]	Concentration (mg ml^{-1})	Activity[c]	
				2 h	7 day
		E	2	−+	++
			1	−	−
		W	2	−	+
			1	−	−
Ekebergia capensis	LF	H	2	−	+
			1	−	−
		E	2	−+	+
			1	−	−
		W	2	−	−+
			1	−	−+
Erythrophleum lasianthum	LF	H	2	−	−
			1	−	−
		E	2	−+	++
			1	−	+
		W	2	−+	++
			1	−	+
Euclea divinorum	BK	H	2	−	−
			1	−	−
		E	2	+	−
			1	−+	−
		W	2	−+	++
			1	−+	++
Heteromorpha trifoliata	LF	H	2	−	−
			1	−	−
		E	2	−+	−
			1	−	−
		W	2	−	+
			1	−	−
Kigelia africana	LF	H	2	−+	−+
			1	−	−+
		E	2	−	−
			1	−	−
		W	2	−+	++
			1	−	+
Leonotis leonurus	LF	H	2	−+	−
			1	−	−
		E	2	−+	++
			1	−	++
		W	2	−	++
			1	−	++
Lippia javanica	LF/TW	H	2	−	−+
			1	−	−
		E	2	−+	+
			1	−	−
		W	2	−	+++
			1	−	+++
Maesa lanceolata	LF	H	2	−+	−
			1	−+	−
		E	2	−	+
			1	−	−+
		W	2	−	−
			1	−	−

Table 3 (*Continued*)

Plant name	Plant part[a]	Extract[b]	Concentration (mg ml^{-1})	Activity[c]	
				2 h	7 day
Melia azedarach	LF	H	2	−+	−
			1	−	−
		E	2	−	−
			1	−	−
		W	2	−+	+++
			1	−	++
Pimpinella caffra	WH	H	2	−	−
			1	−	−
		E	2	−	−
			1	−	−
		W	2	−	++
			1	−	+
Ricinus communis	LF	H	2	−+	++
			1	−+	+
		E	2	−	++
			1	−	+
		W	2	+	++++
			1	−	+++
Sansevieria hyacinthoides	LF	H	2	−	−
			1	−	−
		E	2	−	−
			1	−	−
		W	2	−	+++
			1	−	++
Trema orientalis	BK/WD	H	2	−+	++
			1	−+	+
		E	2	−+	++
			1	−+	++
		W	2	−	+++
			1	−	+++
Tulbaghia violacea	TB	H	2	−	−
			1	−	−
		E	2	+	+++
			1	−+	++
		W	2	−+	++++
			1	−	+++
Zanthoxylum capense	LF	H	2	−	−
			1	−	−
		E	2	−	+
			1	−	+
		W	2	−	−
			1	−	−

[a] Plant part: BK, bark; LF, leaf; RH, rhizome; RT, root; TB, tuber; TW, twig; WD, wood; WH, whole plant.

[b] Extract: H, hexane; E, ethanol; W, water.

[c] Activity: −, same number of nematodes as control; −+, about 80% of nematodes in control, worms move slightly slower; +, small increase (about half number in control), worms move slowly; ++, very small increase (less than 20% of control), worms move slowly; +++, no increase in number, worms move very slowly; ++++, no increase in number, no movement.

Table 4
Anti-amoebic activity in South African medicinal plants

Botanical name	Plant part[a]	Extract[b]	IC_{50}[c] (mg ml^{-1})
Acorus calamus	RH	E	0.3125
		W	2.5
Albizia adianthifolia	LF	E	>5.0
		W	>5.0
Bidens pilosa	LF	E	–
		W	>5.0
Deinbollia oblongifolia	RT	E	>5.0
		W	>5.0
Pittosporum viridiflorum	BK	E	>5.0
		W	–
Schotia brachypetala	RT	E	>5.0
		W	–
Sclerocarya birrea	BK	E	1.25
		W	>5.0
Typha capensis	RH	E	5.0
		W	–
Ziziphus mucronata	LF	E	>5.0
		W	–
Metronidazole			0.32×10^{-3}

[a] Plant part: BK, bark; LF, leaf; RH, rhizome; RT, root; TB, tuber; TW, twig; WD, wood.
[b] Extract: E, ethanol; W, water.
[c] IC_{50}: –, no activity (no effect on amoebae).

matodes. Other species of *Aloe* are known to have a laxative action; for example, *Aloe ferox* (Van Wyk et al., 1997). The main purgative principle of *A. ferox* is the anthrone C-glucoside aloin, or barbaloin (Van Wyk et al., 1997). This compound, or a related compound, may occur in *A. marlothii*. Other plants with marked activity against *C. elegans* included *Artemisia afra*, *Brachylaena discolor*, *Clausena anisata*, *R. communis*, *Trema orientalis* and *T. violacea*. Many chemical constituents have been isolated from different parts of *C. anisata* (Hutchings et al., 1996). The volatile oil of the leaves contains the toxic estragole (Okunade and Olaifa, 1987). The alkaloids imperatorin and xanthoxyletin, isolated from the root or root bark, have molluscicidal activity (Hutchings et al., 1996). *R. communis*, the castor oil plant, contains the fatty acid ricinoleic acid, which stimulates intestinal peristalsis (Van Wyk et al., 1997). This purgative action assists in clearing the body of intestinal parasites, but the direct effect of the plant extract on the nematodes remains unexplained. The bark and wood of *T. orientalis* contain tannin (Watt and Breyer-Brandwijk, 1962). Many chemical constituents of *T. violacea* have been isolated, including several sulphur compounds and steroidal saponins (Burton, 1990), but it is not known whether these compounds are responsible for the biological activity of the plant.

3.3. Anti-amoebic activity

The calculated IC_{50} for the amoebicidal compound metronidazole of 0.202 μg ml^{-1} was close to the values of 0.22 and 0.32 μg ml^{-1} obtained in previously published experiments (Keene et al., 1986; Wright et al., 1988). The activity of plant extracts against *Entamoeba histolytica* is shown in Table 4. Forty-two extracts from 21 genera (18 families) were assayed. *B. latifolia*, *C. inerme*, *C. pulchella*, *Ekebergia capensis*, *H. trifoliata*, *K. africana*, *Leonotis leonurus*, *L. javanica*, *Senna didymobotrya*, *Spirostachys africana*, *Tecomaria capensis* and *T. orientalis* did not exhibit activity in both ethanol and water extracts (results not shown). Wright et al. (1988) found that the extracts of many plant species were inactive, attributing this to the selectivity of the anti-amoebic test. These researchers also reported that the greatest activity was found in methanol extracts, with water extracts of the same plants displaying less activity. In accordance with this, we found here that ethanolic extracts of *A. calamus* and *S. birrea* were more active than water extracts. In total, eight ethanol extracts showed anti-amoebic activity while five water extracts were active.

The high activity of *A. calamus* may be attributed to the presence of the toxic phenylpropanoid β-asarone. Other active plants included *A. adianthifolia*, *D. oblongifolia* and *S. birrea*. Several compounds have been isolated from the bark and roots of *A. adianthifolia* (Hutchings et al., 1996) but little chemical work has been reported on the leaves. Little chemical investigation has been carried out on *D. oblongifolia*. The bark of *S. birrea* contains tannin and traces of alkaloids (Watt and Breyer-Brandwijk, 1962). Galvez et al. (1991, 1993) reported that procyanidin from its bark inhibits peristaltic reflexes in guinea-pig

colon and shows antidiarrhoeal activity on isolated guinea-pig ileum and against various induced diarrhoeas in mice.

It is hoped that the results presented in this study will not only provide useful leads for the discovery of clinically useful antibacterial, anthelmintic and anti-amoebic drugs, but also encourage research on South African plants used in traditional medicine.

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